SECTION IV DRUGS WITH IMPORTANT ACTIONS ON SMOOTH MUSCLE

CHAPTER 16

Histamine, Serotonin, & the Ergot Alkaloids

Bertram G. Katzung, MD, PhD

CASE STUDY

A 35-year-old man visits his family practitioner with a complaint of red, raised, itchy wheals on his arms and legs. Two days earlier, he had eaten a spicy meal at a restaurant he had not previously visited. The following morning, he woke up with the palms of his hands and the soles of his feet red and itchy. During the day, similar raised, itchy lesions appeared on his arms and legs, and some are now appearing on his trunk. He reports a similar, milder episode 2 years ago, from which he recovered without treatment. Physical examination reveals no respiratory symptoms and no evidence of pharyngeal edema. The practitioner makes a diagnosis of urticaria (hives) caused by food allergy and suggests that the patient take an over-the-counter antihistamine. The following day the patient calls saying that the antihistamine has reduced the itching slightly, but the wheals are still present and new ones are appearing. What other therapeutic measures are appropriate?

It has long been known that many tissues contain substances that, when released by various stimuli, cause physiologic effects such as reddening of the skin, pain or itching, and bronchospasm. Later, it was discovered that many of these substances are also present in nervous tissue and have multiple functions. Histamine and serotonin (5-hydroxytryptamine, 5-HT) are biologically active amines that function as neurotransmitters and are found in non-neural tissues, have complex physiologic and pathologic effects through multiple receptor subtypes, and are often released locally. Together with endogenous peptides (see Chapter 17), prostaglandins and leukotrienes (see Chapter 18), and cytokines (see Chapter 55), they constitute the **autacoid group** of drugs.

Because of their broad and largely undesirable peripheral effects, neither histamine nor serotonin has any clinical application in the treatment of disease. However, compounds that *selectively* activate certain receptor subtypes or selectively antagonize the actions of these amines are of considerable clinical usefulness. This chapter therefore emphasizes the basic pharmacology of the agonist amines and the clinical pharmacology of the more selective agonist and antagonist drugs. The ergot alkaloids,

compounds with partial agonist activity at serotonin and several other receptors, are discussed at the end of the chapter.

HISTAMINE

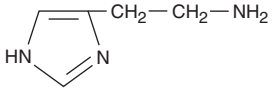
Histamine was synthesized in 1907 and later isolated from mammalian tissues. Early hypotheses concerning the possible physiologic roles of tissue histamine were based on similarities between the effects of intravenously administered histamine and the symptoms of anaphylactic shock and tissue injury. Marked species variation is observed, but in humans histamine is an important mediator of immediate allergic (such as urticaria) and inflammatory reactions, although it plays only a modest role in anaphylaxis. Histamine plays an important role in gastric acid secretion (see Chapter 62) and functions as a neurotransmitter and neuromodulator (see Chapters 6 and 21). Newer evidence indicates that histamine also plays a role in immune functions and chemotaxis of white blood cells.

BASIC PHARMACOLOGY OF HISTAMINE

Chemistry & Pharmacokinetics

Histamine occurs in plants as well as in animal tissues and is a component of some venoms and stinging secretions.

Histamine is formed by decarboxylation of the amino acid L-histidine, a reaction catalyzed in mammalian tissues by the enzyme histidine decarboxylase. Once formed, histamine is either stored or rapidly inactivated. Very little histamine is excreted unchanged. The major metabolic pathways involve conversion to *N*-methylhistamine, methylimidazoleacetic acid, and imidazoleacetic acid (IAA). Certain neoplasms (systemic mastocytosis, urticaria pigmentosa, gastric carcinoid, and occasionally myelogenous leukemia) are associated with increased numbers of mast cells or basophils and with increased excretion of histamine and its metabolites.



Histamine

Most tissue histamine is sequestered and bound in granules (vesicles) in mast cells or basophils; the histamine content of many tissues is directly related to their mast cell content. The bound form of histamine is biologically inactive, but as noted below, many stimuli can trigger the release of mast cell histamine, allowing the free amine to exert its actions on surrounding tissues. Mast cells are especially rich at sites of potential tissue injury—nose, mouth, and feet; internal body surfaces; and blood vessels, particularly at pressure points and bifurcations.

Non-mast cell histamine is found in several tissues, including the brain, where it functions as a neurotransmitter. Strong evidence implicates endogenous neurotransmitter histamine in many brain functions such as neuroendocrine control, cardiovascular regulation, thermal and body weight regulation, and sleep and arousal (see Chapters 21 and 37).

A second important nonneuronal site of histamine storage and release is the enterochromaffin-like (ECL) cells of the fundus of the stomach. ECL cells release histamine, one of the primary gastric acid secretagogues, to activate the acid-producing parietal cells of the mucosa (see Chapter 62).

Storage & Release of Histamine

The stores of histamine in mast cells can be released through several mechanisms.

A. Immunologic Release

Immunologic processes account for the most important pathophysiologic mechanism of mast cell and basophil histamine release. These cells, if sensitized by IgE antibodies attached to their surface membranes, degranulate explosively when exposed to the appropriate antigen (see Figure 55–5, effector phase). This type of release also requires energy and calcium. Degranulation leads to the simultaneous release of histamine, adenosine triphosphate (ATP), and other mediators that are stored together in the granules. Histamine released by this mechanism is a mediator in immediate (type I) allergic reactions, such as hay fever and acute urticaria. Substances released during IgG- or IgM-mediated immune reactions that activate the complement cascade also release histamine from mast cells and basophils.

By a negative feedback control mechanism mediated by H_2 receptors, histamine appears to modulate its own release and that of other mediators from sensitized mast cells in some tissues. In humans, mast cells in skin and basophils show this negative feedback mechanism; lung mast cells do not. Thus, histamine may act to limit the intensity of the allergic reaction in the skin and blood.

Endogenous histamine has a modulating role in a variety of inflammatory and immune responses. Upon injury to a tissue, released histamine causes local vasodilation and leakage of plasma-containing mediators of acute inflammation (complement, C-reactive protein) and antibodies. Histamine has an active chemotactic attraction for inflammatory cells (neutrophils, eosinophils, basophils, monocytes, and lymphocytes). Histamine inhibits the release of lysosome contents and several T- and B-lymphocyte functions. Most of these actions are mediated by H_2 or H_4 receptors. Release of peptides from nerves in response to inflammation is also probably modulated by histamine, in this case acting through presynaptic H_3 receptors.

B. Chemical and Mechanical Release

Certain amines, including drugs such as morphine and tubocurarine, can displace histamine from its bound form within cells. This type of release does not require energy and is not associated with mast cell injury or degranulation. Loss of granules from the mast cell also releases histamine, since sodium ions in the extracellular fluid rapidly displace the amine from the complex. Chemical and mechanical mast cell injury causes degranulation and histamine

release. **Compound 48/80,** an experimental drug, selectively releases histamine from tissue mast cells by an exocytotic degranulation process requiring energy and calcium.

Pharmacodynamics

A. Mechanism of Action

Histamine exerts its biologic actions by combining with specific cellular receptors located on the surface membrane. Four different histamine receptors have been characterized and are designated H_1–H_4; they are described in Table 16–1. Unlike the other amine transmitter receptors discussed previously, no subfamilies have been found within these major types, although different splice variants of several receptor types have been described.

All four receptor types have been cloned and belong to the large superfamily of receptors having seven membrane-spanning regions and coupled with G proteins (GPCR). The structures of the H_1 and H_2 receptors differ significantly and appear to be more closely related to muscarinic and 5-HT_1 receptors, respectively, than to each other. The H_4 receptor has about 40% homology with the H_3 receptor but does not seem to be closely related to any other histamine receptor. All four histamine receptors have been shown to have constitutive activity in some systems; thus, some antihistamines previously considered to be traditional pharmacologic antagonists must now be considered to be inverse agonists (see Chapters 1 and 2). Indeed, many first- and second-generation H_1 blockers (see below) function as inverse agonists. Furthermore, a single molecule may be an agonist at one histamine receptor and an antagonist or inverse agonist at another. For example, clobenpropit, an agonist at H_4 receptors, is an antagonist or inverse agonist at H_3 receptors (Table 16–1).

In the brain, H_1 and H_2 receptors are located on postsynaptic membranes, whereas H_3 receptors are predominantly presynaptic. Activation of H_1 receptors, which are present in endothelium, smooth muscle cells, and nerve endings, usually elicits an increase in phosphoinositol hydrolysis and an increase in inositol trisphosphate (IP_3) and intracellular calcium. Activation of H_2 receptors, present in gastric mucosa, cardiac muscle cells, and some immune cells, increases intracellular cyclic adenosine monophosphate (cAMP) via G_s. Like the β_2 adrenoceptor, under certain circumstances the H_2 receptor may couple to G_q, activating the IP_3-DAG (inositol 1,4,5-trisphosphate-diacylglycerol) cascade. Activation of H_3 receptors decreases transmitter release from histaminergic and other neurons, probably mediated by a decrease in calcium influx through N-type calcium channels in nerve endings. H_4 receptors are found mainly on leukocytes in the bone marrow and circulating blood. H_4 receptors appear to have very important chemotactic effects on eosinophils and mast cells. In this role, they seem to play a part in inflammation and allergy. They may also modulate production of these cell types and they may mediate, in part, the previously recognized effects of histamine on cytokine production.

B. Tissue and Organ System Effects of Histamine

Histamine exerts powerful effects on smooth and cardiac muscle, on certain endothelial and nerve cells, on the secretory cells of the stomach, and on inflammatory cells. However, sensitivity to histamine varies greatly among species. Guinea pigs are exquisitely sensitive; humans, dogs, and cats somewhat less so; and mice and rats very much less so.

1. Nervous system—Histamine is a powerful stimulant of sensory nerve endings, especially those mediating pain and itching. This H_1-mediated effect is an important component of the urticarial response and reactions to insect and nettle stings. Some evidence suggests that local high concentrations can also depolarize efferent (axonal) nerve endings (see Triple Response, item 8 in this list). In the mouse, and probably in humans, respiratory neurons signaling inspiration and expiration are modulated by H_1 receptors. H_1 and H_3 receptors play important roles in appetite and satiety; antipsychotic drugs that block these receptors cause significant weight gain (see Chapter 29). Presynaptic H_3 receptors play important roles in modulating release of several transmitters in the nervous system. H_3 agonists reduce the release of acetylcholine, amine, and peptide transmitters in various areas of the brain and in peripheral nerves.

2. Cardiovascular system—In humans, injection or infusion of histamine causes a decrease in systolic and diastolic blood pressure and an increase in heart rate. The blood pressure changes are caused by the direct vasodilator action of histamine on arterioles and precapillary sphincters; the increase in heart rate involves both stimulatory actions of histamine on the heart and a reflex tachycardia. Flushing, a sense of warmth, and headache may also occur during histamine administration, consistent with the vasodilation.

TABLE 16–1 Histamine receptor subtypes.

Receptor Subtype	Distribution	Postreceptor Mechanism	Partially Selective Agonists	Partially Selective Antagonists or Inverse Agonists
H_1	Smooth muscle, endothelium, brain	G_q, ↑ IP_3, DAG	Histaprodifen	Mepyramine,[1] triprolidine, cetirizine
H_2	Gastric mucosa, cardiac muscle, mast cells, brain	G_s, ↑ cAMP	Amthamine	Cimetidine,[1] ranitidine,[1] tiotidine
H_3	Presynaptic autoreceptors and heteroreceptors: brain, myenteric plexus, other neurons	G_i, ↓ cAMP	*R*-α-Methylhistamine, imetit, immepip	Thioperamide,[1] iodophenpropit, clobenpropit,[1] tiprolisant[1]
H_4	Eosinophils, neutrophils, CD4 T cells	G_i, ↓ cAMP	Clobenpropit, imetit, clozapine	Thioperamide[1]

[1]Inverse agonist.

cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; IP_3, inositol trisphosphate.

Vasodilation elicited by small doses of histamine is caused by H_1-receptor activation and is mediated mainly by release of nitric oxide from the endothelium (see Chapter 19). The decrease in blood pressure is usually accompanied by a reflex tachycardia. Higher doses of histamine activate the H_2-mediated cAMP process of vasodilation and direct cardiac stimulation. In humans, the cardiovascular effects of small doses of histamine can usually be antagonized by H_1-receptor antagonists alone.

Histamine-induced edema results from the action of the amine on H_1 receptors in the vessels of the microcirculation, especially the postcapillary vessels. The effect is associated with the separation of the endothelial cells, which permits the transudation of fluid and molecules as large as small proteins into the perivascular tissue. This effect is responsible for urticaria (hives), which signals the release of histamine in the skin. Studies of endothelial cells suggest that actin and myosin within these cells cause contraction, resulting in separation of the endothelial cells and increased permeability.

Direct cardiac effects of histamine include both increased contractility and increased pacemaker rate. These effects are mediated chiefly by H_2 receptors. In human atrial muscle, histamine can also decrease contractility; this effect is mediated by H_1 receptors. The physiologic significance of these cardiac actions is not clear. Some of the cardiovascular signs and symptoms of anaphylaxis are due to released histamine, although several other mediators are involved and appear to be more important than histamine in humans.

3. Bronchiolar smooth muscle—In both humans and guinea pigs, histamine causes bronchoconstriction mediated by H_1 receptors. In the guinea pig, this effect is the cause of death from histamine toxicity, but in humans with normal airways, bronchoconstriction following small doses of histamine is not marked. However, patients with asthma are very sensitive to histamine. The bronchoconstriction induced in these patients probably represents a hyperactive neural response, since such patients also respond excessively to many other stimuli, and the response to histamine can be blocked by autonomic blocking drugs such as ganglion blocking agents as well as by H_1-receptor antagonists (see Chapter 20). Although methacholine provocation is more commonly used, tests using small doses of inhaled histamine have been used in the diagnosis of bronchial hyperreactivity in patients with suspected asthma or cystic fibrosis. Such individuals may be 100 to 1000 times more sensitive to histamine (and methacholine) than are normal subjects. Curiously, a few species (eg, rabbit) respond to histamine with broncho*dilation*, reflecting the dominance of the H_2 receptor in their airways.

4. Gastrointestinal tract smooth muscle—Histamine causes contraction of intestinal smooth muscle, and histamine-induced contraction of guinea pig ileum is a standard bioassay for this amine. The human gut is not as sensitive as that of the guinea pig, but large doses of histamine may cause diarrhea, partly as a result of this effect. This action of histamine is mediated by H_1 receptors.

5. Other smooth muscle organs—In humans, histamine generally has insignificant effects on the smooth muscle of the eye and genitourinary tract. However, pregnant women suffering anaphylactic reactions may abort as a result of histamine-induced contractions, and in some species the sensitivity of the uterus is sufficient to form the basis for a bioassay.

6. Secretory tissue—Histamine has long been recognized as a powerful stimulant of gastric acid secretion and, to a lesser extent, of gastric pepsin and intrinsic factor production. The effect is caused by activation of H_2 receptors on gastric parietal cells and is associated with increased adenylyl cyclase activity, cAMP concentration, and intracellular Ca^{2+} concentration. Other stimulants of gastric acid secretion such as acetylcholine and gastrin do not increase cAMP even though their maximal effects on acid output can be reduced—but not abolished—by H_2-receptor antagonists. These actions are discussed in more detail in Chapter 62. Histamine also stimulates secretion in the small and large intestine. In contrast, H_3-selective histamine agonists *inhibit* acid secretion stimulated by food or pentagastrin in several species.

Histamine has much smaller effects on the activity of other glandular tissue at ordinary concentrations. Very high concentrations can cause adrenal medullary discharge.

7. Metabolic effects—Recent studies of H_3-receptor knockout mice demonstrate that absence of this receptor results in animals with increased food intake, decreased energy expenditure, and obesity. They also show insulin resistance and increased blood levels of leptin and insulin. It is not yet known whether the H_3 receptor has a similar role in humans, but intensive research is underway to determine whether H_3 agonists are useful in the treatment of obesity.

8. The "triple response"—Intradermal injection of histamine causes a characteristic red spot, edema, and flare response that was first described many years ago. The effect involves three separate cell types: smooth muscle in the microcirculation, capillary or venular endothelium, and sensory nerve endings. At the site of injection, a reddening appears owing to dilation of small vessels, followed soon by an edematous wheal at the injection site and a red irregular flare surrounding the wheal. The flare is said to be caused by an axon reflex. A sensation of itching may accompany these effects.

Similar local effects may be produced by injecting histamine liberators (compound 48/80, morphine, etc) intradermally or by applying the appropriate antigens to the skin of a sensitized person. Although most of these local effects can be prevented by adequate doses of an H_1-receptor–blocking agent, H_2 and H_3 receptors may also be involved.

9. Other effects possibly mediated by histamine receptors—In addition to the local stimulation of peripheral pain nerve endings via H_3 and H_1 receptors, histamine may play a role in nociception in the central nervous system. **Burimamide,** an early candidate for H_2-blocking action, and newer analogs with no effect on H_1, H_2, or H_3 receptors, have been shown to have significant analgesic action in rodents when administered into the central nervous system. The analgesia is said to be comparable to that produced by opioids, but tolerance, respiratory depression,

and constipation have not been reported. Although the mechanism of this action is not known, these compounds may represent an important new class of analgesics.

Other Histamine Agonists

Small substitutions on the imidazole ring of histamine significantly modify the selectivity of the compounds for the histamine receptor subtypes. Some of these are listed in Table 16–1.

CLINICAL PHARMACOLOGY OF HISTAMINE

Clinical Uses

In pulmonary function laboratories, histamine aerosol has been used as a **provocative test** of bronchial hyperreactivity. Histamine has no other current clinical applications.

Toxicity & Contraindications

Adverse effects of histamine release, like those following administration of histamine, are dose related. Flushing, hypotension, tachycardia, headache, wheals, bronchoconstriction, and gastrointestinal upset are noted. These effects are also observed after the ingestion of spoiled fish (scombroid fish poisoning), and there is evidence that histamine produced by bacterial action in the flesh of the fish is the major causative agent.

Histamine should not be given to patients with asthma (except as part of a carefully monitored test of pulmonary function) or to patients with active ulcer disease or gastrointestinal bleeding.

HISTAMINE ANTAGONISTS

The effects of histamine released in the body can be reduced in several ways. **Physiologic antagonists,** especially epinephrine, have smooth muscle actions opposite to those of histamine, but they act at different receptors. This is important clinically because injection of epinephrine can be lifesaving in systemic **anaphylaxis** and in other conditions in which massive release of histamine—and other more important mediators—occurs.

Release inhibitors reduce the degranulation of mast cells that results from immunologic triggering by antigen-IgE interaction. **Cromolyn** and **nedocromil** appear to have this effect (see Chapter 20) and have been used in the treatment of asthma, although the molecular mechanism underlying their action is not fully understood. Beta$_2$-adrenoceptor agonists also appear capable of reducing histamine release.

Histamine **receptor antagonists** represent a third approach to the reduction of histamine-mediated responses. For over 60 years, compounds have been available that competitively antagonize many of the actions of histamine on smooth muscle. However, not until the H_2-receptor antagonist burimamide was described in 1972 was it possible to antagonize the gastric acid-stimulating activity of histamine. The development of selective H_2-receptor antagonists has led to more effective therapy for peptic disease (see Chapter 62).

Selective H_3 and H_4 antagonists are not yet available for clinical use. However, potent and partially selective experimental H_3-receptor antagonists, thioperamide and clobenpropit, have been developed.

■ HISTAMINE RECEPTOR ANTAGONISTS

H_1-RECEPTOR ANTAGONISTS

Compounds that competitively block histamine or act as inverse agonists at H_1 receptors have been used in the treatment of allergic conditions for many years, and in the discussion that follows are referred to as antagonists. Many H_1 antagonists are currently marketed in the USA. A large number are available without prescription, both alone and in combination formulations such as "cold pills" and "sleep aids" (see Chapter 63).

BASIC PHARMACOLOGY OF H_1-RECEPTOR ANTAGONISTS

Chemistry & Pharmacokinetics

The H_1 antagonists are conveniently divided into first-generation and second-generation agents. These groups are distinguished by the relatively strong sedative effects of most of the first-generation drugs. The first-generation agents are also more likely to block autonomic receptors. Second-generation H_1 blockers are less sedating, owing in part to their less complete distribution into the central nervous system. All the H_1 antagonists are stable amines with the general structure illustrated in Figure 16–1. Doses of some of these drugs are given in Table 16–2.

These agents are rapidly absorbed after oral administration, with peak blood concentrations occurring in 1–2 hours. They are widely distributed throughout the body, and the first-generation drugs enter the central nervous system readily. Some of them are extensively metabolized, primarily by microsomal systems in the liver. Several of the second-generation agents are metabolized by the CYP3A4 system and thus are subject to important interactions when other drugs (such as ketoconazole) inhibit this subtype of P450 enzymes. Most of the drugs have an effective duration of action of 4–6 hours following a single dose, but meclizine and several second-generation agents are longer-acting, with a duration of action of 12–24 hours. The newer agents are considerably less lipid-soluble than the first-generation drugs and are substrates of the P-glycoprotein transporter in the blood-brain barrier; as a result they enter the central nervous system with difficulty or not at all. Many H_1 antagonists have active metabolites. The active metabolites of hydroxyzine, terfenadine, and loratadine are available as drugs (cetirizine, fexofenadine, and desloratadine, respectively).

Pharmacodynamics

Both neutral H_1 antagonists and inverse H_1 agonists reduce or block the actions of histamine by reversible competitive binding

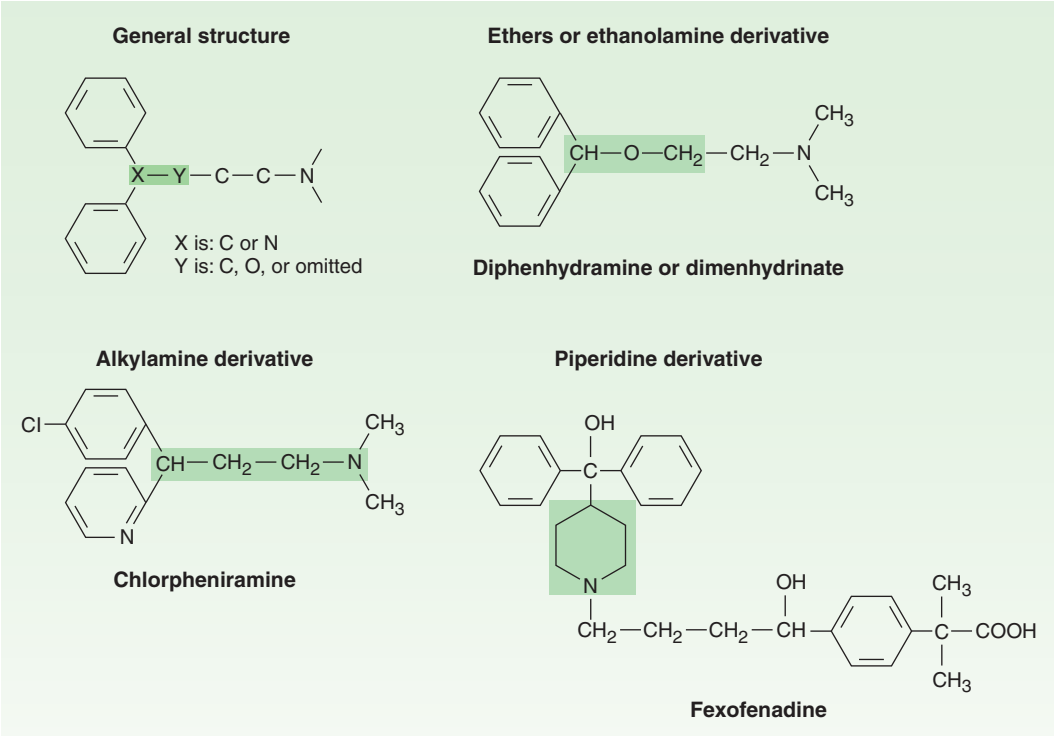


FIGURE 16–1 General structure of H_1-antagonist drugs and examples of the major subgroups. Chemical subgroups are indicated by shading.

to the H_1 receptor. Several have been clearly shown to be inverse agonists, and it is possible that all act by this mechanism. They have negligible potency at the H_2 receptor and little at the H_3 receptor. For example, histamine-induced contraction of bronchiolar or gastrointestinal smooth muscle can be completely blocked by these agents, but the effects on gastric acid secretion and the heart are unmodified.

The first-generation H_1-receptor antagonists have many actions in addition to blockade of the actions of histamine. The large number of these actions probably results from the similarity of the general structure (Figure 16–1) to the structure of drugs that have effects at muscarinic cholinoceptor, α adrenoceptor, serotonin, and local anesthetic receptor sites. Some of these actions are of therapeutic value and some are undesirable.

1. Sedation—A common effect of first-generation H_1 antagonists is sedation, but the intensity of this effect varies among chemical subgroups (Table 16–2) and among patients as well. The effect is sufficiently prominent with some agents to make them useful as "sleep aids" (see Chapter 63) and unsuitable for daytime use. The effect resembles that of some antimuscarinic drugs and is considered very different from the disinhibited sedation produced by sedative-hypnotic drugs. Compulsive use has not been reported. At ordinary dosages, children occasionally (and adults rarely) manifest excitation rather than sedation. At very high toxic dose levels, marked stimulation, agitation, and even convulsions may precede coma. Second-generation H_1 antagonists have little or no sedative or stimulant actions. These drugs (or their active metabolites) also have far fewer autonomic effects than the first-generation antihistamines.

2. Antinausea and antiemetic actions—Several first-generation H_1 antagonists have significant activity in preventing motion sickness (Table 16–2). They are less effective against an episode of motion sickness already present. Certain H_1 antagonists, notably doxylamine (in Bendectin), were used widely in the past in the treatment of nausea and vomiting of pregnancy (see below).

3. Antiparkinsonism effects—Some of the H_1 antagonists, especially **diphenhydramine,** have significant acute suppressant effects on the extrapyramidal symptoms associated with certain antipsychotic drugs. This drug is given parenterally for acute dystonic reactions to antipsychotics.

4. Anticholinoceptor actions—Many first-generation agents, especially those of the ethanolamine and ethylenediamine subgroups, have significant atropine-like effects on peripheral muscarinic receptors. This action may be responsible for some of the (uncertain) benefits reported for nonallergic rhinorrhea but may also cause urinary retention and blurred vision.

5. Adrenoceptor-blocking actions—Alpha-receptor blocking effects can be demonstrated for many H_1 antagonists, especially those in the phenothiazine subgroup, eg, **promethazine.** This action may cause orthostatic hypotension in susceptible individuals. Beta-receptor blockade is not observed.

6. Serotonin-blocking action—Strong blocking effects at serotonin receptors have been demonstrated for some first-generation

TABLE 16–2 Some H_1 antihistaminic drugs in clinical use.

Drugs	Usual Adult Dose	Anticholinergic Activity	Comments
FIRST-GENERATION ANTIHISTAMINES			
Ethanolamines			
Carbinoxamine (Clistin)	4–8 mg	+++	Slight to moderate sedation
Dimenhydrinate (salt of diphenhydramine) (Dramamine)	50 mg	+++	Marked sedation; anti-motion sickness activity
Diphenhydramine (Benadryl, etc)	25–50 mg	+++	Marked sedation; anti-motion sickness activity
Piperazine derivatives			
Hydroxyzine (Atarax, etc)	15–100 mg	nd	Marked sedation
Cyclizine (Marezine)	25–50 mg	–	Slight sedation; anti-motion sickness activity
Meclizine (Bonine, etc)	25–50 mg	–	Slight sedation; anti-motion sickness activity
Alkylamines			
Brompheniramine (Dimetane, etc)	4–8 mg	+	Slight sedation
Chlorpheniramine (Chlor-Trimeton, etc)	4–8 mg	+	Slight sedation; common component of OTC "cold" medication
Phenothiazine derivative			
Promethazine (Phenergan, etc)	10–25 mg	+++	Marked sedation; antiemetic; α block
Miscellaneous			
Cyproheptadine (Periactin, etc)	4 mg	+	Moderate sedation; significant antiserotonin activity
SECOND-GENERATION ANTIHISTAMINES			
Piperidine			
Fexofenadine (Allegra)	60 mg	–	
Miscellaneous			
Loratadine (Claritin), desloratadine (Clarinex)	10 mg (desloratadine, 5 mg)	–	Longer action; used at 5 mg dosage
Cetirizine (Zyrtec)	5–10 mg	–	

nd, no data found.

H_1 antagonists, notably **cyproheptadine**. This drug is promoted as an antiserotonin agent and is discussed with that drug group. Nevertheless, its structure resembles that of the phenothiazine antihistamines, and it is a potent H_1-blocking agent.

7. Local anesthesia—Several first-generation H_1 antagonists are potent local anesthetics. They block sodium channels in excitable membranes in the same fashion as procaine and lidocaine. Diphenhydramine and promethazine are actually more potent than procaine as local anesthetics. They are occasionally used to produce local anesthesia in patients allergic to conventional local anesthetic drugs. A small number of these agents also block potassium channels; this action is discussed below (see Toxicity).

8. Other actions—Certain H_1 antagonists, eg, cetirizine, inhibit mast cell release of histamine and some other mediators of inflammation. This action is not due to H_1-receptor blockade and may reflect an H_4-receptor effect (see below). The mechanism is not fully understood but could play a role in the beneficial effects of these drugs in the treatment of allergies such as rhinitis. A few H_1 antagonists (eg, terfenadine, acrivastine) have been shown to inhibit the P-glycoprotein transporter found in cancer cells, the epithelium of the gut, and the capillaries of the brain. The significance of this effect is not known.

CLINICAL PHARMACOLOGY OF H_1-RECEPTOR ANTAGONISTS

Clinical Uses

First-generation H_1-receptor blockers are among the most extensively promoted and used over-the-counter drugs. The prevalence of allergic conditions and the *relative* safety of the drugs contribute to this heavy use. The fact that they do cause sedation contributes to heavy prescribing and over-the-counter use of second-generation antihistamines.

A. Allergic Reactions

The H_1 antihistaminic agents are often the first drugs used to prevent or treat the symptoms of allergic reactions. In allergic rhinitis (hay fever), the H_1 antagonists are second-line drugs after

glucocorticoids administered by nasal spray. In urticaria, in which histamine is the primary mediator, the H_1 antagonists are the drugs of choice and are often quite effective if given before exposure. However, in bronchial asthma, which involves several mediators, the H_1 antagonists are largely ineffective.

Angioedema may be precipitated by histamine release but appears to be maintained by peptide kinins that are not affected by antihistaminic agents. For atopic dermatitis, antihistaminic drugs such as diphenhydramine are used mostly for their sedative side effect, which reduces awareness of itching.

The H_1 antihistamines used for treating allergic conditions such as hay fever are usually selected with the goal of minimizing sedative effects; in the USA, the drugs in widest use are the alkylamines and the second-generation nonsedating agents. However, the sedative effect and the therapeutic efficacy of different agents vary widely among individuals. In addition, the clinical effectiveness of one group may diminish with continued use, and switching to another group may restore drug effectiveness for as yet unexplained reasons.

The second-generation H_1 antagonists are used mainly for the treatment of allergic rhinitis and chronic urticaria. Several double-blind comparisons with older agents (eg, chlorpheniramine) indicated about equal therapeutic efficacy. However, sedation and interference with safe operation of machinery, which occur in about 50% of subjects taking first-generation antihistamines, occurred in only about 7% of subjects taking second-generation agents. The newer drugs are much more expensive, even in over-the-counter generic formulations.

B. Motion Sickness and Vestibular Disturbances

Scopolamine (see Chapter 8) and certain first-generation H_1 antagonists are the most effective agents available for the prevention of motion sickness. The antihistaminic drugs with the greatest effectiveness in this application are diphenhydramine and promethazine. Dimenhydrinate, which is promoted almost exclusively for the treatment of motion sickness, is a salt of diphenhydramine and has similar efficacy. The piperazines (cyclizine and meclizine) also have significant activity in preventing motion sickness and are less sedating than diphenhydramine in most patients. Dosage is the same as that recommended for allergic disorders (Table 16–2). Both scopolamine and the H_1 antagonists are more effective in preventing motion sickness when combined with ephedrine or amphetamine.

It has been claimed that the antihistaminic agents effective in prophylaxis of motion sickness are also useful in Ménière's syndrome, but efficacy in the latter application is not established.

C. Nausea and Vomiting of Pregnancy

Several H_1-antagonist drugs have been studied for possible use in treating "morning sickness." The piperazine derivatives were withdrawn from such use when it was demonstrated that they have teratogenic effects in rodents. Doxylamine, an ethanolamine H_1 antagonist, was promoted for this application as a component of Bendectin, a prescription medication that also contained pyridoxine. Possible teratogenic effects of doxylamine were widely publicized in the lay press after 1978 as a result of a few case reports of fetal malformation that occurred after maternal ingestion of Bendectin. However, several large prospective studies involving over 60,000 pregnancies, of which more than 3000 involved maternal Bendectin ingestion, disclosed no increase in the incidence of birth defects. Nonetheless, because of the continuing controversy, adverse publicity, and lawsuits, the manufacturer of Bendectin withdrew the product from the market.

Toxicity

The wide spectrum of nonantihistaminic effects of the H_1 antihistamines is described above. Several of these effects (sedation, antimuscarinic action) have been used for therapeutic purposes, especially in over-the-counter remedies (see Chapter 63). Nevertheless, these two effects constitute the most common undesirable actions when these drugs are used to block histamine receptors.

Less common toxic effects of systemic use include excitation and convulsions in children, postural hypotension, and allergic responses. Drug allergy is relatively common after topical use of H_1 antagonists. The effects of severe systemic overdosage of the older agents resemble those of atropine overdosage and are treated in the same way (see Chapters 8 and 58). Overdosage of astemizole or terfenadine may induce cardiac arrhythmias; the same effect may be caused at normal dosage by interaction with enzyme inhibitors (see Drug Interactions). These drugs are no longer marketed in the USA.

Drug Interactions

Lethal ventricular arrhythmias occurred in several patients taking either of the early second-generation agents, terfenadine or astemizole, in combination with ketoconazole, itraconazole, or macrolide antibiotics such as erythromycin. These antimicrobial drugs inhibit the metabolism of many drugs by CYP3A4 and cause significant increases in blood concentrations of the antihistamines. The mechanism of this toxicity involves blockade of the HERG (I_{Kr}) potassium channels in the heart that contribute to repolarization of the action potential (see Chapter 14). The result is prolongation and a change in shape of the action potential, and these changes lead to arrhythmias. Both terfenadine and astemizole were withdrawn from the US market in recognition of these problems. Where still available, terfenadine and astemizole should be considered to be contraindicated in patients taking ketoconazole, itraconazole, or macrolides and in patients with liver disease. Grapefruit juice also inhibits CYP3A4 and has been shown to increase blood levels of terfenadine significantly.

For those H_1 antagonists that cause significant sedation, concurrent use of other drugs that cause central nervous system depression produces additive effects and is contraindicated while driving or operating machinery. Similarly, the autonomic blocking effects of older antihistamines are additive with those of antimuscarinic and α-blocking drugs.

H_2-RECEPTOR ANTAGONISTS

The development of H_2-receptor antagonists was based on the observation that H_1 antagonists had no effect on histamine-induced

acid secretion in the stomach. Molecular manipulation of the histamine molecule resulted in drugs that blocked acid secretion and had no H_1 agonist or antagonist effects. Like the other histamine receptors, the H_2 receptor displays constitutive activity, and some H_2 blockers are inverse agonists.

The high prevalence of peptic ulcer disease created great interest in the therapeutic potential of the H_2-receptor antagonists when first discovered. Although these agents are not the most efficacious available, their ability to reduce gastric acid secretion with very low toxicity has made them extremely popular as over-the-counter preparations. These drugs are discussed in more detail in Chapter 62.

H_3- & H_4-RECEPTOR ANTAGONISTS

Although no selective H_3 or H_4 ligands are presently available for general clinical use, there is great interest in their therapeutic potential. H_3-selective ligands may be of value in sleep disorders, narcolepsy, obesity, and cognitive and psychiatric disorders. Tiprolisant, an inverse H_3-receptor agonist, has been shown to reduce sleep cycles in mutant mice and in humans with narcolepsy. Increased obesity has been demonstrated in both H_1- and H_3-receptor knockout mice. As noted in Chapter 29, several newer antipsychotic drugs have significant affinity for H_3 receptors.

Because of the homology between the H_3 and H_4 receptors, many H_3 ligands also have affinity for the H_4 receptor. H_4 blockers have potential in chronic inflammatory conditions such as asthma, in which eosinophils and mast cells play a prominent role. No selective H_4 ligand is available for use in humans, but in addition to research agents listed in Table 16–1, many H_1-selective blockers (eg, diphenhydramine, cetirizine, loratadine) show some affinity for this receptor. Several studies have suggested that H_4-receptor antagonists may be useful in pruritus, asthma, allergic rhinitis, and pain conditions.

■ SEROTONIN (5-HYDROXYTRYPTAMINE)

Before the identification of 5-hydroxytryptamine (5-HT), it was known that when blood is allowed to clot, a vasoconstrictor (tonic) substance is released from the clot into the serum. This substance was called serotonin. Independent studies established the existence of a smooth muscle stimulant in intestinal mucosa. This was called enteramine. The synthesis of 5-hydroxytryptamine in 1951 led to the identification of serotonin and enteramine as the same metabolite of 5-hydroxytryptophan.

Serotonin is an important neurotransmitter, a local hormone in the gut, a component of the platelet clotting process, and is thought to play a role in migraine headache and several other clinical conditions, including carcinoid syndrome. This syndrome is an unusual manifestation of carcinoid tumor, a neoplasm of enterochromaffin cells. In patients whose tumor is not surgically resectable, a serotonin antagonist may constitute a useful treatment.

BASIC PHARMACOLOGY OF SEROTONIN

Chemistry & Pharmacokinetics

Like histamine, serotonin is widely distributed in nature, being found in plant and animal tissues, venoms, and stings. It is synthesized in biologic systems from the amino acid L-tryptophan by hydroxylation of the indole ring followed by decarboxylation of the amino acid (Figure 16–2). Hydroxylation at C5 by tryptophan hydroxylase-1 is the rate-limiting step and can be blocked by *p*-chlorophenylalanine (PCPA; fenclonine) and by *p*-chloroamphetamine. These agents have been used experimentally to reduce serotonin synthesis in carcinoid syndrome but are too toxic for general clinical use.

After synthesis, the free amine is stored or is rapidly inactivated, usually by oxidation by monoamine oxidase (MAO). In the pineal gland, serotonin serves as a precursor of melatonin, a melanocyte-stimulating hormone. In mammals (including humans), over 90% of the serotonin in the body is found in enterochromaffin cells in the gastrointestinal tract. In the blood, serotonin is found in platelets, which are able to concentrate the amine by means of an active serotonin transporter mechanism (SERT) similar to that in the membrane of serotonergic nerve endings. Once transported into the platelet or nerve ending, 5-HT is concentrated in vesicles by a vesicle-associated transporter (VAT) that is blocked by **reserpine**. Serotonin is also found in the raphe nuclei of the brainstem, which contain cell bodies of serotonergic

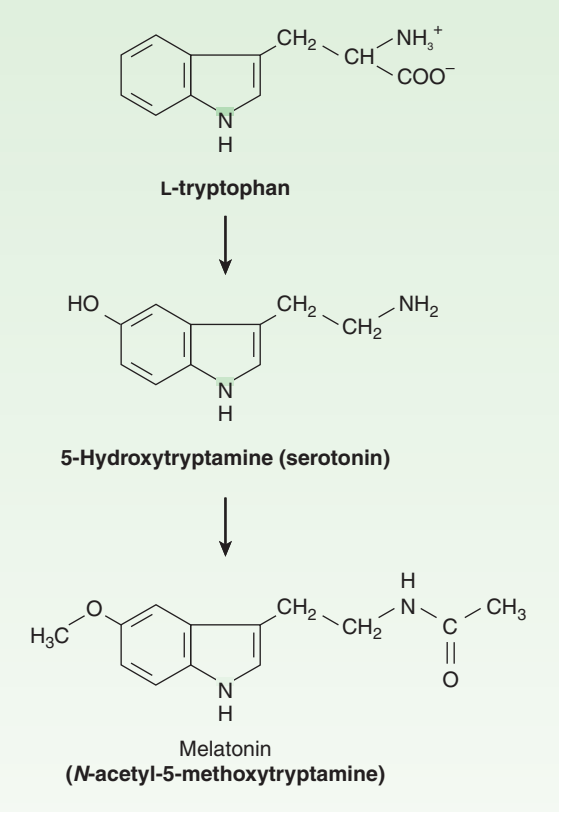


FIGURE 16–2 Synthesis of serotonin and melatonin from L-tryptophan.

neurons that synthesize, store, and release serotonin as a transmitter. Stored serotonin can be depleted by reserpine in much the same manner as this drug depletes catecholamines from vesicles in adrenergic nerves and the adrenal medulla (see Chapter 6).

Brain serotonergic neurons are involved in numerous diffuse functions such as mood, sleep, appetite, and temperature regulation, as well as the perception of pain, the regulation of blood pressure, and vomiting (see Chapter 21). Serotonin also appears to be involved in clinical conditions such as depression, anxiety, and migraine. Serotonergic neurons are also found in the enteric nervous system of the gastrointestinal tract and around blood vessels. In rodents (but not in humans), serotonin is found in mast cells.

The function of serotonin in enterochromaffin cells is not fully understood. These cells synthesize serotonin, store the amine in a complex with adenosine triphosphate (ATP) and other substances in granules, and release serotonin in response to mechanical and neuronal stimuli. This serotonin interacts in a paracrine fashion with several different 5-HT receptors in the gut. Some of the released serotonin diffuses into blood vessels and is taken up and stored in platelets.

Serotonin is metabolized by MAO, and the intermediate product, 5-hydroxyindoleacetaldehyde, is further oxidized by aldehyde dehydrogenase to 5-hydroxyindoleacetic acid (5-HIAA). In humans consuming a normal diet, the excretion of 5-HIAA is a measure of serotonin synthesis. Therefore, the 24-hour excretion of 5-HIAA can be used as a diagnostic test for tumors that synthesize excessive quantities of serotonin, especially carcinoid tumor. A few foods (eg, bananas) contain large amounts of serotonin or its precursors and must be prohibited during such diagnostic tests.

Pharmacodynamics

A. Mechanisms of Action

Serotonin exerts many actions and, like histamine, displays many species differences, making generalizations difficult. The actions of serotonin are mediated through a remarkably large number of cell membrane receptors. The serotonin receptors that have been characterized thus far are listed in Table 16–3. Seven families of 5-HT-receptor subtypes (those given numeric subscripts 1 through 7) have been identified, six involving G protein-coupled receptors of the usual 7-transmembrane serpentine type and one a ligand-gated ion channel. The latter (5-HT_3) receptor is a member of the nicotinic/$GABA_A$ family of Na^+/K^+ channel proteins.

B. Tissue and Organ System Effects

1. Nervous system—Serotonin is present in a variety of sites in the brain. Its role as a neurotransmitter and its relation to the actions of drugs acting in the central nervous system are discussed in Chapters 21 and 30. Serotonin is also a precursor of melatonin in the pineal gland (Figure 16–2; see Box: Melatonin Pharmacology). **Repinotan**, a 5-HT_{1A} agonist currently in clinical trials, appears to have some antinociceptive action at higher doses while reversing opioid-induced respiratory depression.

TABLE 16–3 Serotonin receptor subtypes currently recognized. (See also Chapter 21.)

Receptor Subtype	Distribution	Postreceptor Mechanism	Partially Selective Agonists	Partially Selective Antagonists
5-HT_{1A}	Raphe nuclei, hippocampus	G_i, ↓ cAMP	8-OH-DPAT,[1] repinotan	WAY100635[1]
5-HT_{1B}	Substantia nigra, globus pallidus, basal ganglia	G_i, ↓ cAMP	Sumatriptan, L694247[1]	
5-HT_{1D}	Brain	G_i, ↓ cAMP	Sumatriptan, eletriptan	
5-HT_{1E}	Cortex, putamen	G_i, ↓ cAMP		
5-HT_{1F}	Cortex, hippocampus	G_i, ↓ cAMP	LY3344864[1]	
5-HT_{1P}	Enteric nervous system	G_o, slow EPSP	5-Hydroxyindalpine	Renzapride
5-HT_{2A}	Platelets, smooth muscle, cerebral cortex	G_q, ↑ IP_3	α-Methyl-5-HT, DOI[1]	Ketanserin
5-HT_{2B}	Stomach fundus	G_q, ↑ IP_3	α-Methyl-5-HT, DOI[1]	RS127445[1]
5-HT_{2C}	Choroid, hippocampus, substantia nigra	G_q, ↑ IP_3	α-Methyl-5-HT, DOI,[1] lorcaserin	Mesulergine
5-HT_3	Area postrema, sensory and enteric nerves	Receptor is a Na^+/K^+ ion channel	2-Methyl-5-HT, *m*-chlorophenylbiguanide	Granisetron, ondansetron, others
5-HT_4	CNS and myenteric neurons, smooth muscle	G_s, ↑ cAMP	BIMU8,[1] renzapride, metoclopramide	GR113808[1]
$5\text{-HT}_{5A,B}$	Brain	↓ cAMP		
$5\text{-HT}_{6,7}$	Brain	G_s, ↑ cAMP		Clozapine (5-HT_7)

[1]Research agents; for chemical names see Alexander SPH, Mathie A, Peters JA: Guide to receptors and channels (GRAC). Br J Pharmacol 2009;158 (Suppl 1):S12.

cAMP, cyclic adenosine monophosphate; EPSP, excitatory postsynaptic potential; IP_3, inositol trisphosphate.

Melatonin Pharmacology

Melatonin is *N*-acetyl-5-methoxytryptamine (Figure 16–2), a simple methoxylated and *N*-acetylated product of serotonin found in the pineal gland. It is produced and released primarily at night and has long been suspected of playing a role in diurnal cycles of animals and the sleep-wake behavior of humans. Melatonin receptors have been characterized in the central nervous system and several peripheral tissues. In the brain, MT_1 and MT_2 receptors are found in membranes of neurons in the suprachiasmatic nucleus of the hypothalamus, an area associated—from lesioning experiments—with circadian rhythm. MT_1 and MT_2 are seven-transmembrane G_i protein-coupled receptors. The result of receptor binding is inhibition of adenylyl cyclase. A third receptor, MT_3, is an enzyme; binding to this site has a poorly defined physiologic role, possibly related to intraocular pressure. Activation of the MT_1 receptor results in sleepiness, whereas the MT_2 receptor may be related to the light-dark synchronization of the biologic circadian clock. Melatonin has also been implicated in energy metabolism and obesity, and administration of the agent reduces body weight in certain animal models. However, its role in these processes is poorly understood and there is no evidence that melatonin itself is of any value in obesity in humans. Other studies suggest that melatonin has antiapoptotic effects in experimental models. Recent research implicates melatonin receptors in depressive disorders.

Melatonin is promoted commercially as a sleep aid by the food supplement industry (see Chapter 64). There is an extensive literature supporting its use in ameliorating jet lag. It is used in oral doses of 0.5–5 mg, usually administered at the destination bedtime. **Ramelteon** is a selective MT_1 and MT_2 agonist that is approved for the medical treatment of insomnia. This drug has no addiction liability (it is not a controlled substance), and it appears to be distinctly more efficacious than melatonin (but less efficacious than benzodiazepines) as a hypnotic. It is metabolized by P450 enzymes and should not be used in individuals taking CYP1A2 inhibitors. It has a half-life of 1–3 hours and an active metabolite with a half-life of up to 5 hours. The toxicity of ramelteon is as yet poorly defined, but prolactin levels were elevated in one clinical trial. **Agomelatine**, an MT_1 and MT_2 agonist and a $5\text{-}HT_{2C}$ antagonist, has recently been approved in Europe for use in major depressive disorder.

$5\text{-}HT_3$ receptors in the gastrointestinal tract and in the vomiting center of the medulla participate in the vomiting reflex (see Chapter 62). They are particularly important in vomiting caused by chemical triggers such as cancer chemotherapy drugs. $5\text{-}HT_{1P}$ and $5\text{-}HT_4$ receptors also play important roles in enteric nervous system function.

Like histamine, serotonin is a potent stimulant of pain and itch sensory nerve endings and is responsible for some of the symptoms caused by insect and plant stings. In addition, serotonin is a powerful activator of chemosensitive endings located in the coronary vascular bed. Activation of $5\text{-}HT_3$ receptors on these afferent vagal nerve endings is associated with the **chemoreceptor reflex** (also known as the Bezold-Jarisch reflex). The reflex response consists of marked bradycardia and hypotension, and its physiologic role is uncertain. The bradycardia is mediated by vagal outflow to the heart and can be blocked by atropine. The hypotension is a consequence of the decrease in cardiac output that results from bradycardia. A variety of other agents can activate the chemoreceptor reflex. These include nicotinic cholinoceptor agonists and some cardiac glycosides, eg, ouabain.

2. Respiratory system—Serotonin has a small direct stimulant effect on bronchiolar smooth muscle in normal humans, probably via $5\text{-}HT_{2A}$ receptors. It also appears to facilitate acetylcholine release from bronchial vagal nerve endings. In patients with carcinoid syndrome, episodes of bronchoconstriction occur in response to elevated levels of the amine or peptides released from the tumor. Serotonin may also cause hyperventilation as a result of the chemoreceptor reflex or stimulation of bronchial sensory nerve endings.

3. Cardiovascular system—Serotonin directly causes the contraction of vascular smooth muscle, mainly through $5\text{-}HT_2$ receptors. In humans, serotonin is a powerful vasoconstrictor except in skeletal muscle and the heart, where it dilates blood vessels. At least part of this 5-HT-induced vasodilation requires the presence of vascular endothelial cells. When the endothelium is damaged, coronary vessels are constricted by 5-HT. As noted previously, serotonin can also elicit reflex bradycardia by activation of $5\text{-}HT_3$ receptors on chemoreceptor nerve endings. A triphasic blood pressure response is often seen following injection of serotonin in experimental animals. Initially, there is a decrease in heart rate, cardiac output, and blood pressure caused by the chemoreceptor response. After this decrease, blood pressure increases as a result of vasoconstriction. The third phase is again a decrease in blood pressure attributed to vasodilation in vessels supplying skeletal muscle. Pulmonary and renal vessels seem especially sensitive to the vasoconstrictor action of serotonin.

Studies in knockout mice suggest that 5-HT, acting on $5\text{-}HT_{1A}$, $5\text{-}HT_2$, and $5\text{-}HT_4$ receptors, is needed for normal cardiac development in the fetus. On the other hand, chronic exposure of adults to $5\text{-}HT_{2B}$ agonists is associated with valvulopathy and adult mice lacking the $5\text{-}HT_{2B}$ receptor gene are protected from cardiac hypertrophy. Preliminary studies suggest that $5\text{-}HT_{2B}$ antagonists can prevent development of pulmonary hypertension in animal models.

Serotonin also constricts veins, and venoconstriction with increased capillary filling appears to be responsible for the flush that is observed after serotonin administration or release from a carcinoid tumor. Serotonin has small direct positive chronotropic and inotropic effects on the heart, which are probably of no clinical

Serotonin Syndrome and Similar Syndromes

Excess synaptic serotonin causes a serious, potentially fatal syndrome that is diagnosed on the basis of a history of administration of a serotonergic drug within recent weeks and physical findings (Table 16–4). It has some characteristics in common with neuroleptic malignant syndrome (NMS) and malignant hyperthermia (MH), but its pathophysiology and management are quite different.

As suggested by the drugs that precipitate it, serotonin syndrome occurs when overdose with a single drug, or concurrent use of several drugs, results in excess serotonergic activity in the central nervous system. It is predictable and not idiosyncratic, but milder forms may easily be misdiagnosed. In experimental animal models, many of the signs of the syndrome can be reversed by administration of 5-HT_2 antagonists; however, other 5-HT receptors may be involved as well. Dantrolene is of no value, unlike the treatment of MH.

NMS is idiosyncratic rather than predictable and appears to be associated with hypersensitivity to the parkinsonism-inducing effects of D_2-blocking antipsychotics in certain individuals. MH is associated with a genetic defect in the RyR1 calcium channel of skeletal muscle sarcoplasmic reticulum that permits uncontrolled calcium release from the sarcoplasmic reticulum when precipitating drugs are given (see Chapter 27).

significance. However, prolonged elevation of the blood level of serotonin (which occurs in carcinoid syndrome) is associated with pathologic alterations in the endocardium (subendocardial fibroplasia), which may result in valvular or electrical malfunction.

Serotonin causes blood platelets to aggregate by activating 5-HT_2 receptors. This response, in contrast to aggregation induced during normal clot formation, is not accompanied by the release of serotonin stored in the platelets. The physiologic role of this effect is unclear.

4. Gastrointestinal tract—Serotonin is a powerful stimulant of gastrointestinal smooth muscle, increasing tone and facilitating peristalsis. This action is caused by the direct action of serotonin on 5-HT_2 smooth muscle receptors plus a stimulating action on ganglion cells located in the enteric nervous system (see Chapter 6). 5-HT_{1A} and 5-HT_7 receptors may also be involved in this complex action. Activation of 5-HT_4 receptors in the enteric nervous system causes increased acetylcholine release and thereby mediates a motility-enhancing or "prokinetic" effect of selective serotonin agonists such as cisapride. These agents are useful in several gastrointestinal disorders (see Chapter 62). Overproduction of serotonin (and other substances) in carcinoid tumor is associated with severe diarrhea. Serotonin has little effect on gastrointestinal secretions, and what effects it has are generally inhibitory.

5. Skeletal muscle and the eye—5-HT_2 receptors are present on skeletal muscle membranes, but their physiologic role is not understood. **Serotonin syndrome** is a condition associated with skeletal muscle contractions and precipitated when MAO inhibitors are given with serotonin agonists, especially antidepressants of the selective serotonin reuptake inhibitor class (SSRIs; see Chapter 30). Although the hyperthermia of serotonin syndrome results from excessive muscle contraction, serotonin syndrome is probably caused by a central nervous system effect of these drugs (Table 16–4 and Box: Serotonin Syndrome and Similar Syndromes).

Studies in animal models of glaucoma indicate that 5-HT_{2A} agonists reduce intraocular pressure. This action can be blocked by ketanserin and similar 5-HT_2 antagonists.

CLINICAL PHARMACOLOGY OF SEROTONIN

Serotonin Agonists

Serotonin has no clinical applications as a drug. However, several receptor subtype-selective agonists have proved to be of value. **Buspirone,** a 5-HT_{1A} agonist, has received wide attention for its usefulness as an effective nonbenzodiazepine anxiolytic (see

TABLE 16–4 Characteristics of serotonin syndrome and other hyperthermic syndromes.

Syndrome	Precipitating Drugs	Clinical Presentation	Therapy[1]
Serotonin syndrome	SSRIs, second-generation antidepressants, MAOIs, linezolid, tramadol, meperidine, fentanyl, ondansetron, sumatriptan, MDMA, LSD, St. John's wort, ginseng	Hypertension, hyperreflexia, tremor, clonus, hyperthermia, hyperactive bowel sounds, diarrhea, mydriasis, agitation, coma; onset within hours	**Sedation (benzodiazepines), paralysis, intubation, and ventilation;** consider 5-HT_2 block with cyproheptadine or chlorpromazine
Neuroleptic malignant syndrome	D_2-blocking antipsychotics	Acute severe parkinsonism; hypertension, hyperthermia, normal or reduced bowel sounds, onset over 1–3 days	**Diphenhydramine** (parenteral), cooling if temperature is very high, sedation with benzodiazepines
Malignant hyperthermia	Volatile anesthetics, succinylcholine	Hyperthermia, muscle rigidity, hypertension, tachycardia; onset within minutes	**Dantrolene,** cooling

[1]Precipitating drugs should be discontinued immediately. First-line therapy is in **bold** font.

MAOIs, monoamine oxidase inhibitors; MDMA, methylenedioxy-methamphetamine (ecstasy); SSRIs, selective serotonin reuptake inhibitors.

Chapter 22). **Dexfenfluramine,** another selective 5-HT agonist, was widely used as an appetite suppressant but was withdrawn because of cardiac valve toxicity. Appetite suppression appears to be associated with agonist action at 5-HT_{2C} receptors in the central nervous system.

5-HT$_{1D/1B}$ Agonists & Migraine Headache

The $5\text{-HT}_{1D/1B}$ agonists (**triptans,** eg, **sumatriptan**) are used almost exclusively for migraine headache. Migraine in its "classic" form is characterized by an aura of variable duration that may involve nausea, vomiting, visual scotomas or even hemianopsia, and speech abnormalities; the aura is followed by a severe throbbing unilateral headache that lasts for a few hours to 1–2 days. "Common" migraine lacks the aura phase, but the headache is similar. After a century of intense study, the pathophysiology of migraine is still poorly understood and controversial. Although the symptom pattern and duration of prodrome and headache vary markedly among patients, the severity of migraine headache justifies vigorous therapy in the great majority of cases.

Migraine involves the trigeminal nerve distribution to intracranial (and possibly extracranial) arteries. These nerves release peptide neurotransmitters, especially **calcitonin gene-related peptide** (CGRP; see Chapter 17), an extremely powerful vasodilator. Substance P and neurokinin A may also be involved. Extravasation of plasma and plasma proteins into the perivascular space appears to be a common feature of animal migraine models and is found in biopsy specimens from migraine patients. This effect probably reflects the action of the neuropeptides on the vessels. The mechanical stretching caused by this perivascular edema may be the immediate cause of activation of pain nerve endings in the dura. The onset of headache is sometimes associated with a marked increase in amplitude of temporal artery pulsations, and relief of pain by administration of effective therapy is sometimes accompanied by diminution of the arterial pulsations.

The mechanisms of action of drugs used in migraine are poorly understood, in part because they include such a wide variety of drug groups and actions. In addition to the triptans, these include ergot alkaloids, nonsteroidal anti-inflammatory analgesic agents, β-adrenoceptor blockers, calcium channel blockers, tricyclic antidepressants and SSRIs, and several antiseizure agents. Furthermore, some of these drug groups are effective only for prophylaxis and not for the acute attack.

Two primary hypotheses have been proposed to explain the actions of these drugs. First, the triptans, the ergot alkaloids, and antidepressants may activate $5\text{-HT}_{1D/1B}$ receptors on presynaptic trigeminal nerve endings to inhibit the release of vasodilating peptides, and antiseizure agents may suppress excessive firing of these nerve endings. Second, the vasoconstrictor actions of direct 5-HT agonists (the triptans and ergot) may prevent vasodilation and stretching of the pain endings. It is possible that both mechanisms contribute in the case of some drugs. Sumatriptan and its congeners are currently first-line therapy for acute severe migraine attacks in most patients (Figure 16–3). However, they should not be used in patients at risk for coronary artery disease. Anti-inflammatory analgesics such as aspirin and ibuprofen are often helpful in controlling the pain of migraine. Rarely, parenteral opioids may be needed in refractory cases. For patients with very severe nausea and vomiting, parenteral metoclopramide may be helpful.

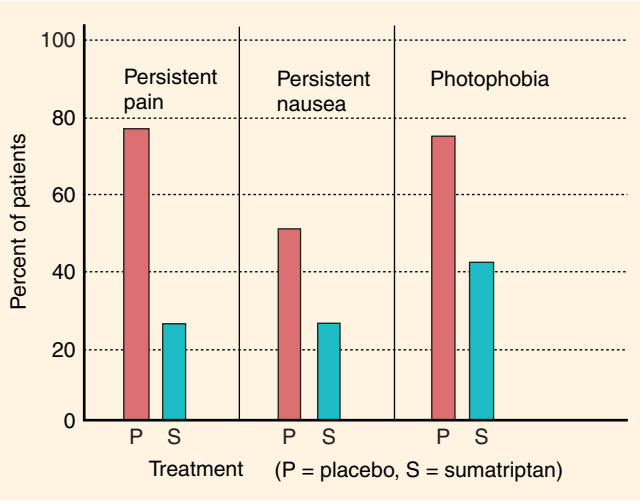
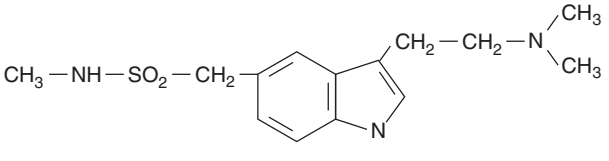


FIGURE 16–3 Effects of sumatriptan (734 patients) or placebo (370 patients) on symptoms of acute migraine headache 60 minutes after injection of 6 mg subcutaneously. All differences between placebo and sumatriptan were statistically significant. (Data from Cady RK et al: Treatment of acute migraine with subcutaneous sumatriptan. JAMA 1991;265:2831.)

Propranolol, amitriptyline, and some calcium channel blockers have been found to be effective for the prophylaxis of migraine in some patients. They are of no value in the treatment of acute migraine. The anticonvulsants **valproic acid** and **topiramate** (see Chapter 24) have also been found to have prophylactic efficacy in many migraine patients. **Flunarizine,** a calcium channel blocker used in Europe, has been reported in clinical trials to effectively reduce the severity of the acute attack and to prevent recurrences. **Verapamil** appears to have modest efficacy as prophylaxis against migraine.

Sumatriptan and the other triptans are selective agonists for 5-HT_{1D} and 5-HT_{1B} receptors; the similarity of the triptan structure to that of the 5-HT nucleus can be seen in the structure below. These receptor types are found in cerebral and meningeal vessels and mediate vasoconstriction. They are also found on neurons and probably function as presynaptic inhibitory receptors.



Sumatriptan

The efficacies of all the triptan 5-HT_1 agonists in migraine are equal to each other and equivalent to or greater than those of other acute drug treatments, eg, parenteral, oral, and rectal ergot alkaloids. The pharmacokinetics of the triptans differ significantly and are set forth in Table 16–5. Most adverse effects are mild and

include altered sensations (tingling, warmth, etc), dizziness, muscle weakness, neck pain, and for parenteral sumatriptan, injection site reactions. Chest discomfort occurs in 1–5% of patients, and chest pain has been reported, probably because of the ability of these drugs to cause coronary vasospasm. They are therefore contraindicated in patients with coronary artery disease and in patients with angina. Another disadvantage is the fact that their duration of effect (especially that of almotriptan, sumatriptan, rizatriptan, and zolmitriptan, Table 16–5) is often shorter than the duration of the headache. As a result, several doses may be required during a prolonged migraine attack, but their adverse effects limit the maximum safe daily dosage. In addition, these drugs are expensive. Naratriptan and eletriptan are contraindicated in patients with severe hepatic or renal impairment or peripheral vascular syndromes; frovatriptan in patients with peripheral vascular disease; and zolmitriptan in patients with Wolff-Parkinson-White syndrome. The brand name triptans are extremely expensive; thus generic sumatriptan should be used whenever possible.

Other Serotonin Agonists in Clinical Use

Cisapride, a 5-HT_4 agonist, was used in the treatment of gastroesophageal reflux and motility disorders. Because of toxicity, it is now available only for compassionate use in the USA. **Tegaserod**, a 5-HT_4 partial agonist, is used for irritable bowel syndrome with constipation. These drugs are discussed in Chapter 62.

Compounds such as **fluoxetine** and other SSRIs, which modulate serotonergic transmission by blocking reuptake of the transmitter, are among the most widely prescribed drugs for the management of depression and similar disorders. These drugs are discussed in Chapter 30.

SEROTONIN ANTAGONISTS

The actions of serotonin, like those of histamine, can be antagonized in several ways. Such antagonism is clearly desirable in those rare patients who have carcinoid tumor and may also be valuable in certain other conditions.

As noted, serotonin synthesis can be inhibited by *p*-chlorophenylalanine and *p*-chloroamphetamine. However, these agents are too toxic for general use. Storage of serotonin can be inhibited by the use of reserpine, but the sympatholytic effects of this drug (see Chapter 11) and the high levels of circulating serotonin that result from release prevent its use in carcinoid. Therefore, receptor blockade is the major therapeutic approach to conditions of serotonin excess.

SEROTONIN-RECEPTOR ANTAGONISTS

A wide variety of drugs with actions at other receptors (eg, α adrenoceptors, H_1-histamine receptors) also have serotonin receptor-blocking effects. **Phenoxybenzamine** (see Chapter 10) has a long-lasting blocking action at 5-HT_2 receptors. In addition, the ergot alkaloids discussed in the last portion of this chapter are partial agonists at serotonin receptors.

Cyproheptadine resembles the phenothiazine antihistaminic agents in chemical structure and has potent H_1-receptor–blocking as well as 5-HT_2–blocking actions. The actions of cyproheptadine are predictable from its H_1 histamine and 5-HT receptor affinities. It prevents the smooth muscle effects of both amines but has no effect on the gastric secretion stimulated by histamine. It also has significant antimuscarinic effects and causes sedation.

The major clinical applications of cyproheptadine are in the treatment of the smooth muscle manifestations of carcinoid tumor and in cold-induced urticaria. The usual dosage in adults is 12–16 mg/d in three or four divided doses. It is of some value in serotonin syndrome, but because it is available only in tablet form, cyproheptadine must be crushed and administered by stomach tube in unconscious patients.

Ketanserin blocks 5-HT_2 receptors on smooth muscle and other tissues and has little or no reported antagonist activity at other 5-HT or H_1 receptors. However, this drug potently blocks vascular α_1 adrenoceptors. The drug blocks 5-HT_2 receptors on platelets and antagonizes platelet aggregation promoted by serotonin. The mechanism involved in ketanserin's hypotensive action probably involves α_1 adrenoceptor blockade more than 5-HT_2 receptor blockade. Ketanserin is available in Europe for the treatment of hypertension and vasospastic conditions but has not been approved in the USA. **Ritanserin,** another 5-HT_2 antagonist, has little or no α-blocking

TABLE 16–5 Pharmacokinetics of triptans.

Drug	Routes	Time to Onset (h)	Single Dose (mg)	Maximum Dose per Day (mg)	Half-Life (h)
Almotriptan	Oral	2.6	6.25–12.5	25	3.3
Eletriptan	Oral	2	20–40	80	4
Frovatriptan	Oral	3	2.5	7.5	27
Naratriptan	Oral	2	1–2.5	5	5.5
Rizatriptan	Oral	1–2.5	5–10	30	2
Sumatriptan	Oral, nasal, subcutaneous, rectal	1.5 (0.2 for subcutaneous)	25–100 (PO), 20 nasal, 6 subcutaneous, 25 rectal	200	2
Zolmitriptan	Oral, nasal	1.5–3	2.5–5	10	2.8

Ergot Poisoning: Not Just an Ancient Disease

As noted in the text, epidemics of ergotism, or poisoning by ergot-contaminated grain, are known to have occurred sporadically in ancient times and through the Middle Ages. It is easy to imagine the social chaos that might result if fiery pain, gangrene, hallucinations, convulsions, and abortions occurred simultaneously throughout a community in which all or most of the people believed in witchcraft, demonic possession, and the visitation of supernatural punishments upon humans for their misdeeds. Fortunately, such beliefs are uncommon today. However, ergotism has not disappeared. A most convincing demonstration of ergotism occurred in the small French village of Pont-Saint-Esprit in 1951. It was described in the *British Medical Journal* in 1951 (Gabbai et al, 1951) and in a later book-length narrative account (Fuller, 1968). Several hundred individuals suffered symptoms of hallucinations, convulsions, and ischemia—and several died—after eating bread made from contaminated flour. Similar events have occurred even more recently when poverty, famine, or incompetence resulted in the consumption of contaminated grain. Ergot toxicity caused by excessive self-medication with pharmaceutical ergot preparations is still occasionally reported.

action. It has been reported to alter bleeding time and to reduce thromboxane formation, presumably by altering platelet function.

Ondansetron is the prototypical 5-HT_3 antagonist. This drug and its analogs are very important in the prevention of nausea and vomiting associated with surgery and cancer chemotherapy. They are discussed in Chapter 62.

Considering the diverse effects attributed to serotonin and the heterogeneous nature of 5-HT receptors, other selective 5-HT antagonists may prove to be clinically useful.

THE ERGOT ALKALOIDS

Ergot alkaloids are produced by *Claviceps purpurea,* a fungus that infects grasses and grains—especially rye—under damp growing or storage conditions. This fungus synthesizes histamine, acetylcholine, tyramine, and other biologically active products in addition to a score or more of unique ergot alkaloids. These alkaloids affect α adrenoceptors, dopamine receptors, 5-HT receptors, and perhaps other receptor types. Similar alkaloids are produced by fungi parasitic to a number of other grass-like plants.

The accidental ingestion of ergot alkaloids in contaminated grain can be traced back more than 2000 years from descriptions of epidemics of ergot poisoning (**ergotism**). The most dramatic effects of poisoning are dementia with florid hallucinations; prolonged vasospasm, which may result in gangrene; and stimulation of uterine smooth muscle, which in pregnancy may result in abortion. In medieval times, ergot poisoning was called **St. Anthony's fire** after the saint whose help was sought in relieving the burning pain of vasospastic ischemia. Identifiable epidemics have occurred sporadically up to modern times (see Box: Ergot Poisoning: Not Just an Ancient Disease) and mandate continuous surveillance of all grains used for food. Poisoning of grazing animals is common in many areas because the fungi may grow on pasture grasses.

In addition to the effects noted above, the ergot alkaloids produce a variety of other central nervous system and peripheral effects. Detailed structure-activity analysis and appropriate semisynthetic modifications have yielded a large number of agents of experimental and clinical interest.

BASIC PHARMACOLOGY OF ERGOT ALKALOIDS

Chemistry & Pharmacokinetics

Two major families of compounds that incorporate the tetracyclic **ergoline** nucleus may be identified; the amine alkaloids and the peptide alkaloids (Table 16–6). Drugs of therapeutic and toxicologic importance are found in both groups.

The ergot alkaloids are variably absorbed from the gastrointestinal tract. The oral dose of ergotamine is about 10 times larger than the intramuscular dose, but the speed of absorption and peak blood levels after oral administration can be improved by administration with caffeine (see below). The amine alkaloids are also absorbed from the rectum and the buccal cavity and after administration by aerosol inhaler. Absorption after intramuscular injection is slow but usually reliable. Semisynthetic analogs such as bromocriptine and cabergoline are well absorbed from the gastrointestinal tract.

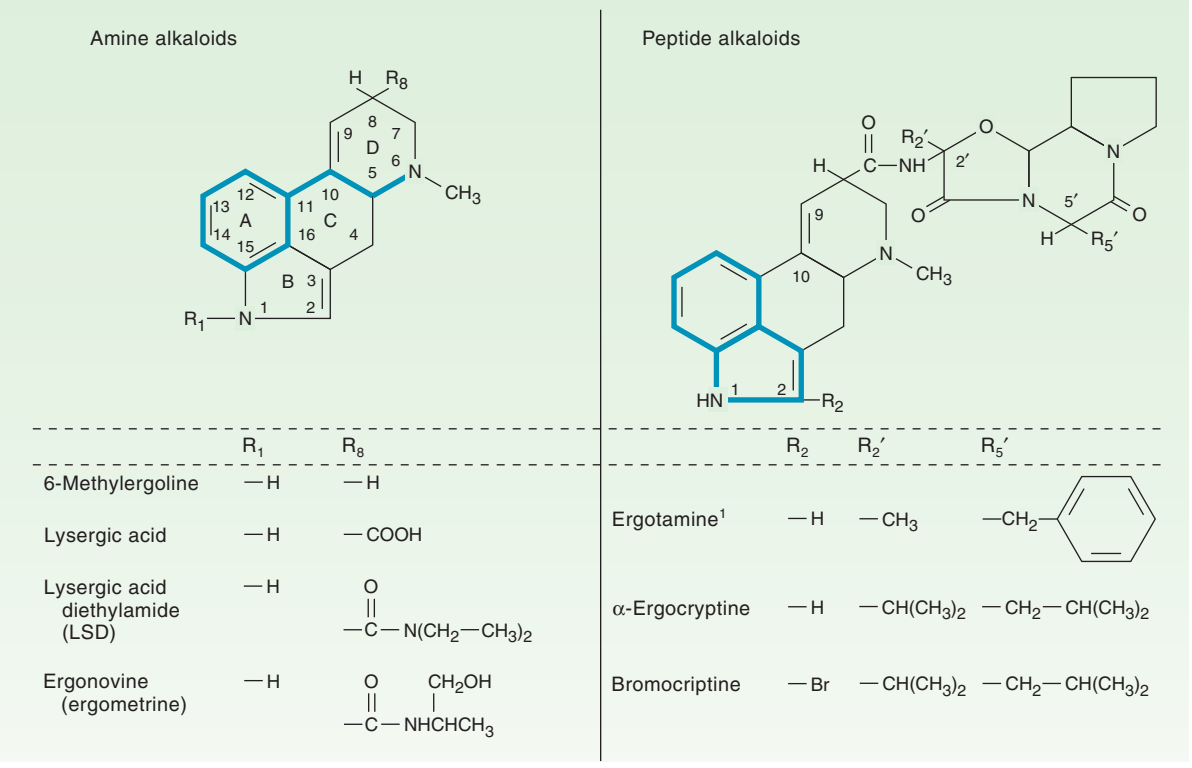
The ergot alkaloids are extensively metabolized in the body. The primary metabolites are hydroxylated in the A ring, and peptide alkaloids are also modified in the peptide moiety.

Pharmacodynamics

A. Mechanism of Action

The ergot alkaloids act on several types of receptors. As shown by the color outlines in Table 16–6, the nuclei of both catecholamines (phenylethylamine, *left panel*) and 5-HT (indole, *right panel*) can be discerned in the ergoline nucleus. Their effects include agonist, partial agonist, and antagonist actions at α adrenoceptors and serotonin receptors (especially 5-HT_{1A} and 5-HT_{1D}; less for 5-HT_2 and 5-HT_3); and agonist or partial agonist actions at central nervous system dopamine receptors (Table 16–7). Furthermore, some members of the ergot family have a high affinity for presynaptic receptors, whereas others are more selective for postjunctional receptors. There is a powerful stimulant effect on the uterus that seems to be most closely associated with agonist or partial agonist effects at 5-HT_2 receptors. Structural variations increase the selectivity of certain members of the family for specific receptor types.

TABLE 16–6 Major ergoline derivatives (ergot alkaloids).



	R_1	R_8	R_2	R_2'	R_5'
6-Methylergoline	—H	—H			
Lysergic acid	—H	—COOH			
Lysergic acid diethylamide (LSD)	—H	—C(=O)—N(CH_2—CH_3)$_2$			
Ergonovine (ergometrine)	—H	—C(=O)—NHCH(CH_2OH)CH_3			
Ergotamine[1]			—H	—CH_3	—CH_2—benzene
α-Ergocryptine			—H	—$CH(CH_3)_2$	—CH_2—$CH(CH_3)_2$
Bromocriptine			—Br	—$CH(CH_3)_2$	—CH_2—$CH(CH_3)_2$

[1]Dihydroergotamine lacks the double bond between carbons 9 and 10.

B. Organ System Effects

1. Central nervous system—As indicated by traditional descriptions of ergotism, certain of the naturally occurring alkaloids are powerful hallucinogens. Lysergic acid diethylamide (LSD; "acid") is a synthetic ergot compound that clearly demonstrates this action. The drug has been used in the laboratory as a potent peripheral 5-HT_2 *antagonist*, but good evidence suggests that its behavioral effects are mediated by *agonist* effects at prejunctional or postjunctional 5-HT_2 receptors in the central nervous system. In spite of extensive research, no clinical value has been discovered for LSD's dramatic central nervous system effects. Abuse of this drug has waxed and waned but is still widespread. It is discussed in Chapter 32.

Dopamine receptors in the central nervous system play important roles in extrapyramidal motor control and the regulation of pituitary prolactin release. The actions of the peptide ergoline **bromocriptine** on the extrapyramidal system are discussed in Chapter 28. Of all the currently available ergot derivatives, bromocriptine, **cabergoline**, and **pergolide** have the highest selectivity for the pituitary dopamine receptors. These drugs directly suppress prolactin secretion from pituitary cells by activating regulatory dopamine receptors (see Chapter 37). They compete for

TABLE 16–7 Effects of ergot alkaloids at several receptors.[1]

Ergot Alkaloid	α Adrenoceptor	Dopamine Receptor	Serotonin Receptor (5-HT_2)	Uterine Smooth Muscle Stimulation
Bromocriptine	–	+++	–	0
Ergonovine	++	– (PA)	+++	
Ergotamine	– – (PA)	0	+ (PA)	+++
Lysergic acid diethylamide (LSD)	0	+++	– – (++ in CNS)	+
Methysergide	+/0	+/0	– – – (PA)	+/0

[1]Agonist effects are indicated by +, antagonist by –, no effect by 0. Relative affinity for the receptor is indicated by the number of + or – signs. PA means partial agonist (both agonist and antagonist effects can be detected).

binding to these sites with dopamine itself and with other dopamine agonists such as apomorphine. They bind with high affinity and dissociate slowly.

2. Vascular smooth muscle—The action of ergot alkaloids on vascular smooth muscle is drug, species, and vessel dependent, so few generalizations are possible. In humans, ergotamine and similar compounds constrict most vessels in nanomolar concentrations (Figure 16–4). The vasospasm is prolonged. This response is partially blocked by conventional α-blocking agents. However, ergotamine's effect is also associated with "epinephrine reversal" (see Chapter 10) and with *blockade* of the response to other α agonists. This dual effect reflects the drug's partial agonist action (Table 16–7). Because ergotamine dissociates very slowly from the α receptor, it produces very long-lasting agonist and antagonist effects at this receptor. There is little or no effect at β adrenoceptors.

Although much of the vasoconstriction elicited by ergot alkaloids can be ascribed to partial agonist effects at α adrenoceptors, some may be the result of effects at 5-HT receptors. Ergotamine, ergonovine, and methysergide all have partial agonist effects at 5-HT_2 vascular receptors. The remarkably specific antimigraine action of the ergot derivatives was originally thought to be related to their actions on vascular serotonin receptors. Current hypotheses, however, emphasize their action on prejunctional neuronal 5-HT receptors.

After overdosage with ergotamine and similar agents, vasospasm is severe and prolonged (see Toxicity, below). This vasospasm is not easily reversed by α antagonists, serotonin antagonists, or combinations of both.

Ergotamine is typical of the ergot alkaloids that have a strong vasoconstrictor spectrum of action. The hydrogenation of ergot alkaloids at the 9 and 10 positions (Table 16–6) yields dihydro derivatives that have reduced serotonin partial agonist and vasoconstrictor effects and increased selective α-receptor–blocking actions.

3. Uterine smooth muscle—The stimulant action of ergot alkaloids on the uterus, as on vascular smooth muscle, appears to combine α agonist, serotonin agonist, and other effects. Furthermore, the sensitivity of the uterus to the stimulant effects of ergot increases dramatically during pregnancy, perhaps because of increasing dominance of α_1 receptors as pregnancy progresses. As a result, the uterus at term is more sensitive to ergot than earlier in pregnancy and far more sensitive than the nonpregnant organ.

In very small doses, ergot preparations can evoke rhythmic contraction and relaxation of the uterus. At higher concentrations, these drugs induce powerful and prolonged contracture. Ergonovine is more selective than other ergot alkaloids in affecting the uterus and is the agent of choice in obstetric applications of the ergot drugs although oxytocin, the peptide hormone, is preferred in most cases.

4. Other smooth muscle organs—In most patients, the ergot alkaloids have little or no significant effect on bronchiolar or urinary smooth muscle. The gastrointestinal tract, on the other hand, is quite sensitive. Nausea, vomiting, and diarrhea may be induced even by low doses in some patients. The effect is consistent with action on the central nervous system emetic center and on gastrointestinal serotonin receptors.

CLINICAL PHARMACOLOGY OF ERGOT ALKALOIDS

Clinical Uses

In spite of their significant toxicities, ergot alkaloids are still widely used in patients with migraine headache or pituitary dysfunction, but only occasionally in the postpartum patient.

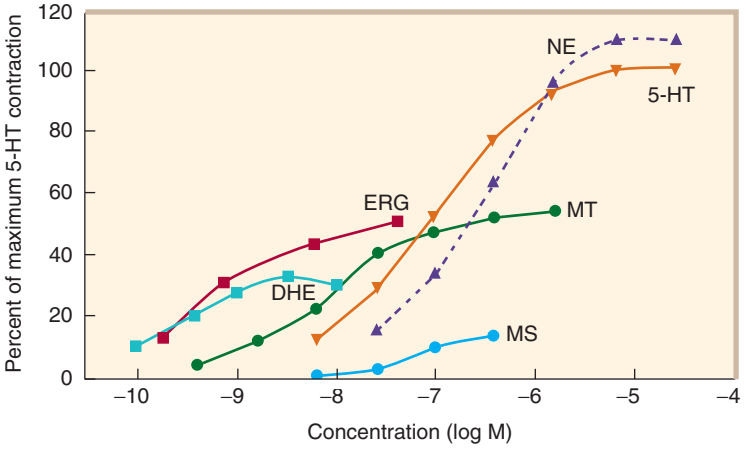


FIGURE 16–4 Effects of ergot derivatives on contraction of isolated segments of human basilar artery strips removed at surgery. All of the ergot derivatives are partial agonists, and all are more potent than the full agonists, norepinephrine and serotonin. DHE, dihydroergotamine; ERG, ergotamine; 5-HT, serotonin; MS, methysergide; MT, methylergometrine; NE, norepinephrine. (Modified and reproduced, with permission, from Müller-Schweinitzer E. In: *5-Hydroxytryptamine Mechanisms in Primary Headaches.* Oleson J, Saxena PR [editors]. Raven Press, 1992.)

A. Migraine

Ergot derivatives are highly specific for migraine pain; they are not analgesic for any other condition. Although the triptan drugs discussed above are preferred by most clinicians and patients, traditional therapy with ergotamine can also be effective when given during the prodrome of an attack; it becomes progressively less effective if delayed. Ergotamine tartrate is available for oral, sublingual, rectal suppository, and inhaler use. It is often combined with caffeine (100 mg caffeine for each 1 mg ergotamine tartrate) to facilitate absorption of the ergot alkaloid.

The vasoconstriction induced by ergotamine is long-lasting and cumulative when the drug is taken repeatedly, as in a severe migraine attack. Therefore, patients must be carefully informed that no more than 6 mg of the oral preparation may be taken for each attack and no more than 10 mg per week. For very severe attacks, ergotamine tartrate, 0.25–0.5 mg, may be given intravenously or intramuscularly. Dihydroergotamine, 0.5–1 mg intravenously, is favored by some clinicians for treatment of intractable migraine. Intranasal dihydroergotamine may also be effective. Methysergide, which was used for migraine prophylaxis in the past, was withdrawn because of toxicity, see below.

B. Hyperprolactinemia

Increased serum levels of the anterior pituitary hormone prolactin are associated with secreting tumors of the gland and also with the use of centrally acting dopamine antagonists, especially the D_2-blocking antipsychotic drugs. Because of negative feedback effects, hyperprolactinemia is associated with amenorrhea and infertility in women as well as galactorrhea in both sexes. Rarely, the prolactin surge that occurs around the end of term pregnancy may be associated with heart failure; cabergoline has been used to treat this cardiac condition successfully.

Bromocriptine is extremely effective in reducing the high levels of prolactin that result from pituitary tumors and has even been associated with regression of the tumor in some cases. The usual dosage of bromocriptine is 2.5 mg two or three times daily. Cabergoline is similar but more potent. Bromocriptine has also been used in the same dosage to suppress physiologic lactation. However, serious postpartum cardiovascular toxicity has been reported in association with the latter use of bromocriptine or pergolide, and this application is discouraged (see Chapter 37).

C. Postpartum Hemorrhage

The uterus at term is extremely sensitive to the stimulant action of ergot, and even moderate doses produce a prolonged and powerful spasm of the muscle quite unlike natural labor. Therefore, ergot derivatives should be used only for control of postpartum uterine bleeding and should never be given before delivery. Oxytocin is the preferred agent for control of postpartum hemorrhage, but if this peptide agent is ineffective, ergonovine maleate, 0.2 mg given intramuscularly, can be tried. It is usually effective within 1–5 minutes and is less toxic than other ergot derivatives for this application. It is given at the time of delivery of the placenta or immediately afterward if bleeding is significant.

D. Diagnosis of Variant Angina

Ergonovine given intravenously produces prompt vasoconstriction during coronary angiography to diagnose variant angina if reactive segments of the coronary arteries are present. In Europe, methylergometrine has been used for this purpose.

E. Senile Cerebral Insufficiency

Dihydroergotoxine, a mixture of dihydro-α-ergocryptine and three similar dihydrogenated peptide ergot alkaloids (ergoloid mesylates), has been promoted for many years for the relief of senility and more recently for the treatment of Alzheimer's dementia. There is no useful evidence that this drug has significant benefit.

Toxicity & Contraindications

The most common toxic effects of the ergot derivatives are gastrointestinal disturbances, including diarrhea, nausea, and vomiting. Activation of the medullary vomiting center and of the gastrointestinal serotonin receptors is involved. Since migraine attacks are often associated with these symptoms before therapy is begun, these adverse effects are rarely contraindications to the use of ergot.

A more dangerous toxic effect—usually associated with overdosage—of agents like ergotamine and ergonovine is prolonged vasospasm. This sign of vascular smooth muscle stimulation may result in gangrene and may require amputation. Bowel infarction has also been reported and may require resection. Peripheral vascular vasospasm caused by ergot is refractory to most vasodilators, but infusion of large doses of nitroprusside or nitroglycerin has been successful in some cases.

Chronic therapy with methysergide was associated with connective tissue proliferation in the retroperitoneal space, the pleural cavity, and the endocardial tissue of the heart. These changes occurred insidiously over months and presented as hydronephrosis (from obstruction of the ureters) or a cardiac murmur (from distortion of the valves of the heart). In some cases, valve damage required surgical replacement. As a result, this drug was withdrawn from the US market. Similar fibrotic change has resulted from the chronic use of 5-HT agonists promoted in the past for weight loss (fenfluramine, dexfenfluramine).

Other toxic effects of the ergot alkaloids include drowsiness and, in the case of methysergide, occasional instances of central stimulation and hallucinations. In fact, methysergide was sometimes used as a substitute for LSD by members of the so-called drug culture.

Contraindications to the use of ergot derivatives consist of the obstructive vascular diseases, especially symptomatic coronary artery disease, and collagen diseases.

There is no evidence that ordinary use of ergotamine for migraine is hazardous in pregnancy. However, most clinicians counsel restraint in the use of the ergot derivatives by pregnant patients. Use to deliberately cause abortion is contraindicated because the high doses required often cause dangerous vasoconstriction.

SUMMARY Drugs with Actions on Histamine and Serotonin Receptors; Ergot Alkaloids

Subclass	Mechanism of Action	Effects	Clinical Applications	Pharmacokinetics, Toxicities, Interactions
H_1 ANTIHISTAMINES				
First generation:				
• Diphenhydramine	Competitive antagonism/inverse agonism at H_1 receptors	Reduces or prevents histamine effects on smooth muscle, immune cells • also blocks muscarinic and α adrenoceptors • highly sedative	IgE immediate allergies, especially hay fever, urticaria • often used as a sedative, antiemetic, and anti-motion sickness drug	Oral and parenteral • duration 4–6 h • *Toxicity:* Sedation when used in hay fever, muscarinic blockade symptoms, orthostatic hypotension • *Interactions:* Additive sedation with other sedatives, including alcohol • some inhibition of CYP2D6, may prolong action of some β blockers
Second generation:				
• Cetirizine	Competitive antagonism/inverse agonism at H_1 receptors	Reduces or prevents histamine effects on smooth muscle, immune cells	IgE immediate allergies, especially hay fever, urticaria	Oral • duration 12–24 h • *Toxicity:* Sedation and arrhythmias in overdose • *Interactions:* Minimal
• *Other first-generation H_1 blockers: Chlorpheniramine is a less sedating H_1 blocker with fewer autonomic effects*				
• *Other second-generation H_1 blockers: Loratadine, desloratadine, and fexofenadine are very similar to cetirizine*				
H_2 ANTIHISTAMINES				
• Cimetidine (see Chapter 62)				
SEROTONIN AGONISTS				
5-$HT_{1B/1D}$:				
• Sumatriptan	Partial agonist at 5-$HT_{1B/1D}$ receptors	Effects not fully understood • may reduce release of calcitonin gene-related peptide and perivascular edema in cerebral circulation	Migraine and cluster headache	Oral, nasal, parenteral • duration 2 h • *Toxicity:* Paresthesias, dizziness, coronary vasoconstriction • *Interactions:* Additive with other vasoconstrictors
• *Other triptans: Similar to sumatriptan except for pharmacokinetics (2–6 h duration of action); much more expensive than generic sumatriptan*				
5-HT_4:				
• Tegaserod (see Chapter 62)				
SEROTONIN BLOCKERS				
5-HT_2:				
• Ketanserin (not available in USA)	Competitive blockade at 5-HT_2 receptors	Prevents vasoconstriction and bronchospasm of carcinoid syndrome	Hypertension • carcinoid syndrome associated with carcinoid tumor	Oral • duration 12–24 h • *Toxicity:* Hypotension
5-HT_3:				
• Ondansetron (see Chapter 62)				
ERGOT ALKALOIDS				
Vasoselective:				
• Ergotamine	Mixed partial agonist effects at 5-HT_2 and α adrenoceptors	Causes marked smooth muscle contraction but blocks α-agonist vasoconstriction	Migraine and cluster headache	Oral, parenteral • duration 12–24 h • *Toxicity:* Prolonged vasospasm causing angina, gangrene; uterine spasm
Uteroselective:				
• Ergonovine	Mixed partial agonist effects at 5-HT_2 and α adrenoceptors	Same as ergotamine • some selectivity for uterine smooth muscle	Postpartum bleeding • migraine headache	Oral, parenteral (methylergonovine) • duration 2–4 h • *Toxicity:* Same as ergotamine
CNS selective:				
• Lysergic acid diethylamide	Central nervous system (CNS) 5-HT_2 and dopamine agonist • 5-HT_2 antagonist in periphery	Hallucinations • psychotomimetic	None • widely abused	Oral • duration several hours • *Toxicity:* Prolonged psychotic state, flashbacks
• *Bromocriptine, pergolide: Ergot derivatives used in Parkinson's disease (see Chapter 28) and prolactinoma (see Chapter 37)*				

PREPARATIONS AVAILABLE

ANTIHISTAMINES (H_1 BLOCKERS)*

Azelastine
Nasal (Astelin): 137 mcg/puff nasal spray
Ophthalmic (Optivar): 0.5 mg/mL solution

Brompheniramine (generic, Brovex)
Oral: 6, 12 mg extended-release tablets; 12 mg chewable tablets; 2, 8, 12 mg/5 mL suspension

Buclizine (Bucladin-S Softabs)
Oral: 50 mg tablets

Carbinoxamine (generic, Histex)
Oral: 4 mg tablets; 8, 10 mg extended-release capsules; 1.5, 3.6, 4 mg/5 mL liquid

Cetirizine (generic, Zyrtec)
Oral: 5, 10 mg tablets; 5, 10 mg chewable tablets; 5 mg/5 mL syrup

Chlorpheniramine (generic, Chlor-Trimeton)
Oral: 2 mg chewable tablets; 4 mg tablets; 2 mg/5 mL syrup
Oral sustained-release: 8, 12, 16 mg tablets; 8, 12 mg capsules

Clemastine (generic, Tavist)
Oral: 1.34, 2.68 mg tablets; 0.67 mg/5 mL syrup

Cyclizine (Marezine)
Oral: 50 mg tablets

Cyproheptadine (generic)
Oral: 4 mg tablets; 2 mg/5 mL syrup

Desloratadine (Clarinex)
Oral: 5 mg tablets; 2.5, 5 mg rapidly disintegrating tablets; 2.5 mg/5 mL syrup

Dimenhydrinate (Dramamine, others)†
Oral: 50 mg tablets; 50 mg chewable tablets; 12.5/5 mL, 12.5 mg/4 mL, 15.62 mg/5 mL liquid
Parenteral: 50 mg/mL for IM or IV injection

Diphenhydramine (generic, Benadryl)
Oral: 12.5, 25 mg chewable tablets; 25, 50 mg tablets, capsules 12.5 mg orally disintegrating tablets; 12.5, 25 mg/5 mL elixir and syrup
Parenteral: 50 mg/mL for injection

Epinastine (Elestat)
Ophthalmic: 0.05% solution

Fexofenadine (generic, Allegra)
Oral: 30, 60, 180 mg tablets; 30 mg rapidly disintegrating tablets; 6 mg/mL suspension

Hydroxyzine (generic, Vistaril)
Oral: 10, 25, 50 mg tablets; 25, 50, 100 mg capsules; 10 mg/5 mL syrup; 25 mg/5 mL suspension
Parenteral: 25, 50 mg/mL for injection

Ketotifen (Zaditor)
Ophthalmic: 0.025% solution

Levocabastine (Livostin)
Ophthalmic: 0.05% solution

Levocetirizine (Xyzal)
Oral: 5 mg tablets

Loratadine (generic, Claritin, Tavist)
Oral: 10 mg tablets; 5 mg chewable tablets; 10 mg rapidly disintegrating tablets; 1 mg/mL syrup

Meclizine (generic, Antivert)
Oral: 12.5, 25, 50 mg tablets; 25 mg capsules; 25 mg chewable tablets

Olopatadine (Patanol)
Ophthalmic: 0.1% solution

Phenindamine (Nolahist)
Oral: 25 mg tablets

Promethazine (generic, Phenergan)
Oral: 12.5, 25, 50 mg tablets; 6.25 mg/5 mL syrups
Parenteral: 25, 50 mg/mL for injection
Rectal: 12.5, 25, 50 mg suppositories

Triprolidine (Zymine)
Oral: 1.25 mg/5 mL liquid

H_2 BLOCKERS

See Chapter 62.

5-HT AGONISTS

Almotriptan (Axert)
Oral: 6.25, 12.5 mg tablets

Eletriptan (Relpax)
Oral: 24.2, 48.5 mg tablets (equivalent to 20, 40 mg base)

Frovatriptan (Frova)
Oral: 2.5 mg tablets

Naratriptan (Amerge)
Oral: 1, 2.5 mg tablets

Rizatriptan
Oral: 5, 10 mg tablets (Maxalt); 5, 10 mg orally disintegrating tablets (Maxalt-MLT)

Sumatriptan (generic, Imitrex)
Oral: 25, 50, 100 mg tablets; generic formulation available
Nasal: 5, 20 mg unit dose spray devices
Parenteral: 4, 6 mg/0.5 mL in SELFdose autoinjection units for subcutaneous injection

Zolmitriptan (Zomig)
Oral: 2.5, 5 mg tablets; 2.5 mg orally disintegrating tablets
Nasal: 5 mg

5-HT ANTAGONISTS

See Chapter 62.

MELATONIN RECEPTOR AGONISTS

Ramelteon (Rozarem)
Oral: 8 mg tablets

ERGOT ALKALOIDS

Dihydroergotamine
Nasal (Migranal): 4 mg/mL nasal spray
Parenteral (D.H.E. 45): 1 mg/mL for injection

Ergonovine (Ergotrate)
Oral: 0.2 mg tablets

Ergotamine mixtures (generic, Cafergot)
Oral: 1 mg ergotamine/100 mg caffeine tablets
Rectal: 2 mg ergotamine/100 mg caffeine suppositories

Ergotamine tartrate (Ergomar)
Sublingual: 2 mg sublingual tablets

Methylergonovine (Methergine)
Oral: 0.2 mg tablets
Parenteral: 0.2 mg/mL for injection

*Several other antihistamines are available only in combination products with, for example, phenylephrine.
†Dimenhydrinate is the chlorotheophylline salt of diphenhydramine.

REFERENCES

Histamine

Arrang J-M, Morisset S, Gbahou F: Constitutive activity of the histamine H_3 receptor. Trends Pharmacol Sci 2007;28:350.

Barnes PJ: Histamine and serotonin. Pulm Pharmacol Ther 2001;14:329.

Bond RA, IJzerman AP: Recent developments in constitutive receptor activity and inverse agonism, and their potential for GPCR drug discovery. Trend Pharmacol Sci 2006;27:92.

deShazo RD, Kemp SF: Pharmacotherapy of allergic rhinitis. www.UpToDate.com, 2010.

Gemkow MJ et al: The histamine H_3 receptor as a therapeutic drug target for CNS disorders. Drug Discov Today 2009;14:509.

Lieberman P: Anaphylaxis. Med Clin North Am 2006;90:77.

Lin J-S et al: An inverse agonist of the histamine H_3 receptor improves wakefulness in narcolepsy: Studies in orexin$^{-/-}$ mice and patients. Neurobiol Dis 2008;30:74.

Preuss H et al: Constitutive activity and ligand selectivity of human, guinea pig, rat, and canine histamine H_2 receptors. J Pharmacol Exp Therap 2007;321:983.

Smits RA, Leurs R, deEdech JP: Major advances in the development of histamine H_4 receptor ligands. Drug Discov Today 2009;14:745.

Thurmond RL, Gelfand EW, Dunford PJ: The role of histamine H_1 and H_4 receptors in allergic inflammation: The search for new antihistamines. Nat Rev Drug Dis 2008;7:41.

Tokita S, Takahashi K, Kotani H: Recent advances in molecular pharmacology of the histamine systems: Physiology and pharmacology of histamine H_3 receptor: Roles in feeding regulation and therapeutic potential for metabolic disorders. J Pharmacol Sci 2006;101:12.

Serotonin

Bajwa ZH, Sabahat A: Acute treatment of migraine in adults. www.UpToDate.com, 2010.

Barrenetxe J, Delagrange P, Martinez JA: Physiologic and metabolic functions of melatonin. J Physiol Biochem 2004;60:61.

Boyer EW, Shannon M: The serotonin syndrome. N Engl J Med 2005;352:1112.

Elangbam CS: Drug-induced valvulopathy: An update. Toxicol Path 2010;38:837.

Frank C: Recognition and treatment of serotonin syndrome. Can Fam Physician 2008;54:988.

Isbister GK, Buckley NA, Whyte IM: Serotonin toxicity: A practical approach to diagnosis and treatment. Med J Aust 2007;187:361.

Loder E: Triptan therapy in migraine. N Engl J Med 2010;363:63.

Porvasnik SL et al: PRX-08066, a novel 5-hydroxytryptamine receptor 2B antagonist, reduces monocrotaline-induced pulmonary hypertension and right ventricular hypertrophy in rats. J Pharmacol Exp Therap 2010;334:364.

Prunet-Marcassus B et al: Melatonin reduces body weight gain in Sprague-Dawley rats with diet-induced obesity. Endocrinology 2003;144:5347.

Raymond JR et al: Multiplicity of mechanisms of serotonin receptor signal transduction. Pharmacol Ther 2001;92:179.

Sack RL: Jet lag. N Engl J Med 2010;362:440.

Ergot Alkaloids: Historical

Fuller JG: *The Day of St. Anthony's Fire*. Macmillan, 1968; Signet, 1969.

Gabbai Dr, Lisbonne Dr, Pourquier Dr: Ergot poisoning at Pont St. Esprit. Br Med J 1951;Sept 15:650.

Ergot Alkaloids: Pharmacology

Dierckx RA et al: Intraarterial sodium nitroprusside infusion in the treatment of severe ergotism. Clin Neuropharmacol 1986;9:542.

Dildy GA: Postpartum hemorrhage: New management options. Clin Obstet Gynecol 2002;45:330.

Mantegani S, Brambilla E, Varasi M: Ergoline derivatives: Receptor affinity and selectivity. Farmaco 1999;54:288.

Porter JK, Thompson FN Jr: Effects of fescue toxicosis on reproduction in livestock. J Animal Sci 1992;70:1594.

Snow V et al: Pharmacologic management of acute attacks of migraine and prevention of migraine headache. Ann Intern Med 2002;137:840.

CASE STUDY ANSWER

The patient demonstrates typical symptoms, signs, and course of urticaria due to food allergy. The failure of the antihistamine to control fully the signs and symptoms is also common. In such cases, addition of oral corticosteroids is often the most effective measure. Because the condition is self-limiting (assuming the patient does not repeat the allergen challenge), a tapering high-dose regimen, using prednisone, is relatively safe and effective. This consists of 50–100 mg prednisone on days 1 and 2, 30–75 mg on days 3 and 4, 15–40 mg on days 5 and 6, 5–10 mg on days 7, 8, and 9, and none thereafter if symptoms do not recur.

This page intentionally left blank

CHAPTER 17

Vasoactive Peptides

Ian A. Reid, PhD

CASE STUDY

During a routine check, a 45-year-old man was found to have high blood pressure (165/100 mm Hg). Blood pressure remained high on two follow-up visits. His physician initially prescribed hydrochlorothiazide, a diuretic commonly used to treat hypertension. Although his blood pressure was reduced by hydrochlorothiazide, it remained at a hypertensive level (145/95 mm Hg), and he was referred to the university hypertension clinic. Your evaluation reveals that the patient has elevated plasma renin activity and aldosterone concentration. Hydrochlorothiazide is therefore replaced with enalapril, an angiotensin-converting enzyme inhibitor. Enalapril lowers the blood pressure to almost normotensive levels. However, after several weeks on the new drug, the patient returns complaining of a persistent cough. In addition, some signs of angioedema are detected. How does enalapril lower blood pressure? Why does it occasionally cause coughing and angioedema? What other drugs could be used to inhibit renin secretion or suppress the renin-angiotensin system, and decrease blood pressure, without the adverse effects of enalapril?

Peptides are used by most tissues for cell-to-cell communication. As noted in Chapters 6 and 21, they play important roles as transmitters in the autonomic and central nervous systems. Several peptides exert important direct effects on vascular and other smooth muscles. These peptides include vasoconstrictors (**angiotensin II, vasopressin, endothelins, neuropeptide Y**, and **urotensin**) and vasodilators (**bradykinin** and related **kinins, natriuretic peptides, vasoactive intestinal peptide, substance P, neurotensin, calcitonin gene-related peptide**, and **adrenomedullin**). This chapter focuses on the smooth muscle actions of the peptides.

ANGIOTENSIN

BIOSYNTHESIS OF ANGIOTENSIN

The pathway for the formation and metabolism of angiotensin II (ANG II) is summarized in Figure 17–1. The principal steps include enzymatic cleavage of angiotensin I (ANG I) from angiotensinogen by renin, conversion of ANG I to ANG II by converting enzyme, and degradation of ANG II by several peptidases.

Renin

Renin is an aspartyl protease enzyme that specifically catalyzes the hydrolytic release of the decapeptide ANG I from angiotensinogen. It is synthesized as a prepromolecule that is processed to prorenin, which has poorly understood actions (see below), and then to active renin, a glycoprotein consisting of 340 amino acids.

Renin in the circulation originates in the kidneys. Enzymes with renin-like activity are present in several extrarenal tissues, including blood vessels, uterus, salivary glands, and adrenal cortex, but no physiologic role for these enzymes has been established. Within the kidney, renin is synthesized and stored in the juxtaglomerular apparatus of the nephron. Specialized granular cells called juxtaglomerular cells are the site of synthesis, storage, and release of renin. The macula densa is a specialized segment of the nephron that is closely associated with the vascular components of the juxtaglomerular apparatus. The vascular and tubular components of the juxtaglomerular apparatus, including the juxtaglomerular cells, are innervated by noradrenergic neurons.

Control of Renin Release

The rate at which renin is released by the kidney is the primary determinant of activity of the renin-angiotensin system. Active

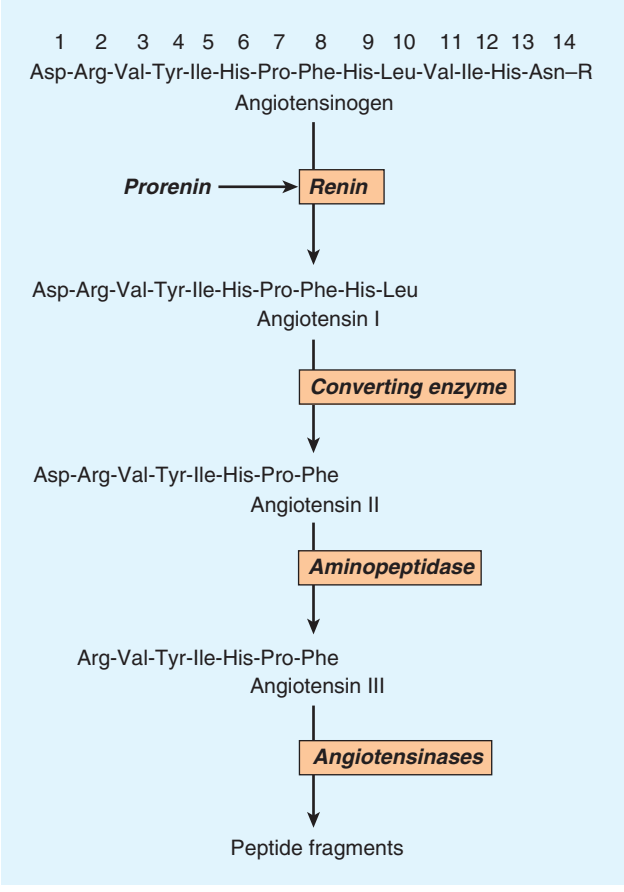


FIGURE 17–1 Chemistry of the renin-angiotensin system. The amino acid sequence of the amino terminal of human angiotensinogen is shown. R denotes the remainder of the protein molecule. See text for additional steps in the formation and metabolism of angiotensin peptides.

renin is released by exocytosis immediately upon stimulation of the juxtaglomerular apparatus. Prorenin is released constitutively, usually at a rate higher than that of active renin, thus accounting for the fact that prorenin can constitute 80–90% of the total renin in the circulation. The significance of circulating prorenin is discussed at the end of this section. Active renin release is controlled by a variety of factors, including a renal vascular receptor, the macula densa, the sympathetic nervous system, and ANG II.

A. Macula Densa

Renin release is controlled in part by the macula densa, a structure that has a close anatomic association with the afferent arteriole. The initial step involves the detection of some function of NaCl concentration in, or delivery to, the distal tubule, possibly by the $Na^+/K^+/2Cl^-$ cotransporter. The macula densa then signals changes in renin release by the juxtaglomerular cells such that there is an inverse relationship between NaCl delivery or concentration and renin release. Potential candidates for signal transmission include prostaglandin E_2 (PGE_2) and nitric oxide, which stimulate renin release, and adenosine, which inhibits it.

B. Renal Baroreceptor

The renal baroreceptor mediates an inverse relationship between renal artery pressure and renin release. The mechanism is not completely understood but it appears that the juxtaglomerular cells are sensitive to stretch and that increased stretch results in decreased renin release. The decrease may result from influx of calcium which, somewhat paradoxically, inhibits renin release. The paracrine factors PGE_2, nitric oxide, and adenosine have also been implicated in the baroreceptor control of renin release.

C. Sympathetic Nervous System

Norepinephrine released from renal sympathetic nerves stimulates renin release indirectly by α-adrenergic activation of the renal baroreceptor and macula densa mechanisms, and directly by an action on the juxtaglomerular cells. In humans, the direct effect is mediated by β_1 adrenoceptors. Through this mechanism, reflex activation of the sympathetic nervous system by hypotension or hypovolemia leads to activation of the renin-angiotensin system.

D. Angiotensin

Angiotensin II inhibits renin release. The inhibition results from increased blood pressure acting by way of the renal baroreceptor and macula densa mechanisms, and from a direct action of the peptide on the juxtaglomerular cells. The direct inhibition is mediated by increased intracellular Ca^{2+} concentration and forms the basis of a short-loop negative feedback mechanism controlling renin release. Interruption of this feedback with drugs that inhibit the renin-angiotensin system (see below) results in stimulation of renin release.

E. Intracellular Signaling Pathways

The release of renin by the juxtaglomerular cells is controlled by interplay among three intracellular messengers: cAMP, cyclic guanosine monophosphate (cGMP), and free cytosolic Ca^{2+} concentration (Figure 17–2). cAMP plays a major role; maneuvers that increase cAMP levels, including activation of adenylyl cyclase, inhibition of cAMP phosphodiesterases, and administration of cAMP analogs, increase renin release. Increases in Ca^{2+} can result from increased entry of extracellular Ca^{2+} or mobilization of Ca^{2+} from intracellular stores, while increases in cGMP levels can result from activation of soluble or particulate guanylyl cyclase. Ca^{2+} and cGMP appear to alter renin release indirectly, primarily by changing cAMP levels.

F. Pharmacologic Alteration of Renin Release

The release of renin is altered by a wide variety of pharmacologic agents. Renin release is stimulated by vasodilators (hydralazine, minoxidil, nitroprusside), β-adrenoceptor agonists, α-adrenoceptor antagonists, phosphodiesterase inhibitors (eg, theophylline, milrinone, rolipram), and most diuretics and anesthetics. This stimulation can be accounted for by the control mechanisms just described. Drugs that inhibit renin release are discussed below.

Many of the peptides reviewed in this chapter also alter renin release. Release is stimulated by adrenomedullin, bradykinin, and

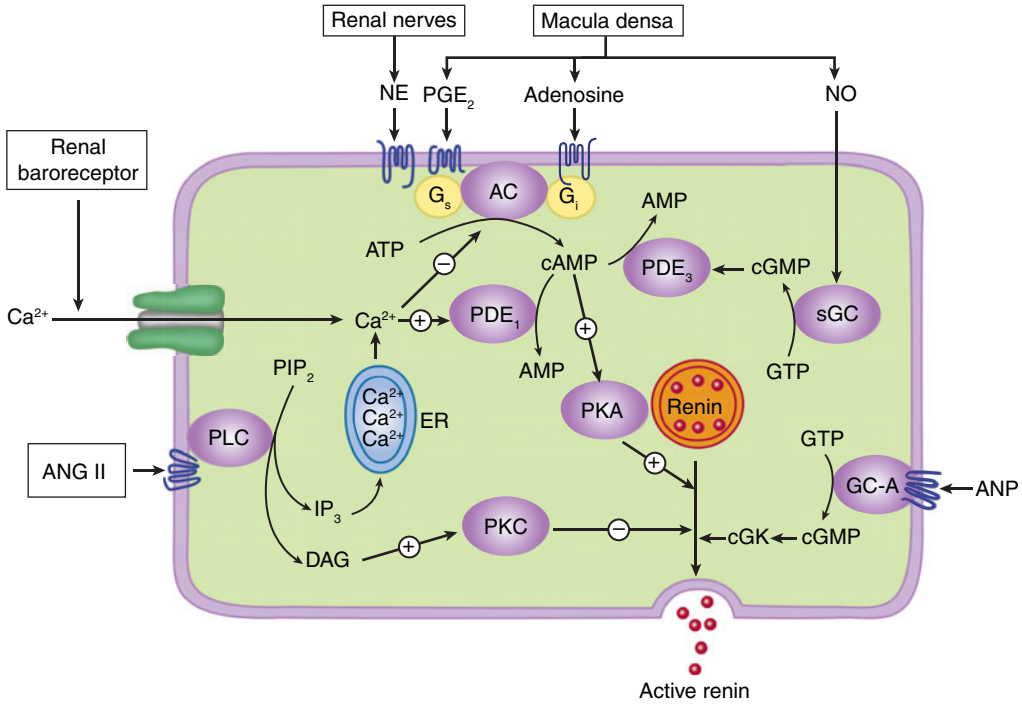


FIGURE 17–2 Major physiologic inputs to renin release and proposed integration with signaling pathways in the juxtaglomerular cell. AC, adenylyl cyclase; ANG II, angiotensin II; ANP, atrial natriuretic peptide; cGK, protein kinase G; DAG, diacylglycerol; GC-A, particulate guanylyl cyclase; ER, endoplasmic reticulum; IP_3, inositol trisphosphate; NE, norepinephrine; NO, nitric oxide; PDE, phosphodiesterase; PKA, protein kinase A; PLC, phospholipase C; sGC, soluble guanylyl cyclase. (Redrawn, with permission, from Castrop H et al: Physiology of kidney renin. Physiol Rev 2010;90:607.)

calcitonin gene-related peptide, and inhibited by atrial natriuretic peptide, endothelin, substance P, and vasopressin.

Angiotensinogen

Angiotensinogen is the circulating protein substrate from which renin cleaves ANG I. It is synthesized in the liver. Human angiotensinogen is a glycoprotein with a molecular weight of approximately 57,000. The 14 amino acids at the amino terminal of the molecule are shown in Figure 17–1. In humans, the concentration of angiotensinogen in the circulation is less than the K_m of the renin-angiotensinogen reaction and is therefore an important determinant of the rate of formation of angiotensin.

The production of angiotensinogen is increased by corticosteroids, estrogens, thyroid hormones, and ANG II. It is also elevated during pregnancy and in women taking estrogen-containing oral contraceptives. The increased plasma angiotensinogen concentration is thought to contribute to the hypertension that may occur in these situations.

Angiotensin I

Although ANG I contains the peptide sequences necessary for all of the actions of the renin-angiotensin system, it has little or no biologic activity. Instead, it must be converted to ANG II by converting enzyme (Figure 17–1). ANG I may also be acted on by plasma or tissue aminopeptidases to form [des-Asp^1]angiotensin I; this in turn is converted to [des-Asp^1]angiotensin II (commonly known as angiotensin III) by converting enzyme.

Converting Enzyme (ACE, Peptidyl Dipeptidase, Kininase II)

Converting enzyme is a dipeptidyl carboxypeptidase with two active sites that catalyzes the cleavage of dipeptides from the carboxyl terminal of certain peptides. Its most important substrates are ANG I, which it converts to ANG II, and bradykinin, which it inactivates (see Kinins, below). It also cleaves enkephalins and substance P, but the physiologic significance of these effects has not been established. The action of converting enzyme is prevented by a penultimate prolyl residue in the substrate, and ANG II is therefore not hydrolyzed by converting enzyme. Converting enzyme is distributed widely in the body. In most tissues, converting enzyme is located on the luminal surface of vascular endothelial cells and is thus in close contact with the circulation.

A homolog of converting enzyme known as ACE2 was recently found to be highly expressed in vascular endothelial cells of the kidneys, heart, and testes. Unlike converting enzyme, ACE2 has only one active site and functions as a carboxypeptidase rather than a dipeptidyl carboxypeptidase. It removes a single amino acid from the C-terminal of ANG I forming ANG 1-9 (Figure 17–3), which is inactive but is converted to ANG 1-7 by ACE. ACE2 also converts ANG

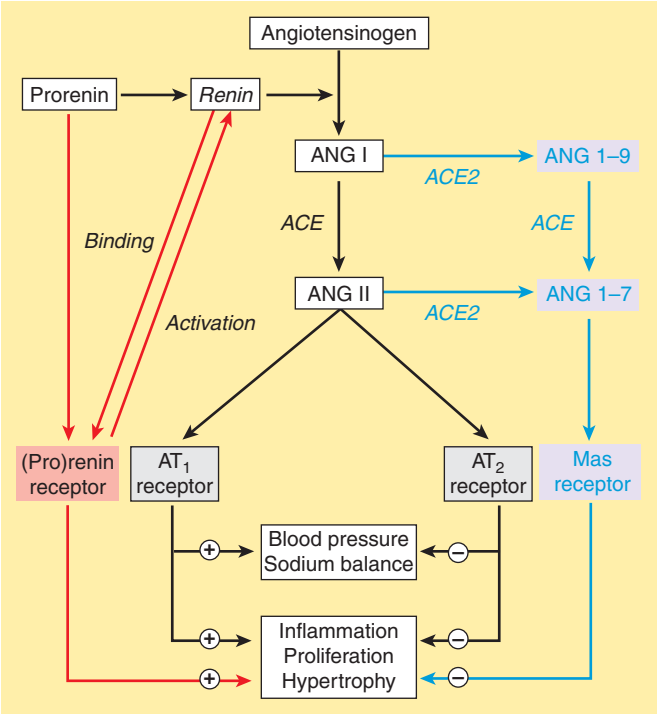


FIGURE 17–3 The renin-angiotensin system showing the established system (black) and recently discovered pathways involving the (pro)renin receptor (red) and ANG 1-7 (blue). (Redrawn, with permission, from Castrop H et al: Physiology of kidney renin. Physiol Rev 2010;90:607.)

II to ANG 1-7. ANG 1-7 has vasodilator activity, apparently mediated by the orphan heterotrimeric guanine nucleotide-binding protein-coupled receptor (Mas receptor). This vasodilation may serve to counteract the vasoconstrictor activity of ANG II. ACE2 also differs from ACE in that it does not hydrolyze bradykinin and is not inhibited by converting enzyme inhibitors (see below). Thus, the enzyme more closely resembles an angiotensinase than a converting enzyme.

Angiotensinase

Angiotensin II, which has a plasma half-life of 15–60 seconds, is removed rapidly from the circulation by a variety of peptidases collectively referred to as angiotensinase. It is metabolized during passage through most vascular beds (a notable exception being the lung). Most metabolites of ANG II are biologically inactive, but the initial product of aminopeptidase action—[des-Asp^{1}]angiotensin II—retains considerable biologic activity.

ACTIONS OF ANGIOTENSIN II

Angiotensin II exerts important actions at vascular smooth muscle, adrenal cortex, kidney, heart, and brain via the receptors described below. Through these actions, the renin-angiotensin system plays a key role in the regulation of fluid and electrolyte balance and arterial blood pressure. Excessive activity of the renin-angiotensin system can result in hypertension and disorders of fluid and electrolyte homeostasis.

Blood Pressure

Angiotensin II is a very potent pressor agent—on a molar basis, approximately 40 times more potent than norepinephrine. The pressor response to intravenous ANG II is rapid in onset (10–15 seconds) and sustained during long-term infusions. A large component of the pressor response is due to direct contraction of vascular—especially arteriolar—smooth muscle. In addition, however, ANG II can also increase blood pressure through actions on the brain and autonomic nervous system. The pressor response to ANG II is usually accompanied by little or no reflex bradycardia because the peptide acts on the brain to reset the baroreceptor reflex control of heart rate to a higher pressure.

Angiotensin II also interacts with the autonomic nervous system. It stimulates autonomic ganglia, increases the release of epinephrine and norepinephrine from the adrenal medulla, and most important, facilitates sympathetic transmission by an action at adrenergic nerve terminals. The latter effect involves both increased release and reduced reuptake of norepinephrine. Angiotensin II also has a less important direct positive inotropic action on the heart.

Adrenal Cortex & Kidney

Angiotensin II acts directly on the zona glomerulosa of the adrenal cortex to stimulate aldosterone synthesis and release. At higher concentrations, ANG II also stimulates glucocorticoid synthesis. Angiotensin II acts on the kidney to cause renal vasoconstriction, increase proximal tubular sodium reabsorption, and inhibit the release of renin.

Central Nervous System

In addition to its central effects on blood pressure, ANG II acts on the central nervous system to stimulate drinking (dipsogenic effect) and increase the secretion of vasopressin and adrenocorticotropic hormone (ACTH). The physiologic significance of the effects of ANG II on drinking and pituitary hormone secretion is not known.

Cell Growth

Angiotensin II is mitogenic for vascular and cardiac muscle cells and may contribute to the development of cardiovascular hypertrophy. It also exerts a variety of important effects on the vascular endothelium. Indeed, overactivity of the renin-angiotensin system has been implicated as one of the most significant factors in the development of hypertensive vascular disease. Considerable evidence now indicates that ACE inhibitors and ANG II receptor antagonists (see below) slow or prevent morphologic changes (remodeling) following myocardial infarction that would otherwise lead to heart failure. The stimulation of vascular and cardiac growth by ANG II is mediated by other pathways, probably receptor and nonreceptor tyrosine kinases such as the Janus tyrosine

kinase Jak2, and by increased transcription of specific genes (see Chapter 2).

ANGIOTENSIN RECEPTORS & MECHANISM OF ACTION

Angiotensin II receptors are widely distributed in the body. Like the receptors for other peptide hormones, ANG II receptors are G protein-coupled and located on the plasma membrane of target cells, and this permits rapid onset of the various actions of ANG II. Two distinct subtypes of ANG II receptors, termed **AT_1** and **AT_2,** have been identified on the basis of their differential affinity for antagonists and their sensitivity to sulfhydryl-reducing agents. AT_1 receptors have a high affinity for the inhibitor losartan and a low affinity for PD 123177 (an experimental nonpeptide antagonist), whereas AT_2 receptors have a high affinity for PD 123177 and a low affinity for losartan. Angiotensin II and saralasin (see below) bind equally to both subtypes. The relative proportion of the two subtypes varies from tissue to tissue: AT_1 receptors predominate in vascular smooth muscle. Most of the known actions of ANG II are mediated by the AT_1 receptor, a G_q protein-coupled receptor. Binding of ANG II to AT_1 receptors in vascular smooth muscle results in activation of phospholipase C and generation of inositol trisphosphate and diacylglycerol (see Chapter 2). These events, which occur within seconds, result in smooth muscle contraction.

The AT_2 receptor has a structure and affinity for ANG II similar to those of the AT_1 receptor. In contrast, however, stimulation of AT_2 receptors causes vasodilation that may serve to counteract the vasoconstriction resulting from AT_1 receptor stimulation. AT_2 receptor-mediated vasodilation appears to be nitric oxide-dependent and may involve the bradykinin B_2 receptor-nitric oxide-cGMP pathway. AT_2 receptors are present at high density in all tissues during fetal development, but they are much less abundant in the adult where they are expressed at high concentration only in the adrenal medulla, reproductive tissues, vascular endothelium, and parts of the brain. AT_2 receptors are up-regulated in pathologic conditions including heart failure and myocardial infarction. The functions of the AT_2 receptor appear to include fetal tissue development, inhibition of growth and proliferation, cell differentiation, apoptosis, and vasodilation.

INHIBITION OF THE RENIN-ANGIOTENSIN SYSTEM

In view of the importance of the renin-angiotensin system in cardiovascular disease, considerable effort has been directed to developing drugs that inhibit the system. A wide variety of agents that block the formation or action of ANG II is now available. Some of these drugs block renin release, but most inhibit the conversion of ANG I to ANG II, block angiotensin AT_1 receptors, or inhibit the enzymatic action of renin.

Drugs That Block Renin Release

Several drugs that interfere with the sympathetic nervous system inhibit the release of renin. Examples are clonidine and propranolol. Clonidine inhibits renin release by causing a centrally mediated reduction in renal sympathetic nerve activity, and it may also exert a direct intrarenal action. Propranolol and other β-adrenoceptor–blocking drugs act by blocking the intrarenal and extrarenal β receptors involved in the neural control of renin release.

Angiotensin-Converting Enzyme Inhibitors

An important class of orally active ACE inhibitors, directed against the active site of ACE, is now extensively used. **Captopril** and **enalapril** are examples of the many potent ACE inhibitors that are available. These drugs differ in their structure and pharmacokinetics, but they are interchangeable in clinical use. ACE inhibitors decrease systemic vascular resistance without increasing heart rate, and they promote natriuresis. As described in Chapters 11 and 13, they are effective in the treatment of hypertension, decrease morbidity and mortality in heart failure and left ventricular dysfunction after myocardial infarction, and delay the progression of diabetic nephropathy.

ACE inhibitors not only block the conversion of ANG I to ANG II but also inhibit the degradation of other substances, including bradykinin, substance P, and enkephalins. The action of ACE inhibitors to inhibit bradykinin metabolism contributes significantly to their hypotensive action (see Figure 11–5) and is apparently responsible for some adverse side effects, including cough and angioedema. These drugs are contraindicated in pregnancy because they cause fetal kidney damage.

Angiotensin Receptor Blockers

Potent peptide antagonists of the action of ANG II are available for research use. The best-known of these is the partial agonist, **saralasin**. Saralasin lowers blood pressure in hypertensive patients but may elicit pressor responses, particularly when circulating ANG II levels are low. Because it must be administered intravenously, saralasin is used only for investigation of renin-dependent hypertension and other hyperreninemic states.

The *non*peptide ANG II receptor blockers (ARBs) are of much greater interest. **Losartan, valsartan, eprosartan, irbesartan, candesartan, olmesartan,** and **telmisartan** are orally active, potent, and specific competitive antagonists of angiotensin AT_1 receptors. The efficacy of these drugs in hypertension is similar to that of ACE inhibitors, but they are associated with a lower incidence of cough. Like ACE inhibitors, ARBs slow the progression of diabetic nephropathy and valsartan has been reported to decrease the incidence of diabetes in patients with impaired glucose tolerance. The antagonists are also effective in the treatment of heart failure and provide a useful alternative when ACE inhibitors are not well tolerated. ARBs are generally well tolerated but should not be used by patients with nondiabetic renal disease or in pregnancy.

Marfan syndrome is a connective tissue disorder associated with aortic disease and other abnormalities involving increased transforming growth factor (TGF)-β signaling. Since ANG II increases TGF-β levels, it was reasoned that blockade of the renin-angiotensin system might be beneficial in Marfan syndrome. Promising initial results have been obtained with losartan, and clinical trials are underway.

The currently available ARBs are selective for the AT_1 receptor. Since prolonged treatment with the drugs disinhibits renin release and increases circulating ANG II levels, there may be increased stimulation of AT_2 receptors. This may be significant in view of the evidence that activation of the AT_2 receptor causes vasodilation and other beneficial effects. AT_2 receptor antagonists such as PD 123177 are available for research but have no clinical applications at this time. However, a selective AT_2 *agonist*, compound 21, lowers blood pressure in hypertensive animals and may be beneficial in human hypertension. The clinical benefits of ARBs are similar to those of ACE inhibitors, and it is not clear if one group of drugs has significant advantages over the other. Combination therapy with an ACE inhibitor plus an ARB has a number of potential advantages and is currently being investigated.

Renin Inhibitors

Cleavage of angiotensinogen by renin (Figures 17–1 and 17–3) is the rate-limiting step in the formation of ANG II and thus represents a logical target for inhibition of the renin-angiotensin system. Drugs that inhibit renin have been available for many years but have been limited by low potency, poor bioavailability, and short duration of action. However, a new class of nonpeptide, low-molecular-weight, orally active inhibitors has recently been developed.

Aliskiren is the most advanced of these and the first to be approved for the treatment of hypertension. In healthy subjects, aliskiren produces a dose-dependent reduction in plasma renin activity and ANG I and II and aldosterone concentrations. In patients with hypertension, many of whom have elevated plasma renin levels, aliskiren suppresses plasma renin activity and causes dose-related reductions in blood pressure similar to those produced by ACE inhibitors and ARBs. The safety and tolerability of aliskiren appear to be comparable to angiotensin antagonists and placebo. Aliskiren is contraindicated in pregnancy.

Inhibition of the renin-angiotensin system with ACE inhibitors or ARBs may be incomplete because the drugs disrupt the negative feedback action of ANG II on renin release and thereby increase plasma renin activity. Other antihypertensive drugs, notably hydrochlorothiazide and other diuretics, also increase plasma renin activity. Aliskiren not only decreases baseline plasma renin activity in hypertensive subjects but also eliminates the rise produced by ACE inhibitors, ARBs, and diuretics and thereby results in a greater antihypertensive effect. Renin inhibition has thus proved to be an important new approach to the treatment of hypertension.

Prorenin Receptors

For many years, prorenin was considered to be an inactive precursor of renin, with no function of its own. Thus the observation noted above in the section on renin that prorenin circulates at high levels was surprising. Recently, however, a receptor that preferentially binds prorenin has been identified. Since it also binds active renin, the receptor is referred to as the (pro)renin receptor.

The receptor is a 350-amino acid protein with a single transmembrane domain. When prorenin binds to the (pro)renin receptor, it undergoes a conformational change and becomes fully active. The catalytic activity of active renin also increases further when it binds to the receptor. The activated prorenin and renin interact with circulating angiotensinogen to form angiotensin (Figure 17–3). However, binding of prorenin to the receptor also activates intracellular signaling pathways that differ depending on the cell type. For example, in mesangial and vascular smooth muscle cells, prorenin binding activates MAP kinases and expression of profibrotic molecules. Thus, elevated prorenin levels (as occur, for example, in diabetes mellitus) could produce a variety of adverse effects via both angiotensin-dependent and independent pathways. Recent research indicates that the (pro)renin receptor is functionally linked to the vacuolar proton-ATPase (ATP6ap2) and is necessary for Wnt signaling pathways involved (independently of renin) in stem cell biology, embryology, and cancer.

A synthetic peptide named handle region peptide (HRP), which consists of the amino acid sequence corresponding to the "handle" region of the prorenin prosegment, has been synthesized and shown to competitively inhibit binding of prorenin to the (pro)renin receptor. HRP has beneficial effects in the kidneys of diabetic rats and there is considerable interest in developing noncompetitive antagonists of the (pro)renin receptor.

This novel receptor could be important in cardiovascular and other diseases, but at the present time its role in human pathology is far from clear.

KININS

BIOSYNTHESIS OF KININS

Kinins are potent vasodilator peptides formed enzymatically by the action of enzymes known as kallikreins or kininogenases acting on protein substrates called kininogens. The kallikrein-kinin system has several features in common with the renin-angiotensin system.

Kallikreins

Kallikreins are present in plasma and in several organs and tissues, including the kidneys, pancreas, intestine, sweat glands, and salivary glands. Plasma prekallikrein can be activated to kallikrein by trypsin, Hageman factor, and possibly kallikrein itself. In general, the biochemical properties of tissue kallikreins are different from those of plasma kallikreins. Kallikreins can convert prorenin to active renin, but the physiologic significance of this action has not been established.

Kininogens

Kininogens—the precursors of kinins and substrates of kallikreins—are present in plasma, lymph, and interstitial fluid. Two kininogens are known to be present in plasma: a low-molecular-weight form (LMW kininogen) and a high-molecular-weight form (HMW kininogen). About 15–20% of the total plasma kininogen is in the HMW form. It is thought that LMW kininogen crosses capillary walls and serves as the substrate for tissue kallikreins, whereas HMW kininogen is confined to the bloodstream and serves as the substrate for plasma kallikrein.

FORMATION OF KININS IN PLASMA & TISSUES

The pathway for the formation and metabolism of kinins is shown in Figure 17–4. Three kinins have been identified in mammals: **bradykinin, lysylbradykinin** (also known as **kallidin**), and **methionyllysylbradykinin.** Each contains bradykinin in its structure.

Each kinin is formed from a kininogen by the action of a different enzyme. Bradykinin is released by plasma kallikrein, lysylbradykinin by tissue kallikrein, and methionyllysylbradykinin by pepsin and pepsin-like enzymes. The three kinins have been found in plasma and urine. Bradykinin is the predominant kinin in plasma, whereas lysylbradykinin is the major urinary form.

PHYSIOLOGIC & PATHOLOGIC EFFECTS OF KININS

Effects on the Cardiovascular System

Kinins produce marked arteriolar dilation in several vascular beds, including the heart, skeletal muscle, kidney, liver, and intestine. In this respect, kinins are approximately 10 times more potent on a molar basis than histamine. The vasodilation may result from a direct inhibitory effect of kinins on arteriolar smooth muscle or may be mediated by the release of nitric oxide or vasodilator prostaglandins such as PGE_2 and PGI_2. In contrast, the predominant effect of kinins on veins is contraction; again, this may result from direct stimulation of venous smooth muscle or from the release of venoconstrictor prostaglandins such as $PGF_{2\alpha}$. Kinins also produce contraction of most visceral smooth muscle.

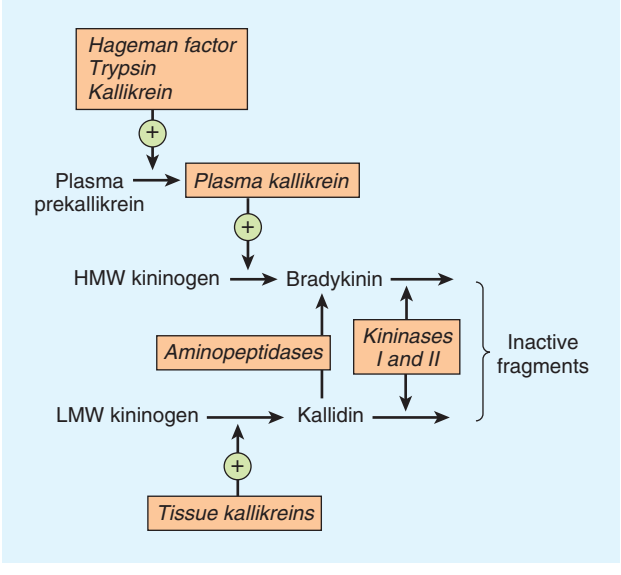


FIGURE 17–4 The kallikrein-kinin system. Kininase II is identical to converting enzyme peptidyl dipeptidase (ACE).

When injected intravenously, kinins produce a rapid but brief fall in blood pressure that is due to their arteriolar vasodilator action. Intravenous infusions of the peptide fail to produce a sustained decrease in blood pressure; prolonged hypotension can only be produced by progressively increasing the rate of infusion. The rapid reversibility of the hypotensive response to kinins is due primarily to reflex increases in heart rate, myocardial contractility, and cardiac output. In some species, bradykinin produces a biphasic change in blood pressure—an initial hypotensive response followed by an increase above the preinjection level. The increase in blood pressure may be due to a reflex activation of the sympathetic nervous system, but under some conditions, bradykinin can directly release catecholamines from the adrenal medulla and stimulate sympathetic ganglia. Bradykinin also increases blood pressure when injected into the central nervous system, but the physiologic significance of this effect is not clear, since it is unlikely that kinins cross the blood-brain barrier. (Note, however, that bradykinin can increase the permeability of the blood-brain barrier to some other substances.) Kinins have no consistent effect on sympathetic or parasympathetic nerve endings.

The arteriolar dilation produced by kinins causes an increase in pressure and flow in the capillary bed, thus favoring efflux of fluid from blood to tissues. This effect may be facilitated by increased capillary permeability resulting from contraction of endothelial cells and widening of intercellular junctions, and by increased venous pressure secondary to constriction of veins. As a result of these changes, water and solutes pass from the blood to the extracellular fluid, lymph flow increases, and edema may result.

The role that endogenous kinins play in the regulation of blood pressure is not clear. They do not appear to participate in the control of blood pressure under resting conditions but may play a role in postexercise hypotension.

Effects on Endocrine & Exocrine Glands

As noted earlier, prekallikreins and kallikreins are present in several glands, including the pancreas, kidney, intestine, salivary glands, and sweat glands, and they can be released into the secretory fluids of these glands. The function of the enzymes in these tissues is not known. Since kinins have such marked effects on smooth muscle, they may modulate the tone of salivary and pancreatic ducts, help regulate gastrointestinal motility, and act as local modulators of blood flow. Kinins also influence the transepithelial transport of water, electrolytes, glucose, and amino acids, and may regulate the transport of these substances in the gastrointestinal tract and kidney. Finally, kallikreins may play a role in the physiologic activation of various prohormones, including proinsulin and prorenin.

Role in Inflammation & Pain

Bradykinin has long been known to produce the four classic symptoms of inflammation—redness, local heat, swelling, and pain. Kinins are rapidly generated after tissue injury and play a pivotal role in the development and maintenance of these inflammatory processes.

Kinins are potent pain-producing substances when applied to a blister base or injected intradermally. They elicit pain by stimulating nociceptive afferents in the skin and viscera.

Other Effects

There is evidence that bradykinin may play a beneficial, protective role in certain cardiovascular diseases and ischemic stroke-induced brain injury. On the other hand, it has been implicated in cancer and some central nervous system diseases.

KININ RECEPTORS & MECHANISMS OF ACTION

The biologic actions of kinins are mediated by specific receptors located on the membranes of the target tissues. Two types of kinin receptors, termed B_1 and B_2, have been defined based on the rank orders of agonist potencies; both are G protein-coupled receptors. (Note that B here stands for bradykinin, not for β adrenoceptor.) Bradykinin displays the highest affinity in most B_2 receptor systems, followed by lys-bradykinin and then by met-lys-bradykinin. One exception is the B_2 receptor that mediates contraction of venous smooth muscle; this appears to be most sensitive to lys-bradykinin. Recent evidence suggests the existence of two B_2-receptor subtypes, which have been termed B_{2A} and B_{2B}.

B_1 receptors appear to have a very limited distribution in mammalian tissues and have few known functional roles. Studies with knockout mice that lack functional B_1 receptors suggest that these receptors participate in the inflammatory response and may also be important in long-lasting kinin effects such as collagen synthesis and cell multiplication. By contrast, B_2 receptors have a widespread distribution that is consistent with the multitude of biologic effects that are mediated by this receptor type. Agonist binding to B_2 receptors sets in motion multiple signal transduction events, including calcium mobilization, chloride transport, formation of nitric oxide, and activation of phospholipase C, phospholipase A_2, and adenylyl cyclase.

METABOLISM OF KININS

Kinins are metabolized rapidly (half-life < 15 seconds) by nonspecific exopeptidases or endopeptidases, commonly referred to as kininases. Two plasma kininases have been well characterized. Kininase I, apparently synthesized in the liver, is a carboxypeptidase that releases the carboxyl terminal arginine residue. Kininase II is present in plasma and vascular endothelial cells throughout the body. It is identical to angiotensin-converting enzyme (ACE, peptidyl dipeptidase), discussed above. Kininase II inactivates kinins by cleaving the carboxyl terminal dipeptide phenylalanyl-arginine. Like angiotensin I, bradykinin is almost completely hydrolyzed during a single passage through the pulmonary vascular bed.

DRUGS AFFECTING THE KALLIKREIN-KININ SYSTEM

Drugs that modify the activity of the kallikrein-kinin system are available, though none are in wide clinical use. Considerable effort has been directed toward developing kinin receptor antagonists, since such drugs have considerable therapeutic potential as anti-inflammatory and antinociceptive agents. Competitive antagonists of both B_1 and B_2 receptors are available for research use. Examples of B_1 receptor antagonists are the peptides [Leu_8-des-Arg_9]bradykinin and Lys[Leu_8-des-Arg_9]bradykinin. The first B_2 receptor antagonists to be discovered were also peptide derivatives of bradykinin. These first-generation antagonists were used extensively in animal studies of kinin receptor pharmacology. However, their half-life is short, and they are almost inactive on the human B_2 receptor.

Icatibant is a second-generation B_2 receptor antagonist. It is a decapeptide with an affinity for the B_2 receptor similar to that of bradykinin and is absorbed rapidly after subcutaneous administration. Icatibant has been shown to be effective in the treatment of hereditary angioedema, an autosomal dominant disorder characterized by recurrent episodes of bradykinin-mediated angioedema of the airways, gastrointestinal tract, extremities, and genitalia. It may also be useful in other conditions including drug-induced angioedema, airway disease, thermal injury, ascites, and pancreatitis.

Recently, a third generation of B_2-receptor antagonists was developed; examples are FR 173657, FR 172357, and NPC 18884. These antagonists block both human and animal B_2 receptors and are orally active. They have been reported to inhibit bradykinin-induced bronchoconstriction in guinea pigs, carrageenin-induced inflammation in rats, and capsaicin-induced nociception in mice. These antagonists have promise for the treatment of inflammatory pain in humans.

SSR240612 is a new, potent, and orally active selective antagonist of B_1 receptors in humans and several animal species. It exhibits analgesic and anti-inflammatory activities in mice and rats and is currently in preclinical development for the treatment of inflammatory and neurogenic pain.

The synthesis of kinins can be inhibited with the kallikrein inhibitor **aprotinin**. Kinin synthesis can also be blocked with **ecallantide,** a newly developed recombinant plasma kallikrein inhibitor which, like the B_2-receptor antagonist icatibant, is effective in the treatment of hereditary angioedema. Actions of kinins mediated by prostaglandin generation can be blocked nonspecifically with inhibitors of prostaglandin synthesis such as aspirin. Conversely, the actions of kinins can be enhanced with ACE inhibitors, which block the degradation of the peptides. Indeed, as noted above, inhibition of bradykinin metabolism by ACE inhibitors contributes significantly to their antihypertensive action.

Selective B_2 agonists are under study and have been shown to be effective in some animal models of human cardiovascular disease. These drugs have potential for the treatment of hypertension, myocardial hypertrophy, and other diseases.

VASOPRESSIN

Vasopressin (**arginine vasopressin, AVP; antidiuretic hormone, ADH**) plays an important role in the long-term control of blood pressure through its action on the kidney to increase water reabsorption. This and other aspects of the physiology of AVP are discussed in Chapters 15 and 37 and will not be reviewed here.

AVP also plays an important role in the short-term regulation of arterial pressure by its vasoconstrictor action. It increases total peripheral resistance when infused in doses less than those required to produce maximum urine concentration. Such doses do not normally increase arterial pressure because the vasopressor activity of the peptide is buffered by a reflex decrease in cardiac output. When the influence of this reflex is removed, eg, in shock, pressor sensitivity to AVP is greatly increased. Pressor sensitivity to AVP is also enhanced in patients with idiopathic orthostatic hypotension. Higher doses of AVP increase blood pressure even when baroreceptor reflexes are intact.

VASOPRESSIN RECEPTORS & ANTAGONISTS

Three subtypes of AVP receptors have been identified; all are G protein-coupled. **V_{1a} receptors** mediate the vasoconstrictor action of AVP; **V_{1b} receptors** mediate release of ACTH by pituitary corticotropes; and **V_2 receptors** mediate the antidiuretic action. V_{1a} effects are mediated by G_q activation of phospholipase C, formation of inositol trisphosphate, and increased intracellular calcium concentration. V_2 effects are mediated by G_s activation of adenylyl cyclase.

AVP analogs selective for vasoconstrictor or antidiuretic activity have been synthesized. The most specific V_1 vasoconstrictor agonist synthesized to date is [Phe^2, Ile^3, Orn^8]vasotocin. Selective V_2 antidiuretic analogs include 1-deamino[D-Arg^8]arginine vasopressin (dDAVP) and 1-deamino[Val^4,D-Arg^8]arginine vasopressin (dVDAVP).

AVP has proved beneficial in the treatment of vasodilatory shock states, at least in part by virtue of its V_{1a} agonist activity. **Terlipressin** (triglycyl lysine vasopressin), a synthetic vasopressin analog that is converted to lysine vasopressin in the body, is also effective. It may have advantages over AVP because it is more selective for V_1 receptors and has a longer half-life.

Antagonists of the vasoconstrictor action of AVP are also available. The peptide antagonist d$(CH2)_5$[$Tyr(Me)^2$]AVP also has antioxytocic activity but does not antagonize the antidiuretic action of AVP. A related antagonist d$(CH2)_5$[$Tyr(Me)^2$,Dab^5]AVP lacks oxytocin antagonism but has less anti-V_1 activity. Recently, nonpeptide, orally active V_{1a}-receptor antagonists have been discovered, examples being **relcovaptan** and **SRX251.**

The V_{1a} antagonists have been particularly useful in revealing the important role that AVP plays in blood pressure regulation in situations such as dehydration and hemorrhage. They have potential as therapeutic agents for the treatment of such diverse diseases and conditions as Raynaud's disease, hypertension, heart failure, brain edema, motion sickness, cancer, preterm labor, and anger reduction. To date, most studies have focused on heart failure; promising results have been obtained with V_2 antagonists such as **tolvaptan**, which is, however, currently approved only for use in hyponatremia. V_{1a} antagonists also have potential, and **conivaptan** (YM087), a drug with both V_{1a} and V_2 antagonist activity, has also been approved for treatment of hyponatremia (see Chapter 15).

NATRIURETIC PEPTIDES

Synthesis & Structure

The atria and other tissues of mammals contain a family of peptides with natriuretic, diuretic, vasorelaxant, and other properties. The family includes atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). The peptides share a common 17-amino-acid disulfide ring with variable C- and N-terminals (Figure 17–5). A fourth peptide, urodilatin, has the same structure as ANP with an extension of four amino acids at the N-terminal. The renal effects of these peptides are discussed in Chapter 15.

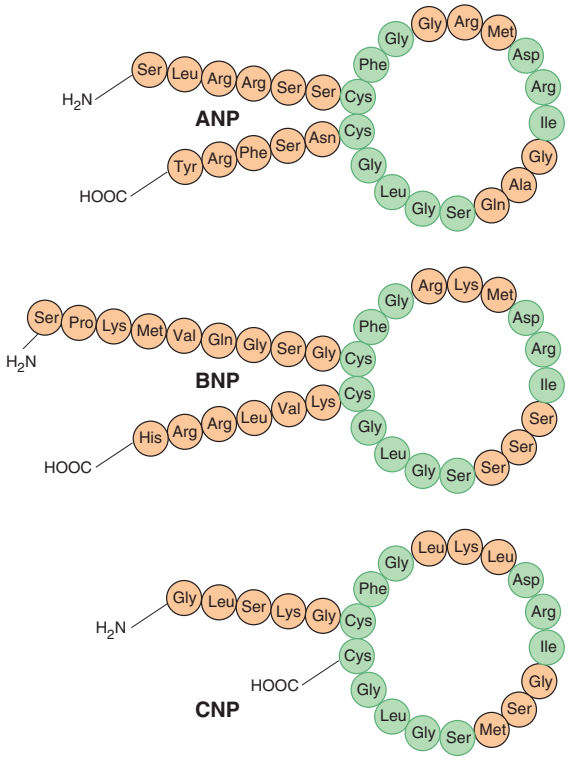


FIGURE 17–5 Structures of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). Sequences common to the peptides are indicated in green.

ANP is derived from the carboxyl terminal end of a common precursor termed preproANP. ANP is synthesized primarily in cardiac atrial cells, but it is also synthesized in ventricular myocardium, by neurons in the central and peripheral nervous systems, and in the lungs.

The most important stimulus to the release of ANP from the heart is atrial stretch via mechanosensitive ion channels. ANP release is also increased by volume expansion, changing from the standing to the supine position, and exercise. ANP release can also be increased by sympathetic stimulation via α_{1A} adrenoceptors, endothelins via the ET_A-receptor subtype (see below), glucocorticoids, and AVP. Plasma ANP concentration increases in several pathologic states, including heart failure, primary aldosteronism, chronic renal failure, and inappropriate ADH secretion syndrome.

Administration of ANP produces prompt and marked increases in sodium excretion and urine flow. Glomerular filtration rate increases, with little or no change in renal blood flow, so that the filtration fraction increases. The ANP-induced natriuresis is due to both the increase in glomerular filtration rate and a decrease in proximal tubular sodium reabsorption. ANP also inhibits the release of renin, aldosterone, and AVP; these changes may also increase sodium and water excretion. Finally, ANP causes vasodilation and decreases arterial blood pressure. Suppression of ANP production or blockade of its action impairs the natriuretic response to volume expansion, and increases blood pressure.

BNP was originally isolated from porcine brain but, like ANP, it is synthesized primarily in the heart. It exists in two forms, having either 26 or 32 amino acids (Figure 17–5). Like ANP, the release of BNP appears to be volume related; indeed, the two peptides may be co-secreted. BNP exhibits natriuretic, diuretic, and hypotensive activities similar to those of ANP but circulates at a lower concentration.

CNP consists of 22 amino acids (Figure 17–5). It is located predominantly in the central nervous system but is also present in several other tissues including the vascular endothelium, kidneys, and intestine. It has not been found in significant concentrations in the circulation. CNP has less natriuretic and diuretic activity than ANP and BNP but is a potent vasodilator and may play a role in the regulation of peripheral resistance.

Urodilatin is synthesized in the distal tubules of the kidneys by alternative processing of the ANP precursor. It elicits potent natriuresis and diuresis, and thus functions as a paracrine regulator of sodium and water excretion. It also relaxes vascular smooth muscle.

Pharmacodynamics & Pharmacokinetics

The biologic actions of the natriuretic peptides are mediated through association with specific high-affinity receptors located on the surface of the target cells. Three receptor subtypes termed **ANP_A, ANP_B,** and **ANP_C** (also known as NPR_1, NPR_2, and NPR_3) have been identified. The ANP_A receptor consists of a 120 kDa membrane-spanning protein with enzymatic activity associated with its intracellular domain. Its primary ligands are ANP and BNP. The ANP_B receptor is similar in structure to the ANP_A receptor, but its primary ligand appears to be CNP. The ANP_A and ANP_B receptors, but not the ANP_C receptor, are guanylyl cyclase enzymes.

The natriuretic peptides have a short half-life in the circulation. They are metabolized in the kidneys, liver, and lungs by the neutral endopeptidase NEP 24.11. Inhibition of this endopeptidase results in increases in circulating levels of the natriuretic peptides, natriuresis, and diuresis. The peptides are also removed from the circulation by binding to ANP_C receptors in the vascular endothelium. This receptor binds the natriuretic peptides with equal affinity. The receptor and bound peptide are internalized, the peptide is degraded enzymatically, and the receptor is returned to the cell surface. Patients with heart failure have high plasma levels of ANP and BNP; the latter has emerged as a diagnostic and prognostic marker in this condition.

VASOPEPTIDASE INHIBITORS

Vasopeptidase inhibitors constitute a new class of cardiovascular drugs that inhibit two metalloprotease enzymes, NEP 24.11 and ACE. They thus simultaneously increase the levels of natriuretic peptides and decrease the formation of ANG II. As a result, they enhance vasodilation, reduce vasoconstriction, and increase sodium excretion, in turn reducing peripheral vascular resistance and blood pressure.

Recently developed vasopeptidase inhibitors include **omapatrilat, sampatrilat,** and **fasidotrilat.** Omapatrilat, which has received the most attention, lowers blood pressure in animal models of hypertension as well as in hypertensive patients, and improves cardiac function in patients with heart failure. Unfortunately, omapatrilat causes a significant incidence of angioedema in addition to cough and dizziness and has not been approved for clinical use.

ENDOTHELINS

The endothelium is the source of a variety of substances with vasodilator (PGI_2 and nitric oxide) and vasoconstrictor activities. The latter include the endothelin family, potent vasoconstrictor peptides that were first isolated from aortic endothelial cells.

Biosynthesis, Structure, & Clearance

Three isoforms of endothelin have been identified: the originally described endothelin, **ET-1,** and two similar peptides, **ET-2** and **ET-3.** Each isoform is a product of a different gene and is synthesized as a prepro form that is processed to a propeptide and then to the mature peptide. Processing to the mature peptides occurs through the action of endothelin-converting enzyme. Each endothelin is a 21-amino-acid peptide containing two disulfide bridges. The structure of ET-1 is shown in Figure 17–6.

Endothelins are widely distributed in the body. ET-1 is the predominant endothelin secreted by the vascular endothelium. It is also produced by neurons and astrocytes in the central nervous system and in endometrial, renal mesangial, Sertoli, breast epithelial, and other cells. ET-2 is produced predominantly in the kidneys and intestine, whereas ET-3 is found in highest concentration

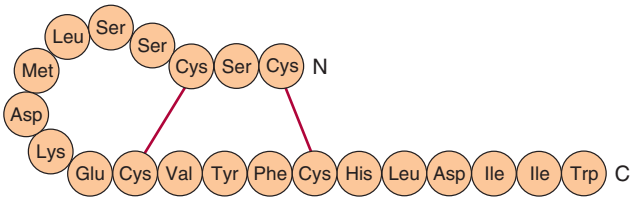


FIGURE 17–6 Structure of human endothelin-1.

in the brain but is also present in the gastrointestinal tract, lungs, and kidneys. Endothelins are present in the blood but in low concentration; they apparently act locally in a paracrine or autocrine fashion rather than as circulating hormones.

The expression of the ET-1 gene is increased by growth factors and cytokines, including transforming growth factor-β (TGF-β) and interleukin 1 (IL-1), vasoactive substances including ANG II and AVP, and mechanical stress. Expression is inhibited by nitric oxide, prostacyclin, and ANP.

Clearance of endothelins from the circulation is rapid and involves both enzymatic degradation by NEP 24.11 and clearance by the ET_B receptor.

Actions

Endothelins exert widespread actions in the body. In particular, they cause potent dose-dependent vasoconstriction in most vascular beds. Intravenous administration of ET-1 causes a rapid and transient decrease in arterial blood pressure followed by a sustained increase. The depressor response results from release of prostacyclin and nitric oxide from the vascular endothelium, whereas the pressor response is due to direct contraction of vascular smooth muscle. Endothelins also exert direct positive inotropic and chronotropic actions on the heart and are potent coronary vasoconstrictors. They act on the kidneys to cause vasoconstriction and decrease glomerular filtration rate and sodium and water excretion. In the respiratory system, they cause potent contraction of tracheal and bronchial smooth muscle. Endothelins interact with several endocrine systems, increasing the secretion of renin, aldosterone, AVP, and ANP. They exert a variety of actions on the central and peripheral nervous systems, the gastrointestinal system, the liver, the urinary tract, the male and female reproductive systems, the eye, the skeletal system, and the skin. Finally, ET-1 is a potent mitogen for vascular smooth muscle cells, cardiac myocytes, and glomerular mesangial cells.

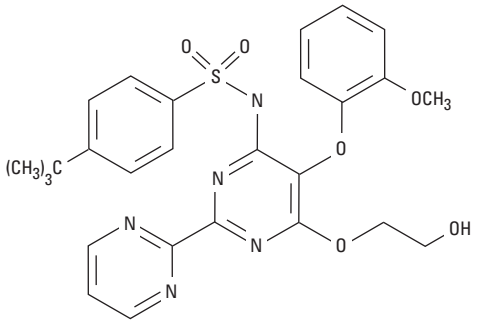
Endothelin receptors are widespread in the body. Two endothelin receptor subtypes, termed **ET_A** and **ET_B,** have been cloned and sequenced. ET_A receptors have a high affinity for ET-1 and a low affinity for ET-3 and are located on smooth muscle cells, where they mediate vasoconstriction (Figure 17–7). ET_B receptors have approximately equal affinities for ET-1 and ET-3 and are primarily located on vascular endothelial cells, where they mediate release of PGI_2 and nitric oxide. Some ET_B receptors are also present on smooth muscle cells and mediate vasoconstriction. Both receptor subtypes belong to the G protein-coupled seven-transmembrane domain family of receptors.

The signal transduction mechanisms triggered by binding of ET-1 to its vascular receptors include stimulation of phospholipase C, formation of inositol trisphosphate, and release of calcium from the endoplasmic reticulum, which results in vasoconstriction. Conversely, stimulation of PGI_2 and nitric oxide synthesis results in decreased intracellular calcium concentration and vasodilation.

INHIBITORS OF ENDOTHELIN SYNTHESIS & ACTION

The endothelin system can be blocked with receptor antagonists and drugs that block endothelin-converting enzyme. Endothelin ET_A or ET_B receptors can be blocked selectively, or both can be blocked with nonselective ET_A-ET_B antagonists.

Bosentan is a nonselective receptor blocker. It is active orally, and blocks both the initial transient depressor (ET_B) and the prolonged pressor (ET_A) responses to intravenous endothelin. Many orally active endothelin receptor antagonists with increased selectivity have been developed and are available for research use. Examples include the selective ET_A antagonists **ambrisentan,** which has been approved by the Food and Drug Administration to treat pulmonary artery hypertension, and **sitaxsentan.**



Bosentan

The formation of endothelins can be blocked by inhibiting endothelin-converting enzyme with phosphoramidon. Phosphoramidon is not specific for endothelin-converting enzyme, but more selective inhibitors including CGS35066 are now available for research. Although the therapeutic potential of these drugs appeared similar to that of the endothelin receptor antagonists (see below), their use has been eclipsed by endothelin antagonists.

Physiologic & Pathologic Roles of Endothelin: Effects of Endothelin Antagonists

Systemic administration of endothelin receptor antagonists or endothelin-converting enzyme inhibitors causes vasodilation and decreases arterial pressure in humans and experimental animals. Intra-arterial administration of the drugs also causes slow-onset forearm vasodilation in humans. These observations provide evidence that the endothelin system participates in the regulation of vascular tone, even under resting conditions. The activity of the

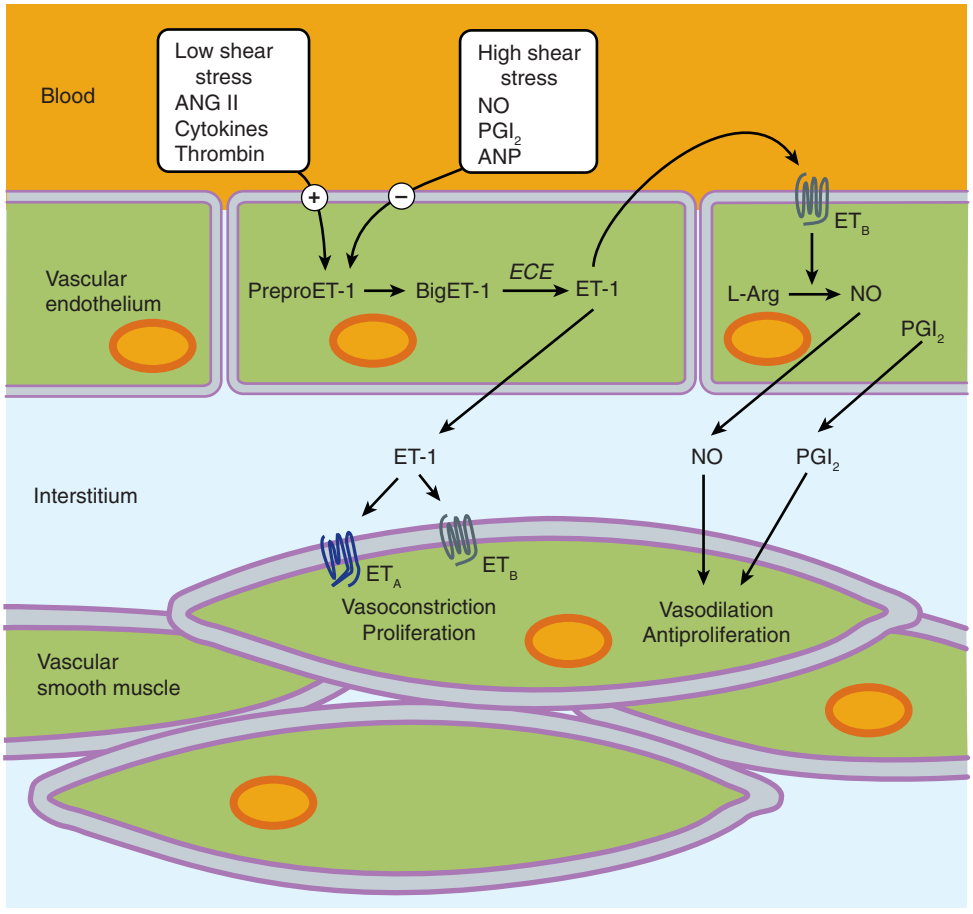


FIGURE 17–7 Generation of endothelin-1 (ET-1) in the vascular endothelium, and its direct and indirect effects on smooth muscle cells mediated by ET_A and ET_B receptors. ANG II, angiotensin II; ANP, atrial natriuretic peptide; ECE, endothelial-converting enzyme; NO, nitric oxide; PGI_2, prostaglandin I_2.

system is higher in males than in females. It increases with age, an effect that can be counteracted by regular aerobic exercise.

Increased production of ET-1 has been implicated in a variety of cardiovascular diseases, including hypertension, cardiac hypertrophy, heart failure, atherosclerosis, coronary artery disease, and myocardial infarction. ET-1 also participates in pulmonary diseases, including asthma and pulmonary hypertension; renal diseases; and several malignancies, including ovarian cancer.

Endothelin antagonists have considerable potential for the treatment of these diseases. Indeed, endothelin antagonism with bosentan, sitaxsentan, and ambrisentan has proved to be a moderately effective and generally well-tolerated treatment for patients with pulmonary arterial hypertension, an important condition with few effective treatments. Other promising targets for these drugs are resistant hypertension, chronic renal disease, connective tissue disease, and subarachnoid hemorrhage. On the other hand, clinical trials of the drugs in the treatment of congestive heart failure have been disappointing.

Endothelin antagonists occasionally cause systemic hypotension, increased heart rate, facial flushing or edema, and headaches. Potential gastrointestinal effects include nausea, vomiting, and constipation. Because of their teratogenic effects, endothelin antagonists are contraindicated in pregnancy. Bosentan has been associated with fatal hepatotoxicity, and patients taking this drug must have monthly liver function tests. Negative pregnancy test results are required for women of child-bearing age to take this drug.

VASOACTIVE INTESTINAL PEPTIDE

Vasoactive intestinal peptide (VIP) is a 28-amino-acid peptide that belongs to the glucagon-secretin family of peptides. VIP is widely distributed in the central and peripheral nervous systems, where it functions as one of the major peptide neurotransmitters. It is present in cholinergic presynaptic neurons in the central nervous system, and in peripheral peptidergic neurons innervating diverse tissues including the heart, lungs, gastrointestinal and urogenital tracts, skin, eyes, ovaries, and thyroid gland. Many blood vessels are innervated by VIP neurons. VIP is also present in key organs of the immune system including the thymus, spleen, and lymph nodes. Although VIP is present in blood, where it

undergoes rapid degradation, it does not appear to function as a hormone. VIP participates in a wide variety of biologic functions including metabolic processes, secretion of endocrine and exocrine glands, cell differentiation, smooth muscle relaxation, and the immune response.

VIP exerts significant effects on the cardiovascular system. It produces marked vasodilation in most vascular beds and in this regard is more potent on a molar basis than acetylcholine. In the heart, VIP causes coronary vasodilation and exerts positive inotropic and chronotropic effects. It may thus participate in the regulation of coronary blood flow, cardiac contraction, and heart rate.

The effects of VIP are mediated by G protein-coupled receptors; two subtypes, **VPAC1** and **VPAC2,** have been cloned from human tissues. Both subtypes are widely distributed in the central nervous system and in the heart, blood vessels, and other tissues. VIP has a high affinity for both receptor subtypes. Binding of VIP to its receptors results in activation of adenylyl cyclase and formation of cAMP, which is responsible for the vasodilation and many other effects of the peptide. Other actions may be mediated by inositol trisphosphate synthesis and calcium mobilization. VIP can also bind with low affinity to the VIP-like peptide pituitary adenylyl cyclase-activating peptide receptor, PAC1.

VIP analogs with longer half-lives than the native peptide are now available for research use. An example is stearyl-Nle^{17}-VIP. These drugs have potential as therapeutic agents for cardiovascular, pulmonary, gastrointestinal, and nervous system diseases. They may also be effective in treating various inflammatory diseases and diabetes. Indeed, some VIP derivatives are currently undergoing preclinical and clinical testing for the treatment of type 2 diabetes and chronic obstructive pulmonary disease. Unfortunately, their use is currently limited by several issues including poor oral availability, rapid metabolism in the blood, and hypotension. VIP receptor antagonists are also being developed.

SUBSTANCE P

Substance P belongs to the **tachykinin** family of peptides, which share the common carboxyl terminal sequence Phe-Gly-Leu-Met. Other members of this family are **neurokinin A** and **neurokinin B.** Substance P is an undecapeptide, while neurokinins A and B are decapeptides.

Substance P is present in the central nervous system, where it is a neurotransmitter (see Chapter 21), and in the gastrointestinal tract, where it may play a role as a transmitter in the enteric nervous system and as a local hormone (see Chapter 6).

Substance P is the most important member of the tachykinin family. It exerts a variety of incompletely understood central actions that implicate the peptide in behavior, anxiety, depression, nausea, and emesis. It is a potent arteriolar vasodilator, producing marked hypotension in humans and several animal species. The vasodilation is mediated by release of nitric oxide from the endothelium. Conversely, substance P causes contraction of venous, intestinal, and bronchial smooth muscle. It also stimulates secretion by the salivary glands and causes diuresis and natriuresis by the kidneys.

The actions of substance P and neurokinins A and B are mediated by three G_q protein-coupled tachykinin receptors designated **NK_1, NK_2,** and **NK_3.** Substance P is the preferred ligand for the NK_1 receptor, the predominant tachykinin receptor in the human brain. However, neurokinins A and B also possess considerable affinity for this receptor. In humans, most of the central and peripheral effects of substance P are mediated by NK_1 receptors. All three receptor subtypes are coupled to inositol trisphosphate synthesis and calcium mobilization.

Several nonpeptide NK_1 receptor antagonists have been developed. These compounds are highly selective and orally active, and enter the brain. Recent clinical trials have shown that these antagonists may be useful in treating depression and other disorders and in preventing chemotherapy-induced emesis. The first of these to be approved for the prevention of chemotherapy-induced and postoperative nausea and vomiting is **aprepitant** (see Chapter 62). **Fosaprepitant** is a prodrug that is converted to aprepitant after intravenous administration and may be a useful parenteral alternative to oral aprepitant.

NEUROTENSIN

Neurotensin (NT) is a tridecapeptide that was first isolated from the central nervous system but subsequently was found to be present in the gastrointestinal tract and in the circulation. It is synthesized as part of a larger precursor that also contains **neuromedin N,** a six-amino-acid NT-like peptide.

In the brain, processing of the precursor leads primarily to the formation of NT and neuromedin N; these are released together from nerve endings. In the gut, processing leads mainly to the formation of NT and a larger peptide that contains the neuromedin N sequence at the carboxyl terminal. Both peptides are secreted into the circulation after ingestion of food. Most of the activity of NT is mediated by the last six amino acids, NT(8-13).

Like many other neuropeptides, NT serves a dual function as a neurotransmitter or neuromodulator in the central nervous system and as a local hormone in the periphery. When administered centrally, NT exerts potent effects including hypothermia, antinociception, and modulation of dopamine and glutamate neurotransmission. When administered into the peripheral circulation, it causes vasodilation, hypotension, increased vascular permeability, increased secretion of several anterior pituitary hormones, hyperglycemia, inhibition of gastric acid and pepsin secretion, and inhibition of gastric motility. It also exerts effects on the immune system.

In the central nervous system, there are close associations between NT and dopamine systems, and NT may be involved in clinical disorders involving dopamine pathways such as schizophrenia, Parkinson's disease, and drug abuse. Consistent with this, it has been shown that central administration of NT produces effects in rodents similar to those produced by antipsychotic drugs.

The effects of NT are mediated by three subtypes of NT receptors, designated **NTR_1, NTR_2,** and **NTR_3,** also known as **NTS_1, NTS_2,** and **NTS_3.** NTR_1 and NTR_2 receptors belong to the G_q protein-coupled superfamily with seven transmembrane domains;

the NTR_3 receptor is a single transmembrane domain protein that belongs to a family of sorting proteins.

NT receptor agonists that cross the blood-brain barrier, all peptide analogs of NT(8-13), have been developed. Conversely, NT receptors can be blocked with the nonpeptide antagonists SR142948A and meclinertant (SR48692). SR142948A is a potent antagonist of the hypothermia and analgesia produced by centrally administered NT. It also blocks the cardiovascular effects of systemic NT. The development of drugs that selectively target or block NT receptors has resulted in potential therapeutic agents for the treatment of schizophrenia and Parkinson's disease, as well as drug abuse.

CALCITONIN GENE-RELATED PEPTIDE

Calcitonin gene-related peptide (**CGRP**) is a member of the calcitonin family of peptides, which also includes calcitonin, adrenomedullin (see below), and amylin. CGRP consists of 37 amino acids. Like calcitonin, CGRP is present in large quantities in the C cells of the thyroid gland. It is also distributed widely in the central and peripheral nervous systems, cardiovascular and respiratory systems, and gastrointestinal tract. In the cardiovascular system CGRP-containing fibers are more abundant around arteries than around veins, and in atria than in ventricles. CGRP fibers are associated with most smooth muscles of the gastrointestinal tract. CGRP is found with substance P (see above) in some of these regions and with acetylcholine in others.

When CGRP is injected into the central nervous system, it produces a variety of effects, including hypertension and suppression of feeding. When injected into the systemic circulation, the peptide causes hypotension and tachycardia. The hypotensive action of CGRP results from the potent vasodilator action of the peptide; indeed, CGRP is the most potent vasodilator yet discovered. It dilates multiple vascular beds, but the coronary circulation is particularly sensitive. The vasodilation is mediated via a nonendothelial mechanism through activation of adenylyl cyclase.

Although CGRP receptors have traditionally been divided into two classes, $CGRP_1$ and $CGRP_2$, it now seems likely that the actions of CGRP are mediated by a single receptor. Specifically, the seven-transmembrane G protein-coupled calcitonin-like protein receptor (CLR) co-assembles with the receptor activity-modifying protein RAMP1 to form a functional single CGRP receptor that can activate both G_s and G_q. Peptide and nonpeptide antagonists of the receptor have been developed. $CGRP_{8-37}$ has been used extensively to investigate the actions of CGRP but displays affinity for related receptors including those for adrenomedullin (see below). Nonpeptide CGRP receptor antagonists target the interface between CLR and RAMP1 and thereby make them more selective for the CGRP receptor. These antagonists display species selectivity and are more selective for human than rodent receptors. Examples are **olcegepant** and **telcagepant.**

Evidence is accumulating that release of CGRP from trigeminal nerves plays a central role in the pathophysiology of migraine. The peptide is released during migraine attacks, and successful treatment of migraine with a selective serotonin agonist normalizes cranial CGRP levels. Clinical trials with olcegepant and telcagepant have demonstrated that CGRP antagonism is an effective, well-tolerated treatment for migraine.

ADRENOMEDULLIN

Adrenomedullin (AM) was first discovered in human adrenal medullary pheochromocytoma tissue. It is a 52-amino-acid peptide with a six-amino-acid ring and a C-terminal amidation sequence. Like CGRP, AM is a member of the calcitonin family of peptides. A related peptide termed adrenomedullin 2, also called intermedin, has been identified in humans and other mammals.

AM is widely distributed in the body. The highest concentrations are found in the adrenal glands, hypothalamus, and anterior pituitary, but high levels are also present in the kidneys, lungs, cardiovascular system, and gastrointestinal tract. AM in plasma apparently originates in the heart and vasculature.

In animals, AM dilates resistance vessels in the kidney, brain, lung, hind limbs, and mesentery, resulting in a marked, long-lasting hypotension. The hypotension in turn causes reflex increases in heart rate and cardiac output. These responses also occur during intravenous infusion of the peptide in healthy human subjects. AM also acts on the kidneys to increase sodium excretion and renin release, and it exerts other endocrine effects including inhibition of aldosterone and insulin secretion. It acts on the central nervous system to increase sympathetic outflow.

The diverse actions of AM are mediated by a receptor closely related to the CGRP receptor (see above). CLR co-assembles with RAMP subtypes 2 and 3, thus forming a receptor-coreceptor system. Binding of AM to CLR activates G_s and triggers cAMP formation in vascular smooth muscle cells, and increases nitric oxide production in endothelial cells. Other signaling pathways are also involved.

Circulating AM levels increase during intense exercise. They also increase in a number of pathologic states, including essential hypertension, cardiac and renal failure, and septic shock. The roles of AM in these states remain to be defined, but it is currently thought that the peptide functions as a physiologic antagonist of the actions of vasoconstrictors including ET-1 and ANG II. By virtue of these actions, AM may protect against cardiovascular overload and injury, and AM may be beneficial in the treatment of some cardiovascular diseases.

NEUROPEPTIDE Y

Neuropeptide Y (NPY) is a member of the family that also includes peptide YY and pancreatic polypeptide. Each of these peptides consists of 36 amino acids.

NPY is one of the most abundant neuropeptides in both the central and peripheral nervous systems. In the sympathetic nervous

system, NPY is frequently localized in noradrenergic neurons and apparently functions both as a vasoconstrictor and as a cotransmitter with norepinephrine. Peptide YY and pancreatic polypeptide are both gut endocrine peptides.

NPY produces a variety of central nervous system effects, including increased feeding (it is one of the most potent orexigenic molecules in the brain), hypotension, hypothermia, respiratory depression, and activation of the hypothalamic-pituitary-adrenal axis. Other effects include vasoconstriction of cerebral blood vessels, positive chronotropic and inotropic actions on the heart, and hypertension. The peptide is a potent renal vasoconstrictor and suppresses renin secretion, but can cause diuresis and natriuresis. Prejunctional neuronal actions include inhibition of transmitter release from sympathetic and parasympathetic nerves. Vascular actions include direct vasoconstriction, potentiation of the action of vasoconstrictors, and inhibition of the action of vasodilators.

These diverse effects are mediated by four subtypes of NPY receptors designated Y_1, Y_2, Y_4, and Y_5. The receptors have been cloned and shown to be G_i protein-coupled receptors linked to mobilization of Ca^{2+} and inhibition of adenylyl cyclase. Y_1 and Y_2 receptors are of major importance in the cardiovascular and other peripheral effects of the peptide. Y_4 receptors have a high affinity for pancreatic polypeptide and may be a receptor for the pancreatic peptide rather than for NPY. Y_5 receptors are found mainly in the central nervous system and may be involved in the control of food intake. They also mediate the activation of the hypothalamic-pituitary-adrenal axis by NPY.

Selective nonpeptide NPY receptor antagonists are now available for research. The first nonpeptide Y_1 receptor antagonist, BIBP3226, is also the most thoroughly studied. It has a short half-life in vivo. In animal models, it blocks the vasoconstrictor and pressor responses to NPY. Structurally related Y_1 antagonists include BIB03304 and H409/22; the latter has been tested in humans. SR120107A and SR120819A are orally active Y_1 antagonists and have a long duration of action. BIIE0246 is the first nonpeptide antagonist selective for the Y_2 receptor; it does not cross the blood-brain barrier. Useful Y_4 antagonists are not available. The Y_5 antagonists MK-0557 and S-2367 have been tested in clinical trials for obesity.

These drugs have been useful in analyzing the role of NPY in cardiovascular regulation. It now appears that the peptide is not important in the regulation of hemodynamics under normal resting conditions but may be of increased importance in cardiovascular disorders including hypertension and heart failure. Other studies have implicated NPY in feeding disorders, obesity, alcoholism, anxiety, depression, epilepsy, pain, cancer, and bone physiology. Y_1 and particularly Y_5 receptor antagonists have potential as antiobesity agents.

UROTENSIN

Urotensin II (UII) was originally identified in fish, but isoforms are now known to be present in the human and other mammalian species. Human UII is an 11-amino acid peptide. Major sites of UII expression in humans include the brain, spinal cord, and kidneys. UII is also present in plasma, and potential sources of this circulating peptide include the heart, lungs, liver, and kidneys. The stimulus to UII release has not been identified but increased blood pressure has been implicated in some studies.

In vitro, UII is a potent constrictor of vascular smooth muscle; its activity depends on the type of blood vessel and the species from which it was obtained. Vasoconstriction occurs primarily in arterial vessels, where UII can be more potent than ET-1, making it the most potent known vasoconstrictor. However, under some conditions, UII may cause vasodilation. In vivo, UII has complex hemodynamic effects, the most prominent being regional vasoconstriction and cardiac depression. In some ways, these effects resemble those produced by ET-1. Nevertheless, the role of the peptide in the normal regulation of vascular tone and blood pressure in humans appears to be minor.

The actions of UII are mediated by a G_q protein-coupled receptor referred to as the UT receptor. UT receptors are widely distributed in the brain, spinal cord, heart, vascular smooth muscle, skeletal muscle, and pancreas. Some effects of the peptide including vasoconstriction are mediated by the phospholipase C, inositol trisphosphate-diacylglycerol signal transduction pathway.

Urantide ("urotensin antagonist peptide"), a penicillamine-substituted derivative of UII, is a UII receptor antagonist. A nonpeptide antagonist, **palosuran,** has also been developed and used in clinical studies.

Although UII appears to play only a minor role in health, evidence is accumulating that it is involved in cardiovascular and other diseases. In particular, it has been reported that plasma UII levels are increased in hypertension, heart failure, atherosclerosis, diabetes mellitus, and renal failure, and palosuran may benefit diabetic patients with renal disease. Nevertheless, the role of UII in disease is poorly understood. Indeed it is possible that rather than contributing to these diseases, UII may actually play a protective role.

SUMMARY Drugs That Interact with Vasoactive Peptide Systems

Subclass	Mechanism of Action	Effects	Clinical Applications
ANGIOTENSIN RECEPTOR ANTAGONISTS			
• Valsartan	Selective competitive antagonist of angiotensin AT_1 receptors	Arteriolar dilation • decreased aldosterone secretion • increased sodium and water excretion	Hypertension
• *Eprosartan, irbesartan, candesartan, olmesartan, telmisartan: Similar to valsartan*			
CONVERTING ENZYME INHIBITORS			
• Enalapril	Inhibits conversion of angiotensin I to angiotensin II	Arteriolar dilation • decreased aldosterone secretion • increased sodium and water excretion	Hypertension • heart failure
• *Captopril and many others: Similar to enalapril*			
RENIN INHIBITORS			
• Aliskiren	Inhibits catalytic activity of renin	Arteriolar dilation • decreased aldosterone secretion • increased sodium and water excretion	Hypertension
KININ INHIBITORS			
• Icatibant	Selective antagonist of kinin B_2 receptors	Blocks effects of kinins on pain, hyperalgesia, and inflammation	Hereditary angioedema
• *Ecallantide: Plasma kallikrein inhibitor*			
VASOPRESSIN AGONISTS			
• Arginine vasopressin	Agonist of vasopressin V_1 (and V_2) receptors	Vasoconstriction	Vasodilatory shock
• *Terlipressin: More selective for V_1 receptor*			
VASOPRESSIN ANTAGONISTS			
• Conivaptan	Antagonist of vasopressin V_1 (and V_2) receptors	Vasodilation	Potential use in hypertension and heart failure • hyponatremia
• *Relcovaptan: Increased selectivity for V_1 receptor*			
NATRIURETIC PEPTIDES			
• Nesiritide	Agonist of natriuretic peptide receptors	Increased sodium and water excretion • vasodilation	Heart failure
• *Uralitide: Synthetic form of urodilatin*			
VASOPEPTIDASE INHIBITORS			
• Omapatrilat	Decreases metabolism of natriuretic peptides and formation of angiotensin II	Vasodilation • increased sodium and water excretion	Hypertension • heart failure[1]
ENDOTHELIN ANTAGONISTS			
• Bosentan	Nonselective antagonist of endothelin ET_A and ET_B receptors	Vasodilation	Pulmonary arterial hypertension
• *Sitaxsentan, ambrisentan: Selective for ET_A receptors*			
VASOACTIVE INTESTINAL PEPTIDE AGONISTS			
• Stearyl-Nle^{17}-VIP	Agonist of VPAC1 and VPAC2 receptors	Vasodilation • multiple metabolic, endocrine, and other effects	Type 2 diabetes • chronic obstructive pulmonary disease[1]

(*continued*)

Subclass	Mechanism of Action	Effects	Clinical Applications
SUBSTANCE P ANTAGONISTS			
• Aprepitant	Selective antagonist of tachykinin NK_1 receptors	Blocks several central nervous system effects of substance P	Prevention of chemotherapy-induced nausea and vomiting
• *Fosaprepitant: Prodrug that is converted to aprepitant*			
NEUROTENSIN AGONISTS			
• PD149163	Agonist of central neurotensin receptors	Interacts with central dopamine systems	Potential for treatment of schizophrenia and Parkinson's disease
NEUROTENSIN ANTAGONISTS			
• Meclinertant	Antagonist of central and peripheral neurotensin receptors	Blocks some central and peripheral (vasodilator) actions of neurotensin	None identified
CALCITONIN GENE-RELATED PEPTIDE ANTAGONISTS			
• Telcagepant, *olcegepant*	Antagonists of the calcitonin gene-related peptide (CGRP) receptor	Blocks some central and peripheral (vasodilator) actions of CGRP	Migraine[1]
NEUROPEPTIDE Y ANTAGONISTS			
• BIBP3226	Selective antagonist of neuropeptide Y_1 receptors	Blocks vasoconstrictor response to neurotensin	Potential antiobesity agent
• *BIIE0246: Selective for Y_2 receptor*			
• *MK-0557: Selective for Y_5 receptor*			
UROTENSIN ANTAGONISTS			
• Palosuran	Antagonist of urotensin receptors	Blocks vasoconstrictor action of urotensin	Diabetic renal failure[1]

[1]Undergoing preclinical or clinical evaluation.

REFERENCES

Angiotensin

Balakumar P, Jagadeesh G: Cardiovascular and renal pathologic implications of prorenin, renin, and the (pro)renin receptor: Promising young players from the old renin-angiotensin-aldosterone system. J Cardiovasc Pharmacol 2010;56:570.

Beierwaltes WH: The role of calcium in the regulation of renin secretion. Am J Physiol Renal Physiol 2010;298:F1.

Castrop H et al: Physiology of kidney renin. Physiol Rev 2010;90:607.

Ichihara A et al: New approaches to blockade of the renin-angiotensin-aldosterone system: Characteristics and usefulness of the direct renin inhibitor aliskiren. J Pharmacol Sci 2009;113:296.

Ichihara A et al: Renin, prorenin and the kidney: A new chapter in an old saga. J Nephrol 2009;22:306.

Kurtz A: Renin release: Sites, mechanisms, and control. Annu Rev Physiol 2011;73:377.

Matt P et al: Recent advances in understanding Marfan syndrome: Should we now treat surgical patients with losartan? J Thorac Cardiovasc Surg 2008;135:389.

McMurray JJ et al: Effect of valsartan on the incidence of diabetes and cardiovascular events. N Engl J Med 2010;362:1477.

Nguyen G: Renin, (pro)renin and receptor: An update. Clin Sci (Lond) 2011;120:169.

Sihn G et al: Physiology of the (pro)renin receptor: Wnt of change? Kidney Int 2010;78:246.

Uresin Y et al: Efficacy and safety of the direct renin inhibitor aliskiren and ramipril alone or in combination in patients with diabetes and hypertension. J Renin Angiotensin Aldosterone Syst 2007;8:190.

Varagic J et al: New angiotensins. J Mol Med 2008;86:663.

Zheng Z et al: A systematic review and meta-analysis of aliskiren and angiotensin receptor blockers in the management of essential hypertension. J Renin Angiotensin Aldosterone Syst 2011;12:102

Kinins

Cicardi M et al: Ecallantide for the treatment of acute attacks in hereditary angioedema. N Engl J Med 2010;363:523.

Cicardi M et al: Icatibant, a new bradykinin-receptor antagonist, in hereditary angioedema. N Engl J Med 2010;363:532.

Costa-Neto CM et al: Participation of kallikrein-kinin system in different pathologies. Int Immunopharmacol 2008;8:135.

Leeb-Lundberg LM et al: International union of pharmacology. XLV. Classification of the kinin receptor family: From molecular mechanisms to pathophysiological consequences. Pharmacol Rev 2005;57:27.

Longhurst HJ: Management of acute attacks of hereditary angioedema: Potential role of icatibant. Vasc Health Risk Manag 2010;6:795.

Vasopressin

Lange M, Ertmer C, Westphal M: Vasopressin vs. terlipressin in the treatment of cardiovascular failure in sepsis. Intensive Care Med 2008;34:821.

Manning M et al: Peptide and non-peptide agonists and antagonists for the vasopressin and oxytocin V1a, V1b, V2 and OT receptors: Research tools and potential therapeutic agents. Prog Brain Res 2008;170:473.

Maybauer MO et al: Physiology of the vasopressin receptors. Best Pract Res Clin Anaesthesiol 2008;22:253.

Natriuretic Peptides

Federico C: Natriuretic peptide system and cardiovascular disease. Heart Views 2010;11:1.

Luss H et al: Renal effects of ularitide in patients with decompensated heart failure. Am Heart J 2008;155:1012.

Saito Y: Roles of atrial natriuretic peptide and its therapeutic use. J Cardiol 2010;56:262.

Vogel MW, Chen HH: Novel natriuretic peptides: New compounds and new approaches. Curr Heart Fail Rep 2011;8:22.

Endothelins

Cartin-Ceba R et al: Safety and efficacy of ambrisentan for the therapy of portopulmonary hypertension. Chest 2011;139:109.

Kasimay O et al: Diet-supported aerobic exercise reduces blood endothelin-1 and nitric oxide levels in individuals with impaired glucose tolerance. J Clin Lipidol 2010;4:427.

Kawanabe Y, Nauli SM: Endothelin. Cell Mol Life Sci 2011;68:195.

Tostes RC et al: Endothelin, sex and hypertension. Clin Sci (Lond) 2008;114:85.

Vasoactive Intestinal Peptide

Onoue S, Misaka S, Yamada S: Structure-activity relationship of vasoactive intestinal peptide (VIP): Potent agonists and potential clinical applications. Naunyn Schmiedebergs Arch Pharmacol 2008;377:579.

White CM et al: Therapeutic potential of vasoactive intestinal peptide and its receptors in neurological disorders. CNS Neurol Disord Drug Targets 2010;9:661.

Substance P

Curran MP, Robinson DM: Aprepitant: A review of its use in the prevention of nausea and vomiting. Drugs 2009;69:1853.

Hokfelt T, Pernow B, Wahren J: Substance P: A pioneer amongst neuropeptides. J Intern Med 2001;249:27.

Mizuta K et al: Expression and coupling of neurokinin receptor subtypes to inositol phosphate and calcium signaling pathways in human airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2008; 294:L523.

Navari RM: Fosaprepitant: A neurokinin-1 receptor antagonist for the prevention of chemotherapy-induced nausea and vomiting. Expert Rev Anticancer Ther 2008;8:1733.

Neurotensin

Boules M et al: Neurotensin agonists: Potential in the treatment of schizophrenia. CNS Drugs 2007;21:13.

Ferraro L et al: Emerging evidence for neurotensin receptor 1 antagonists as novel pharmaceutics in neurodegenerative disorders. Mini Rev Med Chem 2009;9:1429.

Hwang JI et al: Phylogenetic history, pharmacological features, and signal transduction of neurotensin receptors in vertebrates. Ann N Y Acad Sci 2009;1163:169.

Katsanos GS et al: Biology of neurotensin: Revisited study. Int J Immunopathol Pharmacol 2008;21:255.

Calcitonin Gene-Related Peptide

Edvinsson L, Ho TW: CGRP receptor antagonism and migraine. Neurotherapeutics 2010;7:164.

Villalon CM, Olesen J: The role of CGRP in the pathophysiology of migraine and efficacy of CGRP receptor antagonists as acute antimigraine drugs. Pharmacol Ther 2009;124:309.

Walker CS et al: Regulation of signal transduction by calcitonin gene-related peptide receptors. Trends Pharmacol Sci 2010;31:476.

Adrenomedullin

Bell D, McDermott BJ: Intermedin (adrenomedullin-2): A novel counter-regulatory peptide in the cardiovascular and renal systems. Br J Pharmacol 2008;153(Suppl 1):S247.

Yanagawa B, Nagaya N: Adrenomedullin: Molecular mechanisms and its role in cardiac disease. Amino Acids 2007;32:157.

Neuropeptide Y

Brothers SP, Wahlestedt C: Therapeutic potential of neuropeptide Y (NPY) receptor ligands. EMBO Mol Med 2010;2:429.

Parker SL, Balasubramaniam A: Neuropeptide Y Y2 receptor in health and disease. Br J Pharmacol 2008;153:420.

Thorsell A: Brain neuropeptide Y and corticotropin-releasing hormone in mediating stress and anxiety. Exp Biol Med 2010;235:1163.

Urotensin

Cheriyan J et al: The effects of urotensin II and urantide on forearm blood flow and systemic haemodynamics in humans. Br J Clin Pharmacol 2009;68:518.

McDonald J, Batuwangala M, Lambert DG: Role of urotensin II and its receptor in health and disease. J Anesth 2007;21:378.

Russell FD: Urotensin II in cardiovascular regulation. Vasc Health Risk Manag 2008;4:775.

General

Paulis L, Unger T: Novel therapeutic targets for hypertension. Nat Rev Cardiol 2010;7:431.

Takahashi K et al: The renin-angiotensin system, adrenomedullins and urotensin II in the kidney: Possible renoprotection via the kidney peptide systems. Peptides 2009;30:1575.

CASE STUDY ANSWER

Enalapril lowers blood pressure by blocking the conversion of angiotensin I to angiotensin II (ANG II). Since converting enzyme also inactivates bradykinin, enalapril increases bradykinin levels, and this is responsible for adverse side effects such as cough and angioedema. This problem could be avoided by using a renin inhibitor, eg, aliskiren, or an ANG II receptor antagonist, eg, losartan, instead of an ACE inhibitor, to block the renin-angiotensin system.

CHAPTER

18

The Eicosanoids: Prostaglandins, Thromboxanes, Leukotrienes, & Related Compounds

Emer M. Smyth, PhD, & Garret A. FitzGerald, MD

The eicosanoids are oxygenation products of polyunsaturated long-chain fatty acids. They are ubiquitous in the animal kingdom and are also found—together with their precursors—in a variety of plants. They constitute a very large family of compounds that are highly potent and display an extraordinarily wide spectrum of biologic activity. Because of their biologic activity, the eicosanoids, their specific receptor antagonists and enzyme inhibitors, and their plant and fish oil precursors have great therapeutic potential.

ARACHIDONIC ACID & OTHER POLYUNSATURATED PRECURSORS

Arachidonic acid (AA), or 5,8,11,14-eicosatetraenoic acid, the most abundant of the eicosanoid precursors, is a 20-carbon (C20) fatty acid containing four double bonds (designated C20:4–6). AA must first be released or mobilized from the sn-2 position of membrane phospholipids by one or more lipases of the phospholipase A_2 (PLA_2) type (Figure 18–1) for eicosanoid synthesis to occur. At least three classes of phospholipases mediate arachidonate release from membrane lipids: cytosolic (c) PLA_2, secretory (s) PLA_2, and calcium-independent (i) PLA_2. Chemical and physical stimuli activate the Ca^{2+}-dependent translocation of group IVA $cPLA_2$, which has high affinity for AA, to the membrane, where it releases arachidonate. Multiple additional PLA_2 isoforms (group VI $iPLA_2$ and $sPLA_2$ from groups IIA, V, and X) have been characterized. Under nonstimulated conditions, AA liberated by $iPLA_2$ is reincorporated into cell membranes, so there is negligible eicosanoid biosynthesis. While $cPLA_2$ dominates in the acute release of AA, inducible $sPLA_2$ contributes under conditions of sustained or intense stimulation of AA production. AA can also be released by a combination of phospholipase C and diglyceride lipase.

Following mobilization, AA is oxygenated by four separate routes: the cyclooxygenase (COX), lipoxygenase, P450 epoxygenase, and isoeicosanoid pathways (Figure 18–1). Among factors determining the type of eicosanoid synthesized are (1) the substrate lipid species, (2) the type of cell, and (3) the manner in which the cell is stimulated. Distinct but related products can be

ACRONYMS

AA	Arachidonic acid
COX	Cyclooxygenase
DHET	Dihydroxyeicosatrienoic acid
EET	Epoxyeicosatrienoic acid
HETE	Hydroxyeicosatetraenoic acid
HPETE	Hydroxyperoxyeicosatetraenoic acid
LTB, LTC	Leukotriene B, C, etc
LOX	Lipoxygenase
LXA, LXB	Lipoxin A, B
NSAID	Nonsteroidal anti-inflammatory drug
PGE, PGF	Prostaglandin E, F, etc
PLA, PLC	Phospholipase A, C
TXA, TXB	Thromboxane A, B

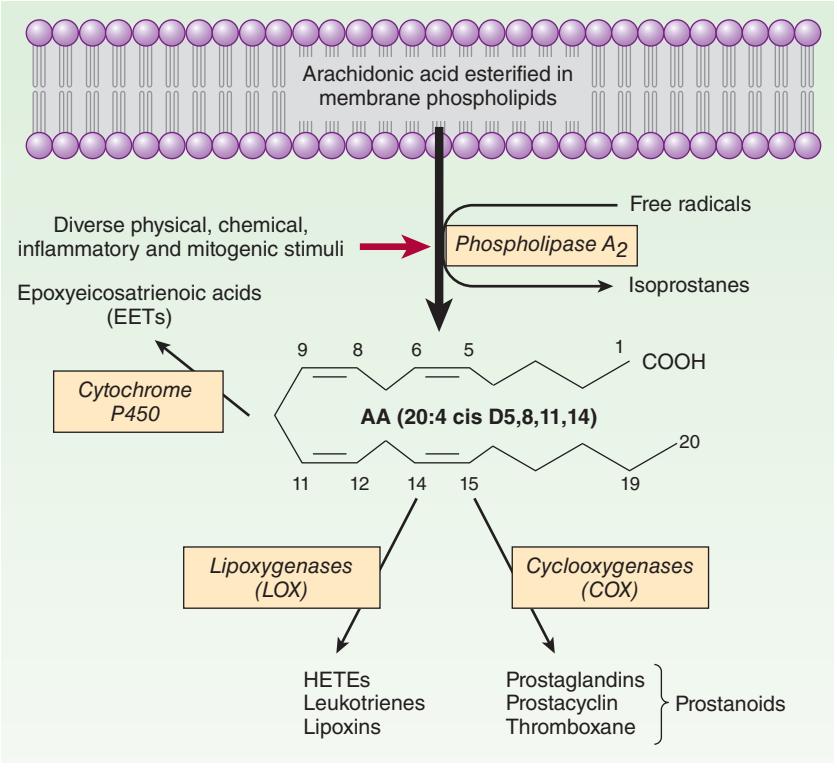


FIGURE 18–1 Pathways of arachidonic acid (AA) release and metabolism.

formed from precursors other than AA. For example, homo-γ-linoleic acid (C20:3–6) or eicosapentaenoic acid (C20:5–3, EPA) yields products that differ quantitatively and qualitatively from those derived from AA. This shift in product formation is the basis for using fatty acids obtained from cold-water fish or from plants as nutritional supplements in humans. For example, thromboxane (TXA_2), a powerful vasoconstrictor and platelet agonist, is synthesized from AA via the COX pathway. COX metabolism of EPA yields TXA_3, which is relatively inactive. 3-Series prostaglandins, such as prostaglandin E_3 (PGE_3), can also act as partial agonists or antagonists thereby reducing the activity of their AA-derived 2-series counterparts. The hypothesis that dietary eicosapentaenoate substitution for arachidonate could reduce the incidence of cardiovascular disease and cancer is a focus of current investigation.

SYNTHESIS OF EICOSANOIDS

Products of Prostaglandin Endoperoxide Synthases (Cyclooxygenases)

Two unique COX isozymes convert AA into prostaglandin endoperoxides. PGH synthase-1 **(COX-1)** is expressed constitutively in most cells. In contrast, PGH synthase-2 **(COX-2)** is inducible; its expression varies depending on the stimulus. COX-2 is an immediate early-response gene product that is markedly up-regulated by shear stress, growth factors, tumor promoters, and cytokines. COX-1 generates prostanoids for "housekeeping" such as gastric epithelial cytoprotection, whereas COX-2 is the major source of prostanoids in inflammation and cancer. This distinction is overly simplistic, however; there are both physiologic and pathophysiologic processes in which each enzyme is uniquely involved and others in which they function coordinately. For example, endothelial COX-2 is the primary source of vascular prostacyclin (PGI_2), whereas renal COX-2-derived prostanoids are important for normal renal development and maintenance of function. Nonsteroidal anti-inflammatory drugs (NSAIDs; see Chapter 36) exert their therapeutic effects through inhibition of the COXs. Indomethacin and sulindac are slightly selective for COX-1. Meclofenamate and ibuprofen are approximately equipotent on COX-1 and COX-2, whereas celecoxib = diclofenac < rofecoxib = lumiracoxib < etoricoxib in inhibition of COX-2 (listed in order of increasing average selectivity). Aspirin acetylates and inhibits both enzymes covalently. Low doses (< 100 mg/d) inhibit preferentially, but not exclusively, platelet COX-1, whereas higher doses inhibit both systemic COX-1 and COX-2.

Both COX-1 and COX-2 promote the uptake of two molecules of oxygen by cyclization of arachidonic acid to yield a C_9–C_{11} endoperoxide C_{15} hydroperoxide (Figure 18–2). This product is PGG_2, which is then rapidly modified by the peroxidase moiety of the COX enzyme to add a 15-hydroxyl group that is essential for biologic activity. This product is PGH_2. Both endoperoxides are highly unstable. Analogous families—PGH_1 and PGH_3 and all

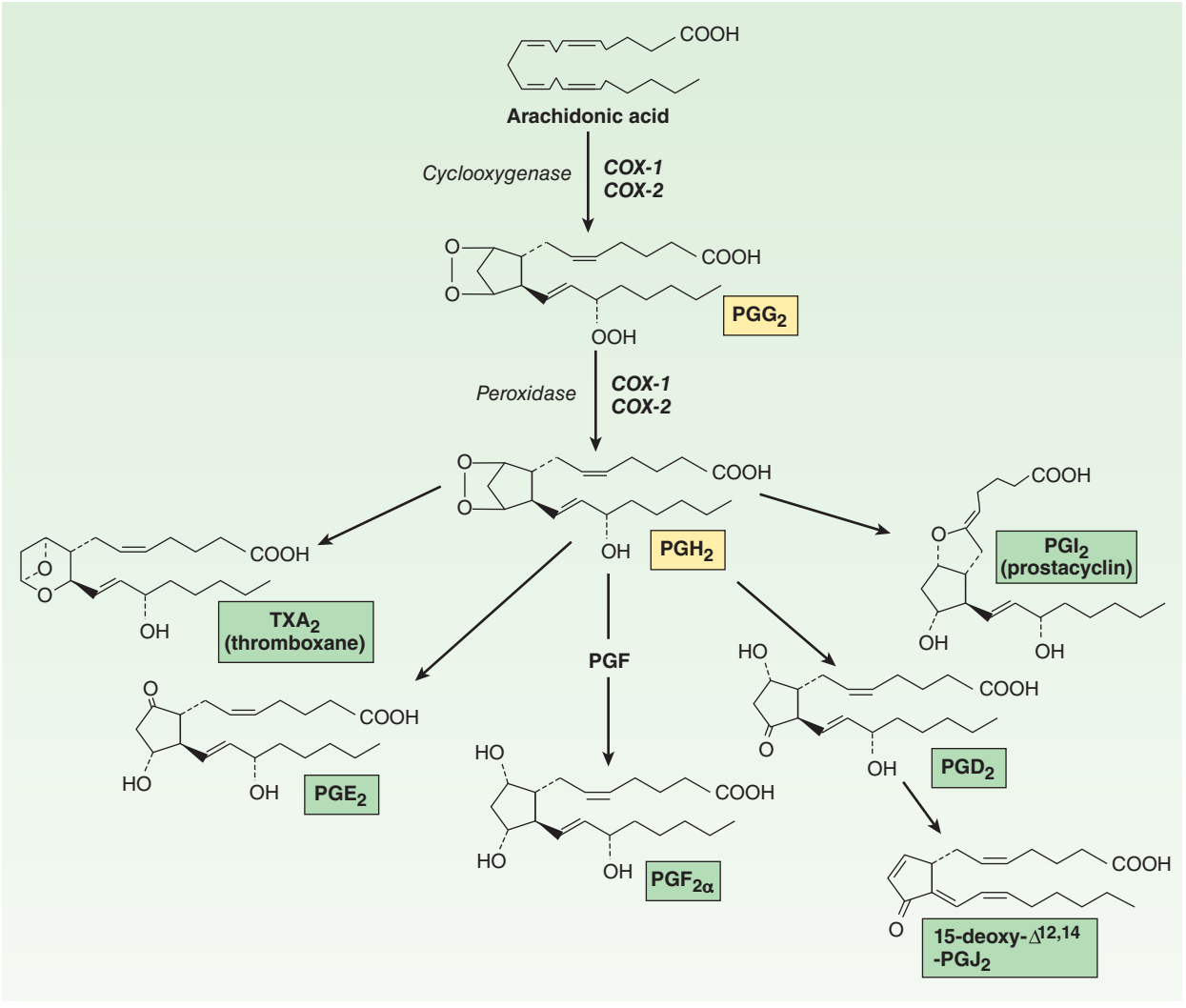


FIGURE 18–2 Prostanoid biosynthesis. Compound names are enclosed in boxes.

their subsequent products—are derived from homo-γ-linolenic acid and eicosapentaenoic acid, respectively.

The prostaglandins, thromboxane, and prostacyclin, collectively termed the prostanoids, are generated from PGH_2 through the action of downstream isomerases and synthases. These terminal enzymes are expressed in a relatively cell-specific fashion, such that most cells make one or two dominant prostanoids. The prostaglandins differ from each other in two ways: (1) in the substituents of the pentane ring (indicated by the last letter, eg, E and F in PGE and PGF) and (2) in the number of double bonds in the side chains (indicated by the subscript, eg, PGE_1, PGE_2). PGH_2 is metabolized by prostacyclin, thromboxane, and PGF synthases (PGIS, TXAS, and PGFS) to PGI_2, TXA_2, and $PGF_{2\alpha}$, respectively. Two additional enzymes, 9,11-endoperoxide reductase and 9-ketoreductase, provide for $PGF_{2\alpha}$ synthesis from PGH_2 and PGE_2, respectively. At least three PGE_2 synthases have been identified: microsomal (m) PGES-1, the more readily inducible mPGES-2, and cytosolic PGES. There are two distinct PGDS isoforms, the lipocalin-type PGDS and the hematopoietic PGDS.

Several products of the arachidonate series are of current clinical importance. **Alprostadil** (PGE_1) may be used for its smooth muscle relaxing effects to maintain the ductus arteriosus patent in some neonates awaiting cardiac surgery and in the treatment of impotence. **Misoprostol,** a PGE_1 derivative, is a cytoprotective prostaglandin used in preventing peptic ulcer and in combination with mifepristone (RU-486) for terminating early pregnancies. **PGE_2** and **PGF_2** are used in obstetrics to induce labor. **Latanoprost** and several similar compounds are topically active $PGF_{2\alpha}$ derivatives used in ophthalmology to treat open-angle glaucoma. **Prostacyclin** (PGI_2, epoprostenol) is synthesized mainly by the vascular endothelium and is a powerful vasodilator and inhibitor of platelet aggregation. It is used clinically to treat pulmonary hypertension and portopulmonary hypertension. In contrast, **thromboxane** (TXA_2) has undesirable properties (aggregation of platelets, vasoconstriction). Therefore TXA_2-receptor antagonists and synthesis inhibitors have been developed for cardiovascular indications, although these (except for aspirin) have yet to establish a place in clinical usage.

All the naturally occurring COX products undergo rapid metabolism to inactive products either by hydration (for PGI_2 and TXA_2) or by oxidation of the key 15-hydroxyl group to the corresponding ketone by prostaglandin 15-hydroxy prostaglandin dehydrogenase (15-PGDH) after cellular uptake via an organic anion transporter polypeptide (OATP 2A1). Further metabolism is by Δ^{13} reduction, β-oxidation, and ω-oxidation. The inactive metabolites can be determined in blood and urine by immunoassay or mass spectrometry as a measure of the in vivo synthesis of their parent compounds.

Products of Lipoxygenase

The metabolism of AA by the **5-, 12-, and 15-lipoxygenases (LOX)** results in the production of hydroperoxyeicosatetraenoic acids (HPETEs), which rapidly convert to hydroxy derivatives (HETEs) and leukotrienes (Figure 18–3). The most actively investigated leukotrienes are those produced by the 5-LOX present in leukocytes (neutrophils, basophils, eosinophils, and monocyte-macrophages) and other inflammatory cells such as mast cells and dendritic cells. This pathway is of great interest since it is associated with asthma, anaphylactic shock, and cardiovascular disease. Stimulation of these cells elevates intracellular Ca^{2+} and releases arachidonate; incorporation of molecular oxygen by 5-LOX, in association with **5-LOX-activating protein (FLAP),** then yields the unstable epoxide leukotriene A_4 (LTA_4). This intermediate is either converted to the dihydroxy leukotriene B_4 (LTB_4), via the action of LTA_4 hydrolase, or is conjugated with glutathione to yield leukotriene C_4 (LTC_4), by LTC_4 synthase. Sequential degradation of the glutathione moiety by peptidases yields LTD_4 and LTE_4. These three products, LTC_4, D_4, and E_4, are called cysteinyl leukotrienes. Although leukotrienes are predominantly generated in leukocytes, non-leukocyte cells (eg, endothelial cells) that express enzymes downstream of 5-LOX/FLAP can take up and convert leukocyte-derived LTA_4 in a process termed transcellular biosynthesis. Transcellular formation of prostaglandins has also been shown; for example, endothelial cells can use platelet PGH_2 to form PGI_2.

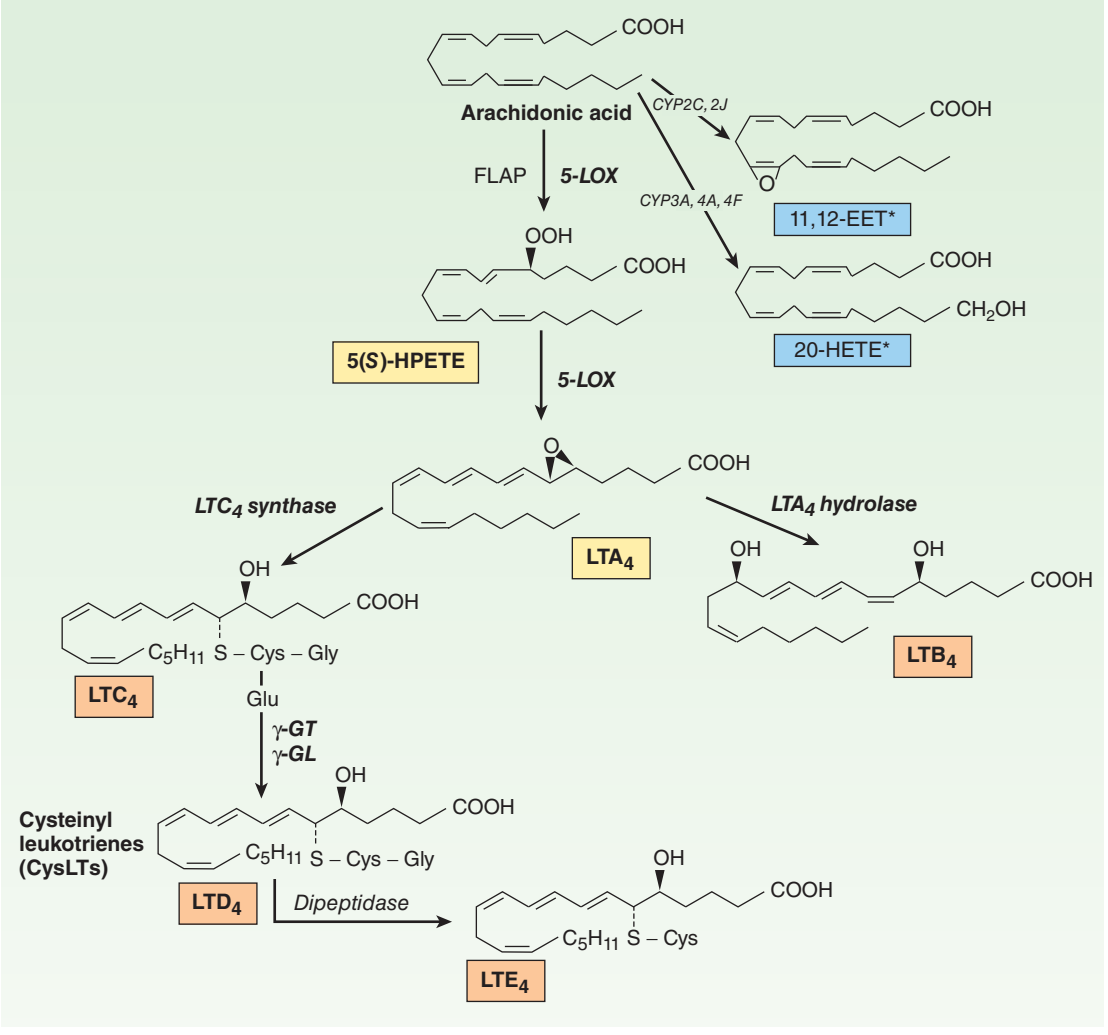


FIGURE 18–3 Leukotriene (LT) biosynthesis. LTC_4, LTD_4, and LTE_4 are known collectively as the cysteinyl (Cys) LTs. FLAP, 5-LOX-activating protein; GT, glutamyl transpeptidase; GL, glutamyl leukotrienase. *Additional products include 5,6-; 8,9-; and 14,15-EET; and 19-, 18-, 17-, and 16-HETE.

LTC_4 and **LTD_4** are potent bronchoconstrictors and are recognized as the primary components of the **slow-reacting substance of anaphylaxis (SRS-A)** that is secreted in asthma and anaphylaxis. There are four current approaches to antileukotriene drug development: 5-LOX enzyme inhibitors, leukotriene-receptor antagonists, inhibitors of FLAP, and phospholipase A_2 inhibitors.

LTA_4, the primary product of 5-LOX, can be converted with appropriate stimulation via 12-LOX in platelets to the **lipoxins** LXA_4 and LXB_4 in vitro. These mediators can also be generated through 5-LOX metabolism of 15-HETE, the product of 15-LOX-2 metabolism of arachidonic acid. 15-LOX-1 prefers linoleic acid as a substrate forming 15S-hydroxyoctadecadienoic acid. The stereochemical isomer, 15(*R*)-HETE, may be derived from the action of aspirin-acetylated COX-2 and further transformed in leukocytes by 5-LOX to 15-epi-LXA_4 or 15-epi-LXB_4, the so-called aspirin-triggered lipoxins. Synthetic lipoxins and epi-lipoxins exert anti-inflammatory actions when applied in vivo. Although these compounds can be formed from endogenous substrates in vitro and when synthesized may have potent biologic effects, the importance of the endogenous compounds in vivo in human biology remains ill defined. 12-HETE, a product of 12-LOX, can also undergo a catalyzed molecular rearrangement to epoxyhydroxyeicosatrienoic acids called **hepoxilins.** Proinflammatory effects of synthetic hepoxilins have been reported although their biologic relevance is unclear.

The LOXs located in epidermal cells are distinct from "conventional" enzymes—arachidonic acid and linoleic acid are apparently not the natural substrates for epidermal LOX. Epidermal accumulation of 12(*R*)-HETE is a feature of psoriasis and ichthyosis and inhibitors of 12(*R*)-LOX are under investigation for the treatment of these proliferative skin disorders.

Epoxygenase Products

Specific isozymes of microsomal cytochrome P450 monooxygenases convert AA to hydroxy- or epoxyeicosatrienoic acids (Figures 18–1 and 18–3). The products are 20-HETE, generated by the CYP hydroxylases (CYP3A, 4A, 4F) and the 5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acids (EETs), which arise from the CYP epoxygenase (2J, 2C). Their biosynthesis can be altered by pharmacologic, nutritional, and genetic factors that affect P450 expression. The biologic actions of the EETs are reduced by their conversion to the corresponding, and biologically less active, dihydroxyeicosatrienoic acids (DHETs) through the action of soluble epoxide hydrolase (sEH). Unlike the prostaglandins, the EETs can be esterified into phospholipids, which then act as storage sites. Intracellular fatty acid-binding proteins promote EET uptake into cells, incorporation into phospholipids, and availability to sEH. EETs are synthesized in endothelial cells and cause vasodilation in a number of vascular beds by activating the smooth muscle large conductance Ca^{2+}-activated K^+ channels. This results in smooth muscle cell hyperpolarization and vasodilation, leading to reduced blood pressure. Substantial evidence indicates that EETs may function as **endothelium-derived hyperpolarizing factors,** particularly in the coronary circulation. Consequently there is interest in inhibitors of soluble sEH as potential antithrombotic and antihypertensive drugs. An exception to the general response to EETs as vasodilators is the pulmonary vasculature where they cause vasoconstriction. It is unclear yet whether this activity of EETs may limit the potential clinical use of sEH inhibitors. Downregulation of pulmonary sEH may contribute to pulmonary hypertension. Anti-inflammatory, antiapoptotic, and proangiogenic actions of the EETs have also been reported.

Isoeicosanoids

The isoeicosanoids, a family of eicosanoid isomers, are formed nonenzymatically by direct free radical-based action on AA and related lipid substrates. Isoprostanes are prostaglandin stereoisomers. Because prostaglandins have many asymmetric centers, they have a large number of potential stereoisomers. COX is not needed for the formation of the isoprostanes, and its inhibition with aspirin or other NSAIDs should not affect the isoprostane pathway. The primary epimerization mechanism is peroxidation of arachidonate by free radicals. Peroxidation occurs while arachidonic acid is still esterified to the membrane phospholipids. Thus, unlike prostaglandins, these stereoisomers are "stored" as part of the membrane. They are then cleaved by phospholipases, circulate, and are excreted in urine. Isoprostanes are present in relatively large amounts (tenfold greater in blood and urine than the COX-derived prostaglandins). They have potent vasoconstrictor effects when infused into renal and other vascular beds and may activate prostanoid receptors. They also may modulate other aspects of vascular function, including leukocyte and platelet adhesive interactions and angiogenesis. It has been speculated that they may contribute to the pathophysiology of inflammatory responses in a manner insensitive to COX inhibitors. A particular difficulty in assessing the likely biologic functions of isoprostanes—several of which have been shown to serve as incidental ligands at prostaglandin receptors—is that while high concentrations of individual isoprostanes may be necessary to elicit a response, multiple compounds are formed coincidentally in vivo under conditions of oxidant stress. Analogous leukotriene and EET isomers have been described.

BASIC PHARMACOLOGY OF EICOSANOIDS

MECHANISMS & EFFECTS OF EICOSANOIDS

Receptor Mechanisms

As a result of their short half-lives, the eicosanoids act mainly in an autocrine and a paracrine fashion, ie, close to the site of their synthesis, and not as circulating hormones. These ligands bind to receptors on the cell surface, and pharmacologic specificity is determined by receptor density and type on different cells (Figure 18–4). A single gene product has been identified for the PGI_2 (IP), $PGF_{2\alpha}$ (FP), and TXA_2 (TP) receptors, while four distinct PGE_2 receptors (EPs 1–4) and two PGD_2 receptors (DP_1 and

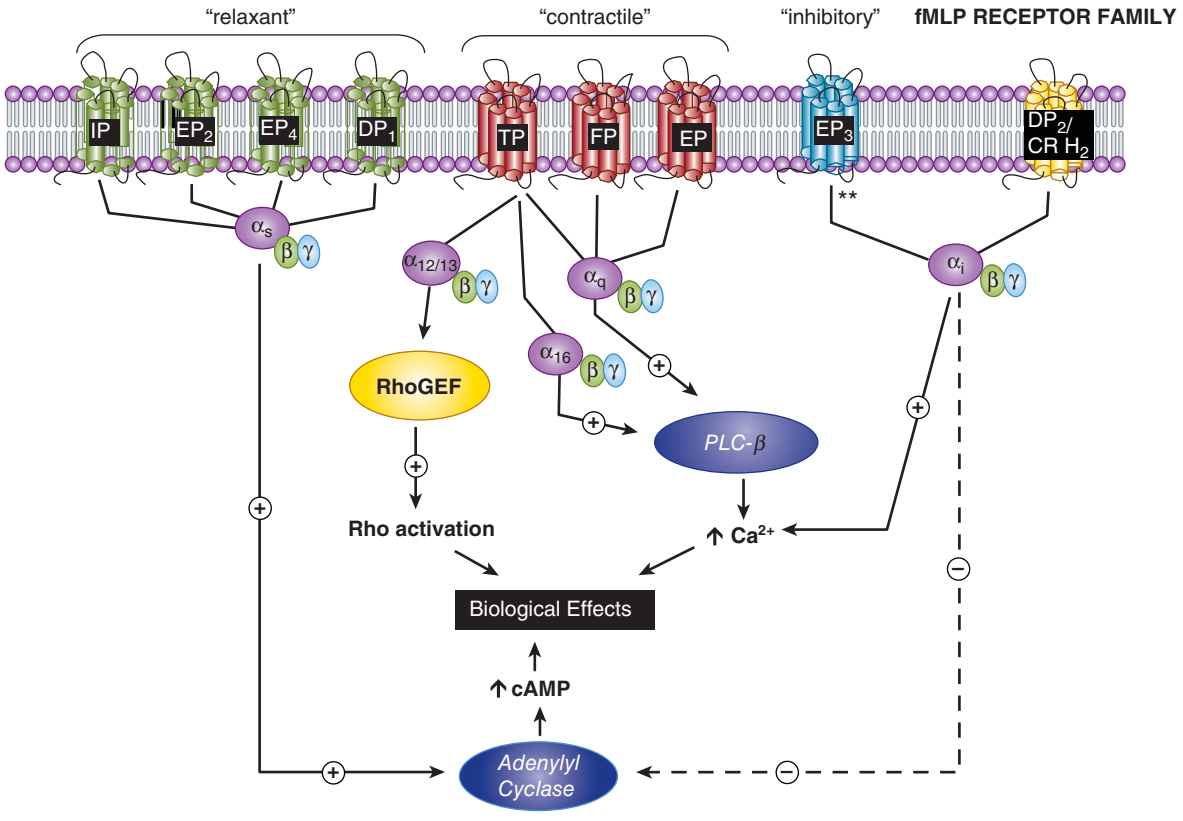


FIGURE 18–4 Prostanoid receptors and their signaling pathways. fMLP, formylated MetLeuPhe, a small peptide receptor; PLC-β, phospholipase C-β. All of the receptors shown are of the seven-transmembrane, G protein-coupled type. The terms "relaxant," "contractile," and "inhibitory" refer to the phylogenetic characterization of their primary effects. **All EP_3 isoforms couple through G_i but some can also activate G_s or $G_{12/13}$ pathways. RhoGEF, rho guanine nucleotide exchange factor. See text for additional details.

DP_2) have been cloned. Additional isoforms of the human TP (α and β), FP (A and B), and EP_3 (I, II, III, IV, V, VI, e, and f) receptors can arise through differential mRNA splicing. Two receptors exist for both LTB_4 (BLT_1 and BLT_2) and the cysteinyl leukotrienes ($cysLT_1$ and $cysLT_2$). The formyl peptide (fMLP)-1 receptor can be activated by lipoxin A4 and consequently has been termed the ALX receptor. All of these receptors are G protein-coupled; properties of the best-studied receptors are listed in Table 18–1.

EP_2, EP_4, IP, and DP_1 receptors activate adenylyl cyclase via G_s. This leads to increased intracellular cAMP levels, which in turn activate specific protein kinases (see Chapter 2). EP_1, FP, and TP activate phosphatidylinositol metabolism, leading to the formation of inositol trisphosphate, with subsequent mobilization of Ca^{2+} stores and an increase of free intracellular Ca^{2+}. TP also couples to multiple G proteins, including $G_{12/13}$ and G_{16}, to stimulate small G protein signaling pathways, and may activate or inhibit adenylyl cyclase via G_s (TPα) or G_i (TPβ), respectively. EP_3 isoforms can couple to both elevation of intracellular calcium and to increased or decreased cAMP. The DP_2 receptor (also known as the chemoattractant receptor-homologous molecule expressed on TH2 cells, or CRTH2), which is unrelated to the other prostanoid receptors, is a member of the fMLP (formylated MetLeuPhe) receptor superfamily. This receptor couples through a G_i-type G protein and leads to inhibition of cAMP synthesis and increases in intracellular Ca^{2+} in a variety of cell types.

LTB_4 also causes inositol trisphosphate release via the BLT_1 receptor, causing activation, degranulation, and superoxide anion generation in leukocytes. The BLT_2 receptor, a low-affinity receptor for LTB_4, is also bound with reasonable affinity by 12(*S*)- and 12(*R*)-HETE, although the biologic relevance of this observation is not clear. $CysLT_1$ and $cysLT_2$ couple to G_q, leading to increased intracellular Ca^{2+}. Studies have also placed G_i downstream of $cysLT_2$. An orphan receptor, GPR17, binds cysLTs and may negatively regulate the function of $cysLT_1$, but its physiologic role remains ill defined. As noted above, the EETs promote vasodilation via paracrine activation of calcium-activated potassium channels on smooth muscle cells leading to hyperpolarization and relaxation. This occurs in a manner consistent with activation of a G_s-coupled receptor, although a specific EET receptor has yet to be identified. EETs may also act in an autocrine manner directly activating endothelial transient receptor potential channels to cause endothelial hyperpolarization, which is then transferred to the smooth muscle cells by gap junctions or potassium ions. Specific receptors for isoprostanes have not been identified, and the biologic importance of their capacity to act as incidental ligands at prostaglandin receptors remains to be established.

TABLE 18–1 Eicosanoid receptors.[1]

Receptor (human)	Endogenous Ligand	Secondary Ligands	G Protein; Second Messenger	Major Phenotype(s) in Knockout Mice
DP_1	PGD_2		G_s; ↑cAMP	↓Allergic asthma
DP_2, CRTH2	PGD_2	$15d\text{-}PGJ_2$	G_i; $\uparrow Ca^{2+}_i$, ↓cAMP	↑Allergic airway inflammation ↓Cutaneous inflammation
EP_1	PGE_2	PGI_2	G_q; $\uparrow Ca^{2+}_i$	↓Colon carcinogenesis
EP_2	PGE_2		G_s; ↑cAMP	Impaired ovulation and fertilization
				Salt-sensitive hypertension
$EP_{3\ I, II, III, IV, V, VI, e, f}$	PGE_2		G_i; ↓cAMP, $\uparrow Ca^{2+}_i$	Resistance to pyrogens
			G_s; ↑cAMP	↓Acute cutaneous inflammation
			G_q; ↑PLC, $\uparrow Ca^{2+}_i$	
EP_4	PGE_2		G_s; ↑cAMP	↓Bone mass/density in aged mice
				↑Bowel inflammatory/immune response
				↓Colon carcinogenesis
				Patent ductus arteriosus
$FP_{A,B}$	$PGF_{2\alpha}$	isoPs	G_q; ↑PLC, $\uparrow Ca^{2+}_i$	Parturition failure
IP	PGI_2	PGE_2	G_s; ↑cAMP	↑Thrombotic response
				↑Response to vascular injury
				↑Atherosclerosis
				↑Cardiac fibrosis
				Salt-sensitive hypertension
				↓Joint inflammation
$TP_{\alpha,\beta}$	TXA_2	isoPs	G_q, $G_{12/13}$, G_{16}; ↑PLC, $\uparrow Ca^{2+}_i$, Rho activation	↑Bleeding time
				↓Response to vascular injury
				↓Atherosclerosis
				↑Survival after cardiac allograft
BLT_1	LTB_4		G_{16}, G_i; $\uparrow Ca^{2+}_i$, ↓cAMP	Some suppression of inflammatory response
BLT_2	LTB_4	12(*S*)-HETE	G_q-like, G_i-like, G_{12}-like, $\uparrow Ca^{2+}_i$	Not known
		12(*R*)-HETE		
$CysLT_1$	LTD_4	LTC_4/LTE_4	G_q; ↑PLC, $\uparrow Ca^{2+}_i$	↓Innate and adaptive immune vascular permeability response
				↑Pulmonary inflammatory and fibrotic response
$CysLT_2$	LTC_4/LTD_4	LTE_4	G_q; ↑PLC, $\uparrow Ca^{2+}_i$	↓Pulmonary inflammatory and fibrotic response

[1]Splice variants for the eicosanoid receptors are indicated where appropriate.

Ca^{2+}_i, intracellular calcium; cAMP, cyclic adenosine 3′,5′-monophosphate; PLC, phospholipase C; isoPs, isoprostanes; $15d\text{-}PGJ_2$, 15-deoxy-$\Delta^{12,14}$-PGJ_2.

Although prostanoids can activate peroxisome proliferator-activated receptors (PPARs) if added in sufficient concentration in vitro, it remains questionable whether these compounds attain concentrations sufficient to function as endogenous nuclear-receptor ligands in vivo.

Effects of Prostaglandins & Thromboxanes

The prostaglandins and thromboxanes have major effects on smooth muscle in the vasculature, airways, and gastrointestinal and reproductive tracts. Contraction of smooth muscle is mediated by the release of calcium, while relaxing effects are mediated by the generation of cAMP. Many of the eicosanoids' contractile effects on smooth muscle can be inhibited by lowering extracellular calcium or by using calcium channel-blocking drugs. Other important targets include platelets and monocytes, kidneys, the central nervous system, autonomic presynaptic nerve terminals, sensory nerve endings, endocrine organs, adipose tissue, and the eye (the effects on the eye may involve smooth muscle).

A. Smooth Muscle

1. Vascular—TXA_2 is a potent vasoconstrictor. It is also a smooth muscle cell mitogen and is the only eicosanoid that has convincingly been shown to have this effect. The mitogenic effect is potentiated by exposure of smooth muscle cells to testosterone, which up-regulates smooth muscle cell TP expression. $PGF_{2\alpha}$ is also a vasoconstrictor but is not a smooth muscle mitogen. Another vasoconstrictor is the isoprostane 8-iso-$PGF_{2\alpha}$, also known as $iPF_{2\alpha}$III, which may act via the TP receptor.

Vasodilator prostaglandins, especially PGI_2 and PGE_2, promote vasodilation by increasing cAMP and decreasing smooth muscle intracellular calcium, primarily via the IP and EP_4 receptors. Vascular PGI_2 is synthesized by both smooth muscle and endothelial cells, with the COX-2 isoform in the latter cell type being the major contributor. In the microcirculation, PGE_2 is a vasodilator produced by endothelial cells. PGI_2 inhibits proliferation of smooth muscle cells, an action that may be particularly relevant in pulmonary hypertension. PGD_2 may also function as a vasodilator—in particular as a dominant mediator of flushing induced by the lipid-lowering drug niacin—although the role of this prostanoid in the cardiovascular system remains under investigation.

2. Gastrointestinal tract—Most of the prostaglandins and thromboxanes activate gastrointestinal smooth muscle. Longitudinal muscle is contracted by PGE_2 (via EP_3) and $PGF_{2\alpha}$ (via FP), whereas circular muscle is contracted strongly by $PGF_{2\alpha}$ and weakly by PGI_2, and is relaxed by PGE_2 (via EP_4). Administration of either PGE_2 or $PGF_{2\alpha}$ results in colicky cramps (see Clinical Pharmacology of Eicosanoids, below). The leukotrienes also have powerful contractile effects.

3. Airways—Respiratory smooth muscle is relaxed by PGE_2 and PGI_2 and contracted by PGD_2, TXA_2, and $PGF_{2\alpha}$. Studies of DP_1 and DP_2 receptor knockout mice suggest an important role of this prostanoid in asthma, although the DP_2 receptor appears more relevant to allergic airway diseases. The cysteinyl leukotrienes are also bronchoconstrictors. They act principally on smooth muscle in peripheral airways and are a thousand times more potent than histamine, both in vitro and in vivo. They also stimulate bronchial mucus secretion and cause mucosal edema. Bronchospasm occurs in about 10% of people taking NSAIDs, possibly because of a shift in arachidonate metabolism from COX metabolism to leukotriene formation.

4. Reproductive—The actions of prostaglandins on reproductive smooth muscle are discussed below under section D, Reproductive Organs.

B. Platelets

Platelet aggregation is markedly affected by eicosanoids. Low concentrations of PGE_2 enhance (via EP_3), whereas higher concentrations inhibit (via IP), platelet aggregation. Both PGD_2 and PGI_2 inhibit aggregation via, respectively, DP_1- and IP-dependent elevation in cAMP generation. Unlike their human counterparts, mouse platelets do not express DP_1. TXA_2 is the major product of COX-1, the only COX isoform expressed in mature platelets. Itself a platelet aggregator, TXA_2 amplifies the effects of other, more potent, platelet agonists such as thrombin. The TP-G_q signaling pathway elevates intracellular Ca^{2+} and activates protein kinase C, facilitating platelet aggregation and TXA_2 biosynthesis. Activation of G_{12}/G_{13} induces Rho/Rho-kinase–dependent regulation of myosin light chain phosphorylation leading to platelet shape change. Mutations in the human TP have been associated with mild bleeding disorders. The platelet actions of TXA_2 are restrained in vivo by PGI_2, which inhibits platelet aggregation by all recognized agonists. Platelet COX-1-derived TXA_2 biosynthesis is increased during platelet activation and aggregation and is irreversibly inhibited by chronic administration of aspirin at low doses. Urinary metabolites of TXA_2 increase in clinical syndromes of platelet activation such as myocardial infarction and stroke. Macrophage COX-2 appears to contribute roughly 10% of the increment in TXA_2 biosynthesis observed in smokers, while the rest is derived from platelet COX-1. A variable contribution, presumably from macrophage COX-2, may be insensitive to the effects of low-dose aspirin. In a single trial comparing low- and high-dose aspirin, no increase in benefit was associated with increased dose; in fact, this study, as well as indirect comparisons across placebo-controlled trials, suggests an inverse dose-response relationship, perhaps reflecting increasing inhibition of PGI_2 synthesis at higher doses of aspirin.

C. Kidney

Both the medulla and the cortex of the kidney synthesize prostaglandins, the medulla substantially more than the cortex. COX-1 is expressed mainly in cortical and medullary collecting ducts and mesangial cells, arteriolar endothelium, and epithelial cells of Bowman's capsule. COX-2 is restricted to the renal medullary interstitial cells, the macula densa, and the cortical thick ascending limb.

The major renal eicosanoid products are PGE_2 and PGI_2, followed by $PGF_{2\alpha}$ and TXA_2. The kidney also synthesizes several hydroxyeicosatetraenoic acids, leukotrienes, cytochrome P450

products, and epoxides. Prostaglandins play important roles in maintaining blood pressure and regulating renal function, particularly in marginally functioning kidneys and volume-contracted states. Under these circumstances, renal cortical COX-2-derived PGE_2 and PGI_2 maintain renal blood flow and glomerular filtration rate through their local vasodilating effects. These prostaglandins also modulate systemic blood pressure through regulation of water and sodium excretion. Expression of medullary COX-2 and mPGES-1 is increased under conditions of high salt intake. COX-2-derived prostanoids increase medullary blood flow and inhibit tubular sodium reabsorption, while COX-1-derived products promote salt excretion in the collecting ducts. Increased water clearance probably results from an attenuation of the action of antidiuretic hormone (ADH) on adenylyl cyclase. Loss of these effects may underlie the systemic or salt-sensitive hypertension often associated with COX inhibition. A common misperception—often articulated in discussion of the cardiovascular toxicity of drugs such as rofecoxib—is that hypertension secondary to NSAID administration is somehow independent of the inhibition of prostaglandins. Loop diuretics, eg, furosemide, produce some of their effect by stimulating COX activity. In the normal kidney, this increases the synthesis of the vasodilator prostaglandins. Therefore, patient response to a loop diuretic is diminished if a COX inhibitor is administered concurrently (see Chapter 15).

There is an additional layer of complexity associated with the effects of renal prostaglandins. In contrast to the medullary enzyme, cortical COX-2 expression is increased by low salt intake, leading to increased renin release. This elevates glomerular filtration rate and contributes to enhanced sodium reabsorption and a rise in blood pressure. PGE_2 is thought to stimulate renin release through activation of EP_4 or EP_2. PGI_2 can also stimulate renin release and this may be relevant to maintenance of blood pressure in volume-contracted conditions and to the pathogenesis of renovascular hypertension. Inhibition of COX-2 may reduce blood pressure in these settings.

TXA_2 causes intrarenal vasoconstriction (and perhaps an ADH-like effect), resulting in a decline in renal function. The normal kidney synthesizes only small amounts of TXA_2. However, in renal conditions involving inflammatory cell infiltration (such as glomerulonephritis and renal transplant rejection), the inflammatory cells (monocyte-macrophages) release substantial amounts of TXA_2. Theoretically, TXA_2 synthase inhibitors or receptor antagonists should improve renal function in these patients, but no such drug is clinically available. Hypertension is associated with increased TXA_2 and decreased PGE_2 and PGI_2 synthesis in some animal models, eg, the Goldblatt kidney model. It is not known whether these changes are primary contributing factors or secondary responses. Similarly, increased TXA_2 formation has been reported in cyclosporine-induced nephrotoxicity, but no causal relationship has been established. $PGF_{2\alpha}$ may elevate blood pressure by regulating renin release in the kidney. Although more research is necessary, FP antagonists have potential as novel antihypertensive drugs.

D. Reproductive Organs

1. Female reproductive organs—Animal studies demonstrate a role for PGE_2 and $PGF_{2\alpha}$ in early reproductive processes such as ovulation, luteolysis, and fertilization. Uterine muscle is contracted by $PGF_{2\alpha}$, TXA_2, and low concentrations of PGE_2; PGI_2 and high concentrations of PGE_2 cause relaxation. $PGF_{2\alpha}$, together with oxytocin, is essential for the onset of parturition. The effects of prostaglandins on uterine function are discussed below (see Clinical Pharmacology of Eicosanoids).

2. Male reproductive organs—Despite the discovery of prostaglandins in seminal fluid, and their uterotropic effects, the role of prostaglandins in semen is still conjectural. The major source of these prostaglandins is the seminal vesicle; the prostate, despite the name "prostaglandin," and the testes synthesize only small amounts. The factors that regulate the concentration of prostaglandins in human seminal plasma are not known in detail, but testosterone does promote prostaglandin production. Thromboxane and leukotrienes have not been found in seminal plasma. Men with a low seminal fluid concentration of prostaglandins are relatively infertile.

Smooth muscle-relaxing prostaglandins such as PGE_1 enhance penile erection by relaxing the smooth muscle of the corpora cavernosa (see Clinical Pharmacology of Eicosanoids).

E. Central and Peripheral Nervous Systems

1. Fever—PGE_2 increases body temperature, predominantly via EP_3, although EP_1 also plays a role, especially when administered directly into the cerebral ventricles. Exogenous $PGF_{2\alpha}$ and PGI_2 induce fever, whereas PGD_2 and TXA_2 do not. Endogenous pyrogens release interleukin-1, which in turn promotes the synthesis and release of PGE_2. This synthesis is blocked by aspirin and other antipyretic compounds.

2. Sleep—When infused into the cerebral ventricles, PGD_2 induces natural sleep (as determined by electroencephalographic analysis) via activation of DP_1 receptors and secondary release of adenosine. PGE_2 infusion into the posterior hypothalamus causes wakefulness.

3. Neurotransmission—PGE compounds inhibit the release of norepinephrine from postganglionic sympathetic nerve endings. Moreover, NSAIDs increase norepinephrine release in vivo, suggesting that the prostaglandins play a physiologic role in this process. Thus, vasoconstriction observed during treatment with COX inhibitors may be due, in part, to increased release of norepinephrine as well as to inhibition of the endothelial synthesis of the vasodilators PGE_2 and PGI_2. PGE_2 and PGI_2 sensitize the peripheral nerve endings to painful stimuli by increasing their terminal membrane excitability. Prostaglandins also modulate pain centrally. Both COX-1 and COX-2 are expressed in the spinal cord and release prostaglandins in response to peripheral pain stimuli. PGE_2, and perhaps also PGD_2, PGI_2, and $PGF_{2\alpha}$, contribute to so-called central sensitization, an increase in excitability of spinal dorsal horn neurons, that augments pain intensity, widens the area of pain perception, and results in pain from normally innocuous stimuli.

F. Inflammation and Immunity

PGE_2 and PGI_2 are the predominant prostanoids associated with inflammation. Both markedly enhance edema formation and leukocyte infiltration by promoting blood flow in the inflamed region. PGE_2 and PGI_2, through activation of EP_2 and IP, respectively, increase vascular permeability and leukocyte infiltration. Through its action as a platelet agonist, TXA_2 can also increase platelet-leukocyte interactions. Although probably not made by lymphocytes, prostaglandins may contribute positively or negatively to lymphocyte function. PGE_2 and TXA_2 may play a role in T-lymphocyte development by regulating apoptosis of immature thymocytes. PGE_2 suppresses the immunologic response by inhibiting differentiation of B lymphocytes into antibody-secreting plasma cells, thus depressing the humoral antibody response. It also inhibits cytotoxic T-cell function, mitogen-stimulated proliferation of T lymphocytes, and the release of cytokines by sensitized TH1 lymphocytes. PGE_2 can modify myeloid cell differentiation promoting type 2 immune-suppressive macrophage and myeloid suppressor cell phenotypes. These effects likely contribute to immune escape in tumors where infiltrating myeloid-derived cells predominantly display type 2 phenotypes. PGD_2, a major product of mast cells, is a potent chemoattractant for eosinophils in which it also induces degranulation and leukotriene biosynthesis. PGD_2 also induces chemotaxis and migration of TH2 lymphocytes mainly via activation of DP_2, although a role for DP_1 has also been established. It remains unclear how these two receptors coordinate the actions of PGD_2 in inflammation and immunity. A degradation product of PGD_2, 15d-PGJ_2, at concentrations actually formed in vivo, may also activate eosinophils via the DP_2 (CRTH2) receptor.

G. Bone Metabolism

Prostaglandins are abundant in skeletal tissue and are produced by osteoblasts and adjacent hematopoietic cells. The major effect of prostaglandins (especially PGE_2, acting on EP_4) in vivo is to increase bone turnover, ie, stimulation of bone resorption and formation. EP_4 receptor deletion in mice results in an imbalance between bone resorption and formation, leading to a negative balance of bone mass and density in older animals. Prostaglandins may mediate the effects of mechanical forces on bones and changes in bone during inflammation. EP_4-receptor deletion and inhibition of prostaglandin biosynthesis have both been associated with impaired fracture healing in animal models. COX inhibitors can also slow skeletal muscle healing by interfering with prostaglandin effects on myocyte proliferation, differentiation, and fibrosis in response to injury. Prostaglandins may contribute to the bone loss that occurs at menopause; it has been speculated that NSAIDs may be of therapeutic value in osteoporosis and bone loss prevention in older women. However, controlled evaluation of such therapeutic interventions has not been carried out. NSAIDs, especially those specific for inhibition of COX-2, delay bone healing in experimental models of fracture.

H. Eye

PGE and PGF derivatives lower intraocular pressure. The mechanism of this action is unclear but probably involves increased outflow of aqueous humor from the anterior chamber via the uveoscleral pathway (see Clinical Pharmacology of Eicosanoids).

I. Cancer

There has been significant interest in the role of prostaglandins, and in particular the COX-2 pathway, in the development of malignancies. Pharmacologic inhibition or genetic deletion of COX-2 restrains tumor formation in models of colon, breast, lung, and other cancers. Large human epidemiologic studies have found that the incidental use of NSAIDs is associated with significant reductions in relative risk for developing these and other cancers. Chronic low-dose aspirin does not appear to have a substantial impact on cancer incidence; however, it is associated with reduced cancer death in a number of studies. In patients with familial polyposis coli, COX inhibitors significantly decrease polyp formation. Polymorphisms in COX-2 have been associated with increased risk of some cancers. Several studies have suggested that COX-2 expression is associated with markers of tumor progression in breast cancer. In mouse mammary tissue, COX-2 is oncogenic whereas NSAID use is associated with a reduced risk of breast cancer in women, especially for hormone receptor-positive tumors. Despite the support for COX-2 as the predominant source of oncogenic prostaglandins, randomized clinical trials have not been performed to determine whether superior anti-oncogenic effects occur with selective inhibition of COX-2, compared with nonselective NSAIDs. Indeed data from animal models and epidemiologic studies in humans are consistent with a role for COX-1 as well as COX-2 in the production of oncogenic prostanoids.

PGE_2, which is considered the principal oncogenic prostanoid, facilitates tumor initiation, progression, and metastasis through multiple biologic effects, increasing proliferation and angiogenesis, inhibiting apoptosis, augmenting cellular invasiveness, and modulating immunosuppression. Augmented expression of mPGES-1 is evident in tumors, and preclinical studies support the potential use of mPGES-1 inhibitors in chemoprevention or treatment. In tumors reduced levels of OATP2A1 and 15-PGDH, which mediate cellular uptake and metabolic inactivation of PGE_2, respectively, likely contribute to sustained PGE_2 activity. The pro- and anti-oncogenic roles of other prostanoids remain under investigation, with TXA_2 emerging as another likely procarcinogenic mediator, deriving either from macrophage COX-2 or platelet COX-1. Studies in mice lacking EP_1, EP_2, or EP_4 receptors confirm reduced disease in multiple carcinogenesis models. EP_3, in contrast, plays no role or may even play a protective role in some cancers. Transactivation of epidermal growth factor receptor (EGFR) has been linked with the oncogenic activity of PGE_2.

Effects of Lipoxygenase & Cytochrome P450-Derived Metabolites

The actions of lipoxygenases generate compounds that can regulate specific cellular responses important in inflammation and immunity. Cytochrome P450-derived metabolites affect nephron transport functions either directly or via metabolism to active compounds (see below). The biologic functions of the various forms of hydroxy- and hydroperoxyeicosaenoic acids are largely unknown, but their pharmacologic potency is impressive.

A. Blood Cells and Inflammation

LTB_4, acting at the BLT_1 receptor, is a potent chemoattractant for T lymphocytes, eosinophils, monocytes, and possibly mast cells; the cysteinyl leukotrienes are potent chemoattractants for eosinophils and T lymphocytes. Cysteinyl leukotrienes may also generate distinct sets of cytokines through activation of mast cell $cysLT_1$ and $cysLT_2$. At higher concentrations, these leukotrienes also promote eosinophil adherence, degranulation, cytokine or chemokine release, and oxygen radical formation. Cysteinyl leukotrienes also contribute to inflammation by increasing endothelial permeability, thus promoting migration of inflammatory cells to the site of inflammation. The leukotrienes have been strongly implicated in the pathogenesis of inflammation, especially in chronic diseases such as asthma and inflammatory bowel disease.

Lipoxins have diverse effects on leukocytes, including activation of monocytes and macrophages and inhibition of neutrophil, eosinophil, and lymphocyte activation. Both lipoxin A and lipoxin B inhibit natural killer cell cytotoxicity.

B. Heart and Smooth Muscle

1. Cardiovascular—12(*S*)-HETE promotes vascular smooth muscle cell proliferation and migration at low concentrations; it may play a role in myointimal proliferation that occurs after vascular injury such as that caused by angioplasty. Its stereoisomer, 12(*R*)-HETE, is not a chemoattractant, but is a potent inhibitor of the Na^+/K^+-ATPase in the cornea. LTC_4 and LTD_4 reduce myocardial contractility and coronary blood flow, leading to cardiac depression. Lipoxin A and lipoxin B exert coronary vasoconstrictor effects in vitro. In addition to their vasodilatory action, EETs may reduce cardiac hypertrophy as well as systemic and pulmonary vascular smooth muscle proliferation and migration.

2. Gastrointestinal—Human colonic epithelial cells synthesize LTB_4, a chemoattractant for neutrophils. The colonic mucosa of patients with inflammatory bowel disease contains substantially increased amounts of LTB_4.

3. Airways—The cysteinyl leukotrienes, particularly LTC_4 and LTD_4, are potent bronchoconstrictors and cause increased microvascular permeability, plasma exudation, and mucus secretion in the airways. Controversies exist over whether the pattern and specificity of the leukotriene receptors differ in animal models and humans. LTC_4-specific receptors have not been found in human lung tissue, whereas both high- and low-affinity LTD_4 receptors are present.

C. Renal System

There is substantial evidence for a role of the epoxygenase products in regulating renal function although their exact role in the human kidney remains unclear. Both 20-HETE and the EETs are generated in renal tissue. 20-HETE, which potently blocks the smooth muscle cell Ca^{2+}-activated K^+ channel and leads to vasoconstriction of the renal arteries, has been implicated in the pathogenesis of hypertension. In contrast, studies support an antihypertensive effect of the EETs because of their vasodilating and natriuretic actions. EETs increase renal blood flow and may protect against inflammatory renal damage by limiting glomerular macrophage infiltration. Inhibitors of soluble epoxide hydrolase, which prolong the biologic activities of the EETs, are being developed as potential new antihypertensive drugs. In vitro studies, and work in animal models, support targeting soluble epoxide hydrolase for blood pressure control, although the potential for pulmonary vasoconstriction and tumor promotion through antiapoptotic actions require careful investigation.

D. Miscellaneous

The effects of these products on the reproductive organs have not been elucidated. Similarly, actions on the nervous system have been suggested but not confirmed. 12-HETE stimulates the release of aldosterone from the adrenal cortex and mediates a portion of the aldosterone release stimulated by angiotensin II but not that by adrenocorticotropic hormone. Very low concentrations of LTC_4 increase and higher concentrations of arachidonate-derived epoxides augment luteinizing hormone (LH) and LH-releasing hormone release from isolated rat anterior pituitary cells.

INHIBITION OF EICOSANOID SYNTHESIS

Corticosteroids block all the known pathways of eicosanoid synthesis, perhaps in part by stimulating the synthesis of several inhibitory proteins collectively called annexins or lipocortins. They inhibit phospholipase A_2 activity, probably by interfering with phospholipid binding, thus preventing the release of arachidonic acid.

The NSAIDs (eg, **indomethacin, ibuprofen**; see Chapter 36) block both prostaglandin and thromboxane formation by reversibly inhibiting COX activity. The traditional NSAIDs are not selective for COX-1 or COX-2. Selective COX-2 inhibitors, which were developed more recently, vary—as do the older drugs—in their degree of selectivity. Indeed, there is considerable variability between (and within) individuals in the selectivity attained by the same dose of the same NSAID. Aspirin is an irreversible COX inhibitor. In platelets, which lack nuclei, COX-1 (the only isoform expressed in mature platelets) cannot be restored via protein biosynthesis, resulting in extended inhibition of TXA_2 biosynthesis.

EP-receptor agonists and antagonists are under evaluation in the treatment of bone fracture and osteoporosis, whereas TP-receptor antagonists are being investigated for usefulness in treatment of cardiovascular syndromes. Direct inhibition of PGE_2 biosynthesis through selective inhibition of the inducible mPGES-1 isoform is also under examination for potential therapeutic efficacy in pain and inflammation, cardiovascular disease, and chemoprevention of cancer.

Although they remain less effective than inhaled corticosteroids, a 5-LOX inhibitor (**zileuton**) and selective antagonists of the $CysLT_1$ receptor for leukotrienes (**zafirlukast, montelukast,** and **pranlukast;** see Chapter 20) are used clinically in mild to moderate asthma. Growing evidence for a role of the leukotrienes in cardiovascular disease has expanded the potential clinical applications of leukotriene modifiers. Conflicting data have been reported in animal studies depending on the disease model used and the

molecular target (5-LOX versus FLAP). Human genetic studies initially demonstrated a link between cardiovascular disease and polymorphisms in the leukotriene biosynthetic enzymes, in particular FLAP, in some populations. However, these results have not been substantiated in more recent, larger studies.

NSAIDs usually do not inhibit lipoxygenase activity at concentrations that inhibit COX activity. In fact, by preventing arachidonic acid conversion via the COX pathway, NSAIDs may cause more substrate to be metabolized through the lipoxygenase pathways, leading to an increased formation of the inflammatory leukotrienes. Even among the COX-dependent pathways, inhibiting the synthesis of one derivative may increase the synthesis of an enzymatically related product. Therefore, drugs that inhibit both COX and lipoxygenase are being developed.

CLINICAL PHARMACOLOGY OF EICOSANOIDS

Several approaches have been used in the clinical application of eicosanoids. First, stable oral or parenteral long-acting analogs of the naturally occurring prostaglandins have been developed (Figure 18–5). Second, enzyme inhibitors and receptor antagonists have been developed to interfere with the synthesis or effects of the eicosanoids. The discovery of COX-2 as a major source of inflammatory prostanoids led to the development of selective COX-2 inhibitors in an effort to preserve the gastrointestinal and renal functions directed through COX-1, thereby reducing toxicity. However, it is apparent that the marked decrease in biosynthesis of PGI_2 that follows COX-2 inhibition occurring without a concurrent inhibition of platelet COX-1-derived TXA_2 removes a protective constraint on endogenous mediators of cardiovascular dysfunction and leads to an increase in cardiovascular events in patients taking selective COX-2 inhibitors. Third, efforts at dietary manipulation—to change the polyunsaturated fatty acid precursors in the cell membrane phospholipids and so change eicosanoid synthesis—is used extensively in over-the-counter products and in diets emphasizing increased consumption of cold-water fish.

Female Reproductive System

Studies with knockout mice have confirmed a role for prostaglandins in reproduction and parturition. COX-1-derived $PGF_{2\alpha}$ appears important for luteolysis, consistent with delayed parturition in COX-1-deficient mice. A complex interplay between $PGF_{2\alpha}$ and oxytocin is critical to the onset of labor. EP_2 receptor-deficient mice demonstrate a preimplantation defect, which underlies some of the breeding difficulties seen in COX-2 knockouts.

A. Abortion

PGE_2 and $PGF_{2\alpha}$ have potent oxytocic actions. The ability of the E and F prostaglandins and their analogs to terminate pregnancy at any stage by promoting uterine contractions has been adapted to common clinical use. Many studies worldwide have established that prostaglandin administration efficiently terminates pregnancy. The drugs are used for first- and second-trimester abortion and for priming or ripening the cervix before abortion. These prostaglandins appear to soften the cervix by increasing proteoglycan content and changing the biophysical properties of collagen.

Dinoprostone, a synthetic preparation of PGE_2, is administered vaginally for oxytocic use. In the USA, it is approved for inducing abortion in the second trimester of pregnancy, for missed abortion, for benign hydatidiform mole, and for ripening of the cervix for induction of labor in patients at or near term.

Dinoprostone stimulates the contraction of the uterus throughout pregnancy. As the pregnancy progresses, the uterus increases its contractile response, and the contractile effect of oxytocin is potentiated as well. Dinoprostone also directly affects the collagenase of the cervix, resulting in softening. The vaginal dose enters the maternal circulation, and a small amount is absorbed directly by the uterus via the cervix and the lymphatic system. Dinoprostone is metabolized in local tissues and on the first pass through the lungs (about 95%). The metabolites are mainly excreted in the urine. The plasma half-life is 2.5–5 minutes.

For the induction of labor, dinoprostone is used either as a gel (0.5 mg PGE_2) or as a controlled-release formulation (10 mg PGE_2) that releases PGE_2 in vivo at a rate of about 0.3 mg/h over 12 hours. An advantage of the controlled-release formulation is a lower incidence of gastrointestinal side effects (< 1%).

For abortifacient purposes, the recommended dosage is a 20-mg dinoprostone vaginal suppository repeated at 3- to 5-hour intervals depending on the response of the uterus. The mean time to abortion is 17 hours, but in more than 25% of cases, the abortion is incomplete and requires additional intervention.

For softening of the cervix at term, the preparations used are either a single vaginal insert containing 10 mg PGE_2 or a vaginal gel containing 0.5 mg PGE_2 administered every 6 hours. The softening of the cervix for induction of labor substantially shortens the time to onset of labor and the delivery time.

Antiprogestins (eg, **mifepristone**) have been combined with an oral oxytocic synthetic analog of PGE_1 (**misoprostol**) to produce early abortion. This regimen is available in the USA and Europe (see Chapter 40). The ease of use and the effectiveness of the combination have aroused considerable opposition in some quarters. The major toxicities are cramping pain and diarrhea. The oral and vaginal routes of administration are equally effective, but the vaginal route has been associated with an increased incidence of sepsis, so the oral route is now recommended.

An analog of $PGF_{2\alpha}$ is also used in obstetrics. This drug, **carboprost tromethamine** (15-methyl-$PGF_{2\alpha}$; the 15-methyl group prolongs the duration of action) is used to induce second-trimester abortions and to control postpartum hemorrhage that is not responding to conventional methods of management. The success rate is approximately 80%. It is administered as a single 250-mcg intramuscular injection, repeated if necessary. Vomiting and diarrhea occur commonly, probably because of gastrointestinal smooth muscle stimulation. In some patients transient bronchoconstriction can occur. Transient elevations in temperature are seen in approximately one eighth of patients.

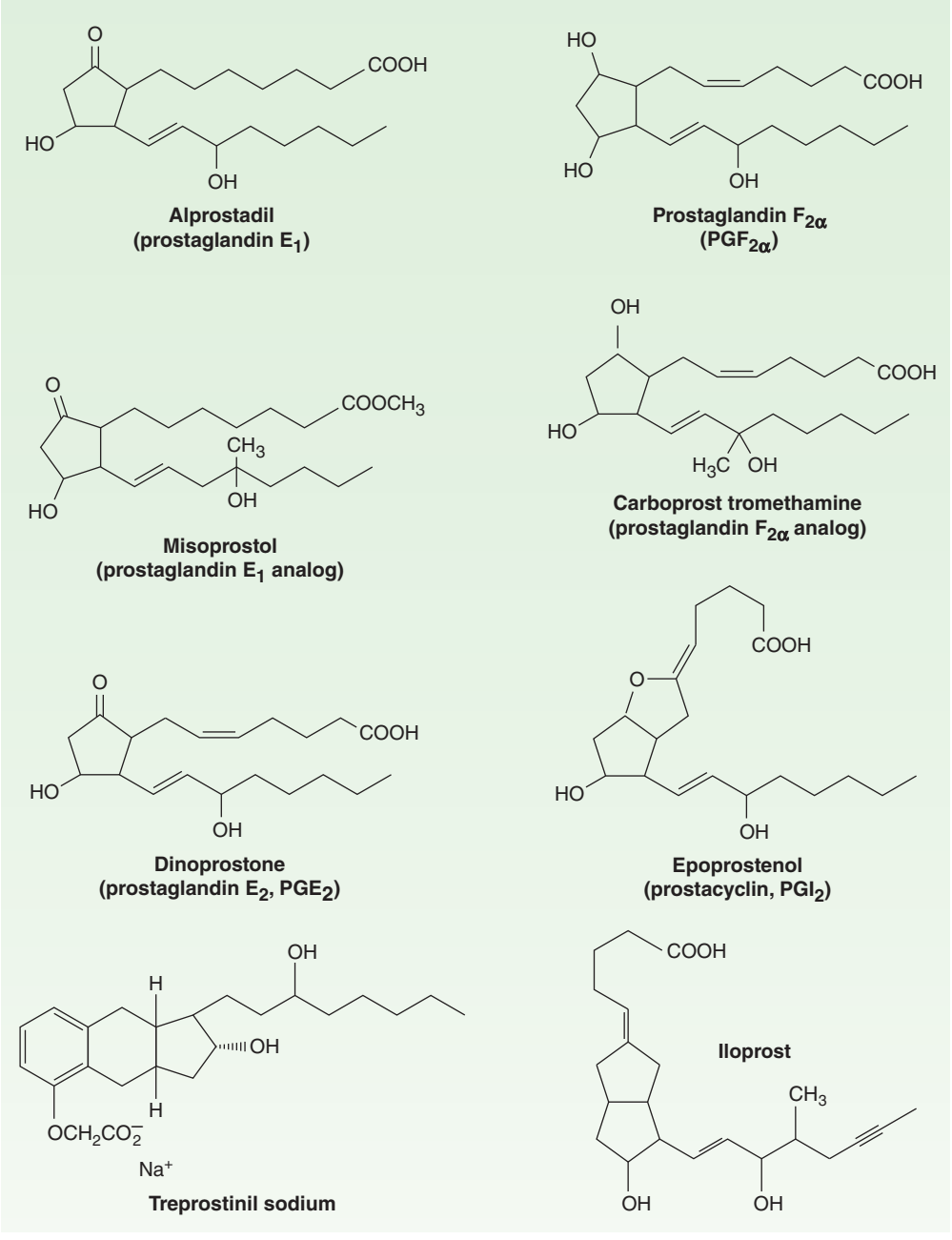


FIGURE 18–5 Chemical structures of some prostaglandins and prostaglandin analogs currently in clinical use.

B. Facilitation of Labor

Numerous studies have shown that PGE_2, $PGF_{2\alpha}$, and their analogs effectively initiate and stimulate labor, but $PGF_{2\alpha}$ is one tenth as potent as PGE_2. There appears to be no difference in the efficacy of PGE_2 and $PGF_{2\alpha}$ when they are administered intravenously; however, the most common usage is local application of PGE_2 analogs (dinoprostone) to promote labor through ripening of the cervix. These agents and oxytocin have similar success rates and comparable induction-to-delivery intervals. The adverse effects of the prostaglandins are moderate, with a slightly higher incidence of nausea, vomiting, and diarrhea than that produced by oxytocin. $PGF_{2\alpha}$ has more gastrointestinal toxicity than PGE_2. Neither drug has significant maternal cardiovascular toxicity in the recommended doses. In fact, PGE_2 must be infused at a rate about 20 times faster than that used for induction of labor to decrease blood pressure and increase heart rate. $PGF_{2\alpha}$ is a bronchoconstrictor and should be used with caution in women with asthma; however, neither asthma attacks nor bronchoconstriction have been observed during the induction of labor. Although both PGE_2 and $PGF_{2\alpha}$ pass the fetoplacental barrier, fetal toxicity is uncommon.

The effects of oral PGE_2 administration (0.5–1.5 mg/h) have been compared with those of intravenous oxytocin and oral

demoxytocin, an oxytocin derivative, in the induction of labor. Oral PGE_2 is superior to the oral oxytocin derivative and in most studies is as efficient as intravenous oxytocin. Oral $PGF_{2\alpha}$ causes too much gastrointestinal toxicity to be useful by this route.

Theoretically, PGE_2 and $PGF_{2\alpha}$ should be superior to oxytocin for inducing labor in women with preeclampsia-eclampsia or cardiac and renal diseases because, unlike oxytocin, they have no antidiuretic effect. In addition, PGE_2 has natriuretic effects. However, the clinical benefits of these effects have not been documented. In cases of intrauterine fetal death, the prostaglandins alone or with oxytocin seem to cause delivery effectively.

C. Dysmenorrhea

Primary dysmenorrhea is attributable to increased endometrial synthesis of PGE_2 and $PGF_{2\alpha}$ during menstruation, with contractions of the uterus that lead to ischemic pain. NSAIDs successfully inhibit the formation of these prostaglandins (see Chapter 36) and so relieve dysmenorrhea in 75–85% of cases. Some of these drugs are available over the counter. Aspirin is also effective in dysmenorrhea, but because it has low potency and is quickly hydrolyzed, large doses and frequent administration are necessary. In addition, the acetylation of platelet COX, causing irreversible inhibition of platelet TXA_2 synthesis, may increase the amount of menstrual bleeding.

Male Reproductive System

Intracavernosal injection or urethral suppository therapy with **alprostadil** (PGE_1) is a second-line treatment for erectile dysfunction. Doses of 2.5–25 mcg are used. Penile pain is a frequent side effect, which may be related to the algesic effects of PGE derivatives; however, only a few patients discontinue the use because of pain. Prolonged erection and priapism are side effects that occur in less than 4% of patients and are minimized by careful titration to the minimal effective dose. When given by injection, alprostadil may be used as monotherapy or in combination with either papaverine or phentolamine.

Renal System

Increased biosynthesis of prostaglandins has been associated with one form of Bartter's syndrome. This is a rare disease characterized by low-to-normal blood pressure, decreased sensitivity to angiotensin, hyperreninemia, hyperaldosteronism, and excessive loss of K^+. There also is an increased excretion of prostaglandins, especially PGE metabolites, in the urine. After long-term administration of COX inhibitors, sensitivity to angiotensin, plasma renin values, and the concentration of aldosterone in plasma return to normal. Although plasma K^+ rises, it remains low, and urinary wasting of K^+ persists. Whether an increase in prostaglandin biosynthesis is the cause of Bartter's syndrome or a reflection of a more basic physiologic defect is not yet known.

Cardiovascular System

The vasodilator effects of PGE compounds have been studied extensively in hypertensive patients. These compounds also promote sodium diuresis. Practical application will require derivatives with oral activity, longer half-lives, and fewer adverse effects.

A. Pulmonary Hypertension

PGI_2 lowers peripheral, pulmonary, and coronary vascular resistance. It has been used to treat primary pulmonary hypertension as well as secondary pulmonary hypertension, which sometimes occurs after mitral valve surgery. In addition, prostacyclin has been used successfully to treat portopulmonary hypertension, which arises secondary to liver disease. The first commercial preparation of PGI_2 (**epoprostenol**) approved for treatment of primary pulmonary hypertension improves symptoms, prolongs survival, and delays or prevents the need for lung or lung-heart transplantation. Side effects include flushing, headache, hypotension, nausea, and diarrhea. The extremely short plasma half-life (3–5 minutes) of epoprostenol necessitates continuous intravenous infusion through a central line for long-term treatment, which is its greatest limitation. Several prostacyclin analogs with longer half-lives have been developed and used clinically. **Iloprost** (half-life about 30 minutes) is usually inhaled six to nine times per day, although it has been delivered by intravenous administration outside the USA. **Treprostinil** (half-life about 4 hours) may be delivered by subcutaneous or intravenous infusion.

B. Peripheral Vascular Disease

A number of studies have investigated the use of PGE_1 and PGI_2 compounds in Raynaud's phenomenon and peripheral arterial disease. However, these studies are mostly small and uncontrolled, and these therapies do not have an established place in the treatment of peripheral vascular disease.

C. Patent Ductus Arteriosus

Patency of the fetal ductus arteriosus depends on COX-2-derived PGE_2 acting on the EP_4 receptor. At birth, reduced PGE_2 levels, a consequence of increased PGE_2 metabolism, allow ductus arteriosus closure. In certain types of congenital heart disease (eg, transposition of the great arteries, pulmonary atresia, pulmonary artery stenosis), it is important to maintain the patency of the neonate's ductus arteriosus until corrective surgery can be carried out. This can be achieved with alprostadil (PGE_1). Like PGE_2, PGE_1 is a vasodilator and an inhibitor of platelet aggregation, and it contracts uterine and intestinal smooth muscle. Adverse effects include apnea, bradycardia, hypotension, and hyperpyrexia. Because of rapid pulmonary clearance (the half-life is about 5–10 minutes in healthy adults and neonates), the drug must be continuously infused at an initial rate of 0.05–0.1 mcg/kg/min, which may be increased to 0.4 mcg/kg/min. Prolonged treatment has been associated with ductal fragility and rupture.

In delayed closure of the ductus arteriosus, COX inhibitors are often used to inhibit synthesis of PGE_2 and so close the ductus. Premature infants in whom respiratory distress develops due to failure of ductus closure can be treated with a high degree of success with indomethacin. This treatment often precludes the need for surgical closure of the ductus.

Blood

As noted above, eicosanoids are involved in thrombosis because TXA_2 promotes platelet aggregation while PGI_2, and perhaps also

PGE_2 and PGD_2, are platelet antagonists. Chronic administration of low-dose aspirin (81 mg/d) selectively and irreversibly inhibits platelet COX-1 without modifying the activity of systemic COX-1 or COX-2 (see Chapter 34). Because their effects are reversible within the typical dosing interval, nonselective NSAIDs (eg, ibuprofen) do not reproduce this effect, although naproxen, because of its variably prolonged half-life, may provide antiplatelet benefit in some individuals. TXA_2, in addition to activating platelets, amplifies the response to other platelet agonists; hence inhibition of its synthesis inhibits secondary aggregation of platelets induced by adenosine diphosphate, by low concentrations of thrombin and collagen, and by epinephrine. Not surprisingly, selective COX-2 inhibitors do not alter platelet TXA_2 biosynthesis and are not platelet inhibitors. However, COX-2-derived PGI_2 generation is substantially suppressed during selective COX-2 inhibition, removing a restraint on the cardiovascular action of TXA_2, and other platelet agonists. It is highly likely that selective depression of PGI_2 generation contributes to the increased thrombotic events in humans treated with selective COX-2 inhibitors.

Overview analyses have shown that low-dose aspirin reduces the secondary incidence of heart attack and stroke by about 25%. However, low-dose aspirin also elevates the low risk of serious gastrointestinal bleeding about twofold over placebo. Low-dose aspirin also reduces the incidence of first myocardial infarction. However, in this case, the benefit versus risk of gastrointestinal bleeding is less clear. The effects of aspirin on platelet function are discussed in greater detail in Chapter 34.

Respiratory System

PGE_2 is a powerful bronchodilator when given in aerosol form. Unfortunately, it also promotes coughing, and an analog that possesses only the bronchodilator properties has been difficult to obtain.

$PGF_{2\alpha}$ and TXA_2 are both strong bronchoconstrictors and were once thought to be primary mediators in asthma. Polymorphisms in the genes for PGD_2 synthase, both DP receptors, and the TP receptor have been linked with asthma in humans. DP antagonists, particularly those directed against DP_2, are being investigated as potential treatments for allergic diseases including asthma. However, the cysteinyl leukotrienes—LTC_4, LTD_4, and LTE_4—probably dominate during asthmatic constriction of the airways. As described in Chapter 20, leukotriene-receptor inhibitors (eg, **zafirlukast, montelukast)** are effective in asthma. A lipoxygenase inhibitor (**zileuton**) has also been used in asthma but is not as popular as the receptor inhibitors. It remains unclear whether leukotrienes are partially responsible for acute respiratory distress syndrome.

Corticosteroids and cromolyn are also useful in asthma. Corticosteroids inhibit eicosanoid synthesis and thus limit the amounts of eicosanoid mediator available for release. Cromolyn appears to inhibit the release of eicosanoids and other mediators such as histamine and platelet-activating factor from mast cells.

Gastrointestinal System

The word "cytoprotection" was coined to signify the remarkable protective effect of the E prostaglandins against peptic ulcers in animals at doses that do not reduce acid secretion. Since then, numerous experimental and clinical investigations have shown that the PGE compounds and their analogs protect against peptic ulcers produced by either steroids or NSAIDs. **Misoprostol** is an orally active synthetic analog of PGE_1. The FDA-approved indication is for prevention of NSAID-induced peptic ulcers. The drug is administered at a dosage of 200 mcg four times daily with food. This and other PGE analogs (eg, enprostil) are cytoprotective at low doses and inhibit gastric acid secretion at higher doses. Misoprostol use is low, probably because of its adverse effects including abdominal discomfort and occasional diarrhea. Dose-dependent bone pain and hyperostosis have been described in patients with liver disease who were given long-term PGE treatment.

Selective COX-2 inhibitors were developed in an effort to spare gastric COX-1 so that the natural cytoprotection by locally synthesized PGE_2 and PGI_2 is undisturbed (see Chapter 36). However, this benefit is seen only with highly selective inhibitors and may be offset by increased cardiovascular toxicity.

Immune System

Cells of the immune system contribute substantially to eicosanoid biosynthesis during an immune reaction. T and B lymphocytes are not primary synthetic sources; however, they may supply arachidonic acid to monocyte-macrophages for eicosanoid synthesis. In addition, there is evidence for eicosanoid-mediated cell-cell interaction by platelets, erythrocytes, leukocytes, and endothelial cells.

The eicosanoids modulate the effects of the immune system. PGE_2 and PGI_2 limit T-lymphocyte proliferation in vitro, as do corticosteroids. PGE_2 also inhibits B-lymphocyte differentiation and the antigen-presenting function of myeloid-derived cells, suppressing the immune response. T-cell clonal expansion is attenuated through inhibition of interleukin-1 and interleukin-2 and class II antigen expression by macrophages or other antigen-presenting cells. The leukotrienes, TXA_2, and platelet-activating factor stimulate T-cell clonal expansion. These compounds stimulate the formation of interleukin-1 and interleukin-2 as well as the expression of interleukin-2 receptors. The leukotrienes also promote interferon-γ release and can replace interleukin-2 as a stimulator of interferon-γ. PGD_2 induces chemotaxis and migration of TH2 lymphocytes. These in vitro effects of the eicosanoids agree with in vivo findings in animals with acute organ transplant rejection, as described below.

A. Cell-Mediated Organ Transplant Rejection

Acute organ transplant rejection is a cell-mediated immune response (see Chapter 55). Administration of PGI_2 to renal transplant patients has reversed the rejection process in some cases. Experimental in vitro and in vivo data show that PGE_2 and PGI_2 can attenuate T-cell proliferation and rejection, which can also be seen with drugs that inhibit TXA_2 and leukotriene formation. In organ transplant patients, urinary excretion of metabolites of TXA_2 increases during acute rejection. Corticosteroids, the first-line drugs used for treatment of acute rejection because of their lymphotoxic effects, inhibit both phospholipase and COX-2 activity.

B. Inflammation

Aspirin has been used to treat arthritis of all types for approximately 100 years, but its mechanism of action—inhibition of COX activity—was not discovered until 1971. COX-2 appears to be the form of the enzyme most associated with cells involved in the inflammatory process, although, as outlined above, COX-1 also contributes significantly to prostaglandin biosynthesis during inflammation. Aspirin and other anti-inflammatory agents that inhibit COX are discussed in Chapter 36.

C. Rheumatoid Arthritis

In rheumatoid arthritis, immune complexes are deposited in the affected joints, causing an inflammatory response that is amplified by eicosanoids. Lymphocytes and macrophages accumulate in the synovium, whereas leukocytes localize mainly in the synovial fluid. The major eicosanoids produced by leukocytes are leukotrienes, which facilitate T-cell proliferation and act as chemoattractants. Human macrophages synthesize the COX products PGE_2 and TXA_2 and large amounts of leukotrienes.

D. Infection

The relationship of eicosanoids to infection is not well defined. The association between the use of the anti-inflammatory steroids and increased risk of infection is well established. However, NSAIDs do not seem to alter patient responses to infection.

Glaucoma

Latanoprost, a stable long-acting $PGF_{2\alpha}$ derivative, was the first prostanoid used for glaucoma. The success of latanoprost has stimulated development of similar prostanoids with ocular hypotensive effects, and **bimatoprost**, **travoprost,** and **unoprostone** are now available. These drugs act at the FP receptor and are administered as drops into the conjunctival sac once or twice daily. Adverse effects include irreversible brown pigmentation of the iris and eyelashes, drying of the eyes, and conjunctivitis.

DIETARY MANIPULATION OF ARACHIDONIC ACID METABOLISM

Because arachidonic acid is derived from dietary linoleic and α-linolenic acids, which are essential fatty acids, the effects of dietary manipulation on arachidonic acid metabolism have been extensively studied. Two approaches have been used. The first adds corn, safflower, and sunflower oils, which contain linoleic acid (C18:2), to the diet. The second approach adds oils containing eicosapentaenoic (C20:5) and docosahexaenoic acids (C22:6), so-called omega-3 fatty acids, from cold-water fish. Both types of diet change the phospholipid composition of cell membranes by replacing arachidonic acid with the dietary fatty acids. Diets high in fish oils have been shown to impact ex vivo indices of platelet and leukocyte function, blood pressure, and triglycerides with different dose-response relationships. There is an abundance of epidemiologic data relating diets high in fatty fish to a reduction in the incidence of myocardial infarction and sudden cardiac death although there is more ambiguity about stroke. Of course, epidemiologic data may confound such diets with a reduction in saturated fats and other elements of a "healthy" lifestyle. In addition, some data from prospective randomized trials suggest that such dietary interventions may reduce the incidence of sudden death. Experiments in vitro suggest that fish oils protect against experimentally induced arrhythmogenesis, platelet aggregation, vasomotor spasm, and dyslipidemias.

PREPARATIONS AVAILABLE

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ARE LISTED IN CHAPTER 36.

Alprostadil
Penile injection (Caverject, Edex): 5, 10, 20, 40 mcg sterile powder for reconstitution
Parenteral (Prostin VR Pediatric): 500 mcg/mL ampules

Bimatoprost (Lumigan)
Ophthalmic drops: 0.03% solution

Carboprost tromethamine (Hemabate)
Parenteral: 250 mcg carboprost and 83 mcg tromethamine per mL ampules

Dinoprostone [prostaglandin E_2](Prostin E2, Prepidil, Cervidil)
Vaginal: 20 mg suppositories, 0.5 mg gel, 10 mg controlled-release system

Epoprostenol [prostacyclin] (Flolan)
Intravenous: 0.5, 1.5 mg powder to reconstitute

Iloprost (Ventavis)
Inhalation: 10 mcg/mL solution

Latanoprost (Xalatan)
Topical: 0.005% ophthalmic solution

Misoprostol (generic, Cytotec)
Oral: 100 and 200 mcg tablets

Montelukast (Singulair)
Oral: 4, 5 mg chewable tablets, 10 mg tablets, 4 mg granules

Travoprost (Travatan)
Ophthalmic solution: 0.004%

Treprostinil (Remodulin)
Parenteral: 1, 2.5, 5, 10 mg/mL for intravenous infusion or subcutaneous

Zafirlukast (Accolate)
Oral: 10, 20 mg tablets

Zileuton (Zyflo)
Oral: 600 mg tablets

REFERENCES

Breyer RM et al: Prostanoid receptors: Subtypes and signaling. Annu Rev Pharmacol Toxicol 2001;41:661.

Brink C et al: International Union of Pharmacology XXXVII. Nomenclature for leukotriene and lipoxin receptors. Pharmacol Rev 2003;55:195.

Cheng HF, Harris RC: Cyclooxygenases, the kidney, and hypertension. Hypertension 2004;43:525.

Christin-Maitre S, Bouchard P, Spitz IM: Medical termination of pregnancy. N Engl J Med 2000;342:946.

Grosser T, Fries S, FitzGerald GA: Biological basis for the cardiovascular consequences of COX-2 inhibition: Therapeutic challenges and opportunities. J Clin Invest 2006;116:4.

Hao CM, Breyer MD: Physiological regulation of prostaglandins in the kidney. Annu Rev Physiol 2008;70:357.

Hata AN, Breyer RM: Pharmacology and signaling of prostaglandin receptors: Multiple roles in inflammation and immune modulation. Pharmacol Ther 2004;103:147.

Leonhardt A et al: Expression of prostanoid receptors in human ductus arteriosus. Br J Pharmacol 2003;138:655.

Martel-Pelletier J et al: Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. Ann Rheum Dis 2003;62:501.

Matsuoka T, Narumiya S: Prostaglandin receptor signaling in disease. Scientific World Journal 2007;7:1329.

Narumiya S, FitzGerald GA: Genetic and pharmacological analysis of prostanoid receptor function. J Clin Invest 2001;108:25.

Rokach J et al: Nomenclature of isoprostanes: A proposal. Prostaglandins 1997;54:853.

Smith WL, Dewitt DL, Garavito MR: Cyclooxygenases: Structural, cellular, and molecular biology. Annu Rev Biochem 2000;69:145.

Smyth EM, FitzGerald GA: Prostaglandin mediators. In: Bradshaw RD, Dennis EA (editors): *Handbook of Cell Signaling*. Elsevier, 2009.

Steiropoulos P, Trakada G, Bouros D: Current pharmacological treatment of pulmonary arterial hypertension. Curr Clin Pharmacol 2008;3:11.

Wang D, Dubois RN: Prostaglandins and cancer. Gut 2006;55:115.

Wang D, Mann JR, DuBois RN: The role of prostaglandins and other eicosanoids in the gastrointestinal tract. Gastroenterology 2005;128:1445.

This page intentionally left blank

CHAPTER 19

Nitric Oxide

Samie R. Jaffrey, MD, PhD

Nitric oxide (NO) is a gaseous signaling molecule that readily diffuses across cell membranes and regulates a wide range of physiologic and pathophysiologic processes including cardiovascular, inflammatory, and neuronal functions. Nitric oxide should not be confused with nitrous oxide (N_2O), an anesthetic gas, nor with nitrogen dioxide (NO_2), a toxic pulmonary irritant gas.

DISCOVERY OF ENDOGENOUSLY GENERATED NITRIC OXIDE

Because NO is an environmental pollutant, the finding that NO is synthesized by cells and activates specific intracellular signaling pathways was unexpected. The first indication that NO is generated in cells came from studies of cultured macrophages, which showed that treatment with inflammatory mediators, such as bacterial endotoxin, resulted in the production of nitrate and nitrite, molecules that are byproducts of NO breakdown. Similarly, injection of endotoxin in animals elevated urinary nitrite and nitrate.

The second indication came from studies of vascular regulation. Several molecules, such as acetylcholine, were known to cause relaxation of blood vessels. This effect occurred only when the vessels were prepared so that the luminal endothelial cells covering the smooth muscle of the vessel wall were retained. Subsequent studies showed that endothelial cells respond to these vasorelaxants by releasing a soluble **endothelial-derived relaxing factor (EDRF).** EDRF acts on vascular muscle to elicit relaxation. These findings prompted an intense search for the identity of EDRF.

At the same time, it was observed that exogenous application of NO or organic nitrates, which are metabolized to NO, elicit a variety of effects including inhibition of platelet aggregation and vasorelaxation. These effects were particularly intriguing, since they appeared to involve the activation of highly specific cellular responses, rather than more general cytotoxic responses. Comparison of the biochemical and pharmacological properties of EDRF and NO provided initial evidence that NO is the major bioactive component of EDRF. These findings also made it clear that exogenously applied NO and NO-releasing compounds (nitrates, nitrites, nitroprusside; see Chapters 11 and 12) elicited their effects by recruiting physiologic signaling pathways that normally mediate the actions of endogenously generated NO.

NITRIC OXIDE SYNTHESIS, SIGNALING MECHANISMS, & INACTIVATION

Synthesis

NO, written as $NO^{\bullet}$ to indicate an unpaired electron in its chemical structure, or simply NO, is a highly reactive signaling molecule that is made by any of three closely related NO synthase (NOS, EC 1.14.13.49) isoenzymes, each of which is encoded by a separate gene and named for the initial cell type from which it was isolated (Table 19–1). These enzymes, neuronal NOS (nNOS or NOS-1), macrophage or inducible NOS (iNOS or NOS-2), and endothelial NOS (eNOS or NOS-3), despite their names, are each expressed in a wide variety of cell types, often with an overlapping distribution. These isoforms generate NO from the amino acid L-arginine in an O_2- and NADPH-dependent reaction (Figure 19–1). This enzymatic reaction involves enzyme-bound cofactors, including heme, tetrahydrobiopterin, and flavin adenine dinucleotide (FAD). In the case of nNOS and eNOS, NO synthesis is triggered by agents and processes that increase cytosolic calcium concentrations. Cytosolic calcium forms complexes with calmodulin, an abundant calcium-binding protein, which then binds and activates eNOS and nNOS. On the other hand, iNOS is not regulated by calcium, but is constitutively active. In macrophages and several other cell types, inflammatory mediators induce the transcriptional activation of the iNOS gene, resulting in accumulation of iNOS and increased synthesis of NO.

Signaling Mechanisms

NO mediates its effects by covalent modification of proteins. There are three major targets of NO (Figure 19–1):

***1. Metalloproteins*—**NO interacts with metals, especially iron in heme. The major target of NO is soluble guanylyl cyclase (sGC),

TABLE 19–1 Properties of the three isoforms of nitric oxide synthase (NOS).

	Isoform Names		
Property	**NOS-1**	**NOS-2**	**NOS-3**
Other names	nNOS (neuronal NOS)	iNOS (inducible NOS)	eNOS (endothelial NOS)
Tissue	Neurons, skeletal muscle	Macrophages, smooth muscle cells	Endothelial cells, neurons
Expression	Constitutive	Transcriptional induction	Constitutive
Calcium regulation	Yes	No	Yes

a heme-containing enzyme that generates cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). NO binds to the heme in sGC, resulting in enzyme activation and elevation in intracellular cGMP levels. cGMP activates protein kinase G (PKG), which phosphorylates specific proteins. In blood vessels, NO-dependent elevations in cGMP and PKG activity result in the phosphorylation of proteins that lead to reduced cytosolic calcium levels and subsequently reduced contraction of vascular smooth muscle. Interaction of NO with other metalloproteins mediates some of the cytotoxic effects of NO associated with NO overproduction, eg, by activated macrophages. For example, NO inhibits metalloproteins involved in cellular respiration, such as the citric acid cycle enzyme aconitase and the electron transport chain protein cytochrome oxidase. Inhibition of heme-containing cytochrome P450 enzymes by NO is a major pathogenic mechanism in inflammatory liver disease.

***2. Thiols*—**NO reacts with thiols (compounds containing the –SH group) to form nitrosothiols. In proteins, the thiol moiety is found in the amino acid cysteine. This posttranslational modification, termed *S*-nitrosylation or *S*-nitrosation, requires either metals or O_2 to catalyze the formation of the nitrosothiol adduct. *S*-nitrosylation is highly specific, with only certain cysteine residues in proteins becoming *S*-nitrosylated. *S*-nitrosylation can alter the function, stability, or localization of target proteins. Although the physiologic roles of protein nitrosylation are not fully

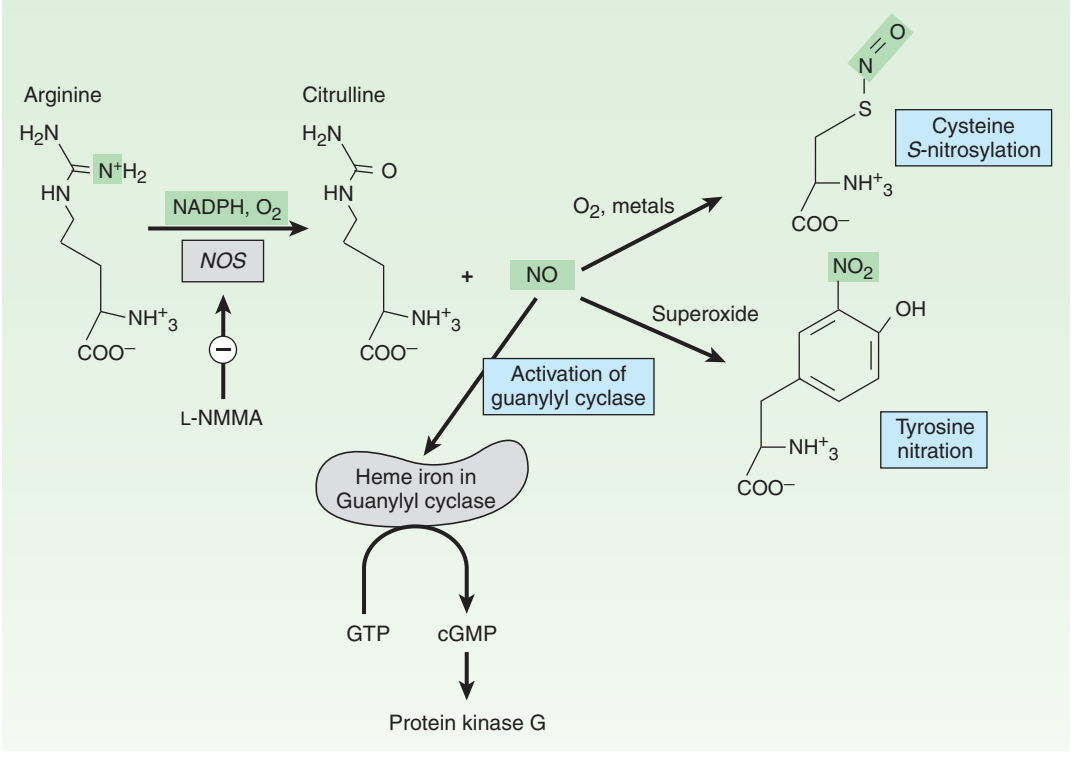


FIGURE 19–1 Synthesis and reactions of nitric oxide (NO). L-NMMA inhibits nitric oxide synthase. NO complexes with the iron in hemoproteins (eg, guanylyl cyclase), resulting in the activation of cyclic guanosine monophosphate (cGMP) synthesis and cGMP target proteins such as protein kinase G. Under conditions of oxidative stress, NO can react with superoxide to nitrate tyrosine. GTP, guanosine triphosphate.

TABLE 19–2 Oxides of nitrogen.

Name	Structure	Known Function
Nitric oxide (NO)	$N{=}O^{\bullet}$	Vasodilator, platelet inhibitor, immune regulator, neurotransmitter
Peroxynitrite (NO_3^-)	$O{=}N{-}O{-}O^-$	Oxidant and nitrating agent
Nitroxyl anion (NO^-)	$N^-{=}O$	Can form from nonspecific donation of an electron from metals to $NO^{\bullet}$ Exhibits NO-like effects, possibly by first being oxidized to NO
Nitrous oxide (N_2O)	$N^-{=}N^+{=}O$	Anesthetic
Dinitrogen trioxide (N_2O_3)	$O{=}N{-}N^+{=}O$ with O^-	Auto-oxidation product of NO that can nitrosylate protein thiols
Nitrite (NO_2^-)	$O{=}N{=}O^-$	Stable oxidation product of $NO^{\bullet}$ Slowly metabolized to nitrosothiols, and decomposes to NO at acidic pH
Nitrate (NO_3^-)	$O{=}N^+{-}O^-$ with O^-	Stable oxidation product of $NO^{\bullet}$

established, major targets of *S*-nitrosylation are H-ras, a regulator of cell proliferation that is activated by *S*-nitrosylation, and the metabolic enzyme glyceraldehyde-3-phosphate dehydrogenase, which is inhibited when it is *S*-nitrosylated. Denitrosylation of proteins is poorly understood but may involve enzymes, such as thioredoxin, or chemical reduction by intracellular reducing agents such as glutathione, an abundant intracellular sulfhydryl-containing compound. Glutathione can also be *S*-nitrosylated under physiologic conditions to generate *S*-nitrosoglutathione. *S*-nitrosoglutathione may serve as an endogenous stabilized form of NO or as a carrier of NO. Vascular glutathione is decreased in diabetes mellitus and atherosclerosis, and the resulting deficiency of *S*-nitrosoglutathione may account for the increased incidence of cardiovascular complications in these conditions.

***3. Tyrosine nitration*—**NO undergoes both oxidative and reductive reactions, resulting in a variety of oxides of nitrogen that can nitrosylate thiols and nitrate tyrosines (described below) or are stable oxidation products (Table 19–2). NO reacts very efficiently with superoxide to form peroxynitrite ($ONOO^-$), a highly reactive oxidant that leads to DNA damage, nitration of tyrosine, and oxidation of cysteine to disulfides or to various sulfur oxides (SO_x). Several cellular enzymes synthesize superoxide, and the activity of these enzymes, as well as NO synthesis, is increased in numerous inflammatory and degenerative diseases, resulting in an increase in peroxynitrite levels. Numerous proteins are susceptible to peroxynitrite-catalyzed tyrosine nitration, and this irreversible modification can be associated with either activation or inhibition of protein function. Detection of tyrosine nitration in tissue is often used as a marker of oxidative stress and tissue damage, although a direct causal role of tyrosine nitration in the pathogenesis of any disease has not been definitively established. Peroxynitrite-mediated protein modification is mitigated by intracellular levels of glutathione, which can protect against tissue damage by scavenging peroxynitrite. Factors that regulate the biosynthesis and decomposition of glutathione may have important consequences on the toxicity of NO.

Inactivation

NO is highly labile due to its rapid reaction with metals, O_2, and reactive oxygen species. NO can react with heme and hemoproteins, including oxyhemoglobin, which oxidizes NO to nitrate. The reaction of NO with hemoglobin may also lead to *S*-nitrosylation of hemoglobin, resulting in transport of NO throughout the vasculature. NO is also inactivated by reaction with O_2 to form nitrogen dioxide. NO reacts with superoxide, which results in the formation of the highly reactive oxidizing species, peroxynitrite. Scavengers of superoxide anion such as superoxide dismutase may protect NO, enhancing its potency and prolonging its duration of action.

■ PHARMACOLOGIC MANIPULATION OF NITRIC OXIDE

Inhibitors of Nitric Oxide Synthesis

The primary strategy to reduce NO generation in cells is to use NOS inhibitors. The majority of these inhibitors are arginine analogs that bind to the NOS arginine-binding site. Since each of the NOS isoforms has high structural similarity, most of these inhibitors do not exhibit selectivity for individual NOS isoforms. In inflammatory disorders and sepsis (see below), inhibition of the iNOS isoform is potentially beneficial, whereas nNOS-specific inhibitors may be useful for the treatment of neurodegenerative conditions. However, administration of nonselective NOS inhibitors leads to concurrent inhibition of eNOS, which impairs its homeostatic signaling and also results in vasoconstriction and

potential ischemic damage. Thus, NOS isoform-selective inhibitors are being designed that exploit subtle differences in substrate binding sites between the isoforms, as well as newer isoform-selective inhibitors that prevent NOS dimerization, the conformation required for enzymatic activity. The efficacy of NOS isoform-selective inhibitors in medical conditions is under investigation.

Nitric Oxide Donors

NO donors, which release NO or related NO species, are used to elicit smooth muscle relaxation. Different classes of NO donors have differing biologic properties, depending on the nature of the NO species released and the mechanism that is responsible for its release.

1. Organic nitrates—Nitroglycerin, which dilates veins and coronary arteries, is metabolized to NO by mitochondrial aldehyde reductase, an enzyme enriched in venous smooth muscle, accounting for the potent venodilating activity of this molecule. Venous dilation decreases cardiac preload, which along with coronary artery dilation accounts for the antianginal effects of nitroglycerin. Other organic nitrates, such as isosorbide dinitrate, are metabolized to an NO-releasing species through a poorly understood enzymatic pathway. Unlike NO, organic nitrates have less significant effects on aggregation of platelets, which appear to lack the enzymatic pathways necessary for rapid metabolic activation. Organic nitrates exhibit rapid tolerance during continuous administration. This nitrate tolerance may derive from the generation of reactive oxygen species that inhibit mitochondrial aldehyde reductase, endogenous NO synthesis, and other pathways (see Chapter 12).

2. Organic nitrites—Organic nitrites, such as the antianginal inhalant amyl nitrite, also require metabolic activation to elicit vasorelaxation, although the responsible enzyme has not been identified. Nitrites are arterial vasodilators and do not exhibit the rapid tolerance seen with nitrates. Amyl nitrite is abused for euphoric effects and combining it with phosphodiesterase inhibitors, such as sildenafil, can cause lethal hypotension. Amyl nitrite has been largely replaced by nitrates, such as nitroglycerin, which are more easily administered.

3. Sodium nitroprusside—Sodium nitroprusside, which dilates arterioles and venules, is used for rapid pressure reduction in arterial hypertension. In response to light as well as chemical or enzymatic mechanisms in cell membranes, sodium nitroprusside breaks down to generate five cyanide molecules and a single NO. See Chapter 11 for additional details.

4. NO gas inhalation—NO itself can be used therapeutically. Inhalation of NO results in reduced pulmonary artery pressure and improved perfusion of ventilated areas of the lung. Inhaled NO is used for pulmonary hypertension, acute hypoxemia, and cardiopulmonary resuscitation, and there is evidence of short-term improvements in pulmonary function. Inhaled NO is stored as a compressed gas mixture with nitrogen, which does not readily react with NO, and further diluted to the desired concentration upon administration. NO can react with O_2 to form nitrogen dioxide, a pulmonary irritant that can cause deterioration of lung function (see Chapter 56). Additionally, NO can induce the formation of methemoglobin, a form of hemoglobin containing Fe^{3+} rather than Fe^{2+}, which does not bind O_2 (see also Chapter 12). Therefore, nitrogen dioxide and methemoglobin levels are monitored during inhaled NO treatment.

5. Alternate strategies—Another mechanism to potentiate the actions of NO is to inhibit the phosphodiesterase enzymes that degrade cGMP. Inhibitors of type 5 phosphodiesterase such as sildenafil result in prolongation of the duration of NO-induced cGMP elevations in a variety of tissues (see Chapter 12).

NITRIC OXIDE IN DISEASE

VASCULAR EFFECTS

NO has a significant effect on vascular smooth muscle tone and blood pressure. Numerous endothelium-dependent vasodilators, such as acetylcholine and bradykinin, act by increasing intracellular calcium levels in endothelial cells, leading to the synthesis of NO. NO diffuses to vascular smooth muscle leading to vasorelaxation (Figure 19–2). Mice with a knockout mutation in the eNOS gene display increased vascular tone and elevated mean arterial pressure, indicating that eNOS is a fundamental regulator of blood pressure.

Apart from being a vasodilator and regulating blood pressure, NO also has antithrombotic effects. Both endothelial cells and platelets contain eNOS, which acts via an NO-cGMP pathway to inhibit platelet activation, an initiator of thrombus formation. Thus, in diseases associated with endothelial dysfunction, the associated decrease in NO generation leads to an increased propensity for abnormal platelet function and thrombosis. NO may have an additional inhibitory effect on blood coagulation by enhancing fibrinolysis via an effect on plasminogen.

NO also protects against atherogenesis. A major antiatherogenic mechanism of NO involves the inhibition of proliferation and migration of vascular smooth muscle cells. In animal models, myointimal proliferation following angioplasty can be blocked by NO donors, by NOS gene transfer, and by NO inhalation. NO reduces endothelial adhesion of monocytes and leukocytes, which are early steps in the development of atheromatous plaques. This effect is due to the inhibitory effect of NO on the expression of adhesion molecules on the endothelial surface. In addition, NO may act as an antioxidant, blocking the oxidation of low-density lipoproteins and thus preventing or reducing the formation of foam cells in the vascular wall. Plaque formation is also affected by NO-dependent reduction in endothelial cell permeability to lipoproteins. The importance of eNOS in cardiovascular disease is supported by experiments showing increased atherosclerosis in animals treated with eNOS inhibitors. Atherosclerosis risk factors,

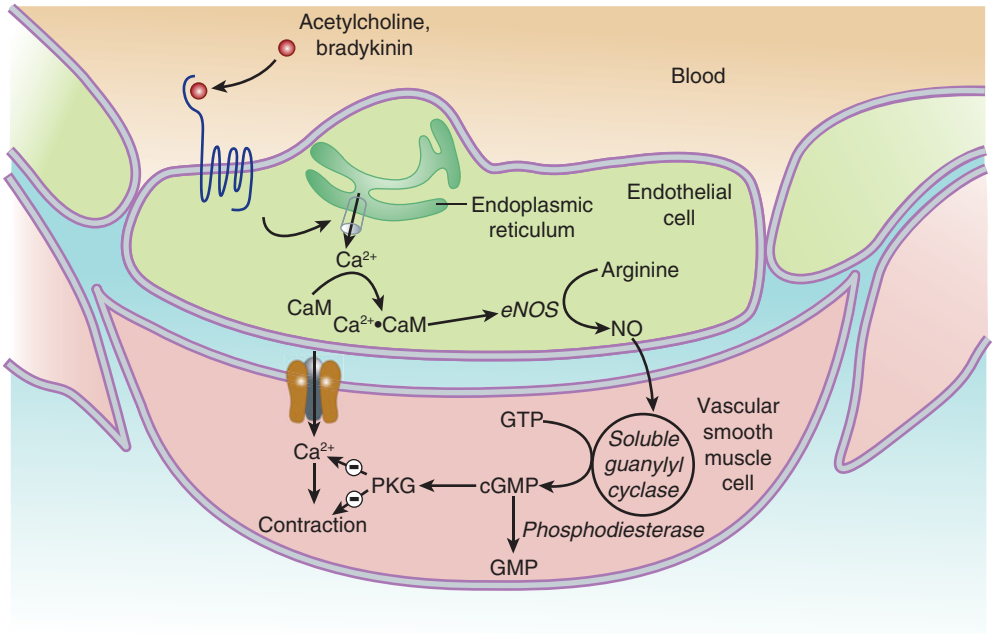


FIGURE 19–2 Regulation of vasorelaxation by endothelial-derived nitric oxide (NO). Endogenous vasodilators, eg, acetylcholine and bradykinin, activate NO synthesis in the luminal endothelial cells, leading to calcium (Ca^{2+}) efflux from the endoplasmic reticulum into the cytoplasm. Calcium binds to calmodulin (CaM), which activates endothelial NO synthase (eNOS), resulting in NO synthesis from L-arginine. NO diffuses into smooth muscle cells, where it activates soluble guanylyl cyclase and cyclic guanosine monophosphate (cGMP) synthesis from guanosine triphosphate (GTP). cGMP binds and activates protein kinase G (PKG), resulting in an overall reduction in calcium influx, and inhibition of calcium-dependent muscle contraction. PKG can also block other pathways that lead to muscle contraction. cGMP signaling is terminated by phosphodiesterases, which convert cGMP to GMP.

such as smoking, hyperlipidemia, diabetes, and hypertension, are associated with decreased endothelial NO production, and thus enhance atherogenesis.

SEPTIC SHOCK

Sepsis is a systemic inflammatory response caused by infection. Endotoxin components from the bacterial wall along with endogenously generated tumor necrosis factor-α and other cytokines induce synthesis of iNOS in macrophages, neutrophils, and T cells, as well as hepatocytes, smooth muscle cells, endothelial cells, and fibroblasts. This widespread generation of NO results in exaggerated hypotension, shock, and, in some cases, death. This hypotension is reduced or reversed by NOS inhibitors in humans as well as in animal models (Table 19–3). A similar reversal of hypotension is produced by compounds that prevent the action of NO, such as the sGC inhibitor methylene blue. Furthermore, knockout mice lacking a functional iNOS gene are more resistant to endotoxin than wild-type mice. However, despite the ability of NOS inhibitors to ameliorate hypotension in sepsis, there is no overall improvement in survival in patients with gram-negative sepsis treated with NOS inhibitors. The absence of benefit may reflect the inability of the NOS inhibitors used in these trials to differentiate between NOS isoforms, or may reflect concurrent inhibition of beneficial aspects of iNOS signaling.

INFECTION & INFLAMMATION

The generation of NO has both beneficial and detrimental roles in the host immune response and in inflammation. The host response to infection or injury involves the recruitment of leukocytes and the release of inflammatory mediators, such as tumor

TABLE 19–3 Some inhibitors of nitric oxide synthesis or action.

Inhibitor	Mechanism	Comment
N^{ω}-Monomethyl-L-arginine (L-NMMA)	Competitive inhibitor, binds arginine-binding site in NOS	Nonselective NOS inhibitor
N^{ω}-Nitro-L-arginine methyl ester (L-NAME)	Competitive inhibitor, binds arginine-binding site in NOS	Nonselective NOS inhibitor
7-Nitroindazole	Competitive inhibitor, binds both tetrahydrobiopterin and arginine-binding sites in NOS	Partially selective for NOS-1 in vivo
BBS-2	Inhibits iNOS dimerization	Also weakly inhibits nNOS and eNOS
Hemoglobin	NO scavenger	

NOS, nitric oxide SYNTHASE; BBS-2, a pyrimide imidazole.

necrosis factor and interleukin-1. This leads to induction of iNOS in leukocytes, fibroblasts, and other cell types. The NO that is produced, along with peroxynitrite that forms from its interaction with superoxide, is an important microbicide. NO also appears to play an important protective role in the body via immune cell function. When challenged with foreign antigens, TH1 cells (see Chapter 55) respond by synthesizing NO, which has roles in TH1 cells. The importance of NO in TH1 cell function is demonstrated by the impaired protective response to injected parasites in animal models after inhibition of iNOS. NO also stimulates the synthesis of inflammatory prostaglandins by activating cyclooxygenase isoenzyme 2 (COX-2). Through its effects on COX-2, its direct vasodilatory effects, and other mechanisms, NO generated during inflammation contributes to the erythema, vascular permeability, and subsequent edema associated with acute inflammation.

However, in both acute and chronic inflammatory conditions, prolonged or excessive NO production may exacerbate tissue injury. Indeed, psoriasis lesions, airway epithelium in asthma, and inflammatory bowel lesions in humans all demonstrate elevated levels of NO and iNOS, suggesting that persistent iNOS induction may contribute to disease pathogenesis. Moreover, these tissues also exhibit increased levels of nitrotyrosine, indicating excessive formation of peroxynitrite. In several animal models of arthritis, increasing NO production by dietary L-arginine supplementation exacerbates arthritis, whereas protection is seen with iNOS inhibitors. Thus, inhibition of the NO pathway may have a beneficial effect on a variety of acute and chronic inflammatory diseases.

THE CENTRAL NERVOUS SYSTEM

NO has an important role in the central nervous system as a neurotransmitter (see Chapter 21). Unlike classic transmitters such as glutamate or dopamine, which are stored in synaptic vesicles and released in the synaptic cleft upon vesicle fusion, NO is not stored, but rather is synthesized on demand and immediately diffuses to neighboring cells. NO synthesis is induced at postsynaptic sites in neurons, most commonly upon activation of the NMDA subtype of glutamate receptor, which results in calcium influx and activation of nNOS. In several neuronal subtypes, eNOS is also present and activated by neurotransmitter pathways that lead to calcium influx. NO synthesized postsynaptically may function as a retrograde messenger and diffuse to the presynaptic terminal to enhance the efficiency of neurotransmitter release, thereby regulating synaptic plasticity, the process of synapse strengthening that underlies learning and memory. Because aberrant NMDA receptor activation and excessive NO synthesis is linked to excitotoxic neuronal death in several neurologic diseases, including stroke, amyotrophic lateral sclerosis, and Parkinson's disease, therapy with NOS inhibitors may reduce neuronal damage in these conditions. However, clinical trials have not clearly supported the benefit of NOS inhibition, which may reflect nonselectivity of the inhibitors, resulting in inhibition of the beneficial effects of eNOS.

THE PERIPHERAL NERVOUS SYSTEM

Nonadrenergic, noncholinergic (NANC) neurons are widely distributed in peripheral tissues, especially the gastrointestinal and reproductive tracts (see Chapter 6). Considerable evidence implicates NO as a mediator of certain NANC actions, and some NANC neurons appear to release NO. Penile erection is thought to be caused by the release of NO from NANC neurons; NO promotes relaxation of the smooth muscle in the corpora cavernosa—the initiating factor in penile erection—and inhibitors of NOS have been shown to prevent erection caused by pelvic nerve stimulation in the rat. An established approach in treating erectile dysfunction is to enhance the effect of NO signaling by inhibiting the breakdown of cGMP by the phosphodiesterase (PDE isoform 5) present in the smooth muscle of the corpora cavernosa with drugs such as sildenafil, tadalafil, and vardenafil (see Chapter 12).

RESPIRATORY DISORDERS

NO is administered by inhalation (see Preparations Available) to newborns with hypoxic respiratory failure associated with pulmonary hypertension. The current treatment for severely defective gas exchange in the newborn is with extracorporeal membrane oxygenation (ECMO), which does not directly affect pulmonary vascular pressures. NO inhalation dilates pulmonary vessels, resulting in decreased pulmonary vascular resistance and reduced pulmonary artery pressure. Inhaled NO also improves oxygenation by reducing mismatch of ventilation and perfusion in the lung. Inhalation of NO results in dilation of pulmonary vessels in areas of the lung with better ventilation, thereby redistributing pulmonary blood flow away from poorly ventilated areas. NO inhalation does not typically exert pronounced effects on the systemic circulation. Inhaled NO has also been shown to improve cardiopulmonary function in adult patients with pulmonary artery hypertension.

An additional approach for treating pulmonary hypertension is to potentiate the actions of NO in pulmonary vascular beds. Due to the enrichment of PDE-5 in pulmonary vascular beds, PDE-5 inhibitors such as sildenafil and tadalafil induce vasodilation and marked reductions in pulmonary hypertension (see also Chapter 12).

SUMMARY Nitric Oxide

Subclass	Mechanism of Action	Effects	Clinical Applications	Pharmacokinetics, Toxicity, Interactions
NITRIC OXIDE (NO)				
	NO activates soluble guanylyl cyclase to elevate cGMP levels in vascular smooth muscle	Vasodilator • relaxes other smooth muscle • inhalation of NO leads to increased blood flow to parts of the lung exposed to NO and decreased pulmonary vascular resistance	Hypoxic respiratory failure and pulmonary hypertension	Inhaled gas • *Toxicity:* Methemoglobinemia

PREPARATIONS AVAILABLE

Nitric Oxide (INOmax)
Inhalation: 100, 800 ppm gas

REFERENCES

Chen Z, Stamler JS: Bioactivation of nitroglycerin by the mitochondrial aldehyde dehydrogenase. Trends Cardiovasc Med 2006;16:259.

Griffiths MJ, Evans TW: Inhaled nitric oxide therapy in adults. N Engl J Med 2005;353:2683.

Guix FX et al: The physiology and pathophysiology of nitric oxide in the brain. Prog Neurobiol 2005;76:126.

McMillan K et al: Allosteric inhibitors of inducible nitric oxide synthase dimerization discovered via combinatorial chemistry. Proc Nat Acad Sci USA 2000;97:1506.

Moncada S, Higgs EA: The discovery of nitric oxide and its role in vascular biology. Br J Pharmacol 2006;147:S193.

Napoli C, Ignarro LJ: Nitric oxide-releasing drugs. Annu Rev Pharmacol Toxicol 2003;43:97.

Paige JS, Jaffrey, SR: Pharmacologic manipulation of nitric oxide signaling: Targeting NOS dimerization and protein-protein interactions. Curr Topics Med Chem 2007;7:97.

Ramani GV, Park MH: Update on the clinical utility of sildenafil in the treatment of pulmonary arterial hypertension. Drug Des Devel Ther 2010;4:61.

Wimalawansa SJ: Nitric oxide: New evidence for novel therapeutic indications. Expert Opin Pharmacother 2008;9:1935.

This page intentionally left blank

CHAPTER 20

Drugs Used in Asthma

Homer A. Boushey, MD

CASE STUDY

A 10-year-old girl with a history of poorly controlled asthma presents to the emergency department with severe shortness of breath and audible inspiratory and expiratory wheezing. She is pale, refuses to lie down, and appears extremely frightened. Her pulse is 120 bpm and respirations 32/min. Her mother states that the girl has just recovered from a mild case of flu and had seemed comfortable until this afternoon. The girl uses an inhaler (albuterol) but "only when really needed" because her parents are afraid that she will become too dependent on medication. She administered two puffs from her inhaler just before coming to the hospital, but "the inhaler doesn't seem to have helped." What emergency measures are indicated? How should her long-term management be altered?

Asthma is characterized clinically by recurrent bouts of shortness of breath, chest tightness, and wheezing, often associated with coughing; physiologically by widespread, reversible narrowing of the bronchial airways and a marked increase in bronchial responsiveness to inhaled stimuli; and pathologically by lymphocytic, eosinophilic inflammation of the bronchial mucosa. It is also characterized pathologically by "remodeling" of the bronchial mucosa, with thickening of the lamina reticularis beneath the airway epithelium and hyperplasia of the cells of all structural elements of the airway wall—vessels, smooth muscle, and secretory glands and goblet cells.

In mild asthma, symptoms occur only occasionally, as on exposure to allergens or certain pollutants, on exercise, or after viral upper respiratory infection. More severe forms of asthma are associated with frequent attacks of wheezing dyspnea, especially at night, or with chronic airway narrowing, causing chronic respiratory impairment. These consequences of asthma are regarded as largely preventable, because effective treatments for relief of acute bronchoconstriction ("short-term relievers") and for reduction in symptoms and prevention of attacks ("long-term controllers") are available. The persistence of high medical costs for asthma care, driven largely by the costs of emergency department or hospital treatment of asthma exacerbations, are believed to reflect underutilization of the treatments available.

The causes of airway narrowing in acute asthmatic attacks, or "asthma exacerbations," include contraction of airway smooth muscle, inspissation of viscid mucus plugs in the airway lumen, and thickening of the bronchial mucosa from edema, cellular infiltration, and hyperplasia of secretory, vascular, and smooth muscle cells. Of these causes of airway obstruction, contraction of smooth muscle is most easily reversed by current therapy; reversal of the edema and cellular infiltration requires sustained treatment with anti-inflammatory agents.

Short-term relief is thus most effectively achieved by agents that relax airway smooth muscle, of which β-adrenoceptor stimulants (see Chapter 9) are the most effective and most widely used. Theophylline, a methylxanthine drug, and antimuscarinic agents (see Chapter 8) are sometimes also used for reversal of airway constriction.

Long-term control is most effectively achieved with an anti-inflammatory agent such as an inhaled corticosteroid. It can also be achieved, though less effectively, with a leukotriene pathway antagonist or an inhibitor of mast cell degranulation, such as cromolyn or nedocromil. Finally, clinical trials have established the efficacy of treatment for severe asthma with a humanized monoclonal antibody, omalizumab, which is specifically targeted against IgE, the antibody responsible for allergic sensitization.

The distinction between "short-term relievers" and "long-term controllers" is blurred. Inhaled corticosteroids, regarded as long-term controllers, produce modest immediate bronchodilation. Theophylline, regarded as a bronchodilator, inhibits some lymphocyte functions and modestly reduces airway mucosal inflammation. Theophylline may also enhance the anti-inflammatory action of inhaled corticosteroids. This is also true of long-acting β-adrenoceptor

stimulants, like salmeterol and formoterol, which are effective in improving asthma control when added to inhaled corticosteroid treatment, though neither is anti-inflammatory when taken as a single agent.

This chapter presents the basic pharmacology of the methylxanthines, cromolyn, leukotriene pathway inhibitors, and monoclonal anti-IgE antibody—agents whose medical use is almost exclusively for pulmonary disease. The other classes of drugs listed above are discussed in relation to the therapy of asthma.

PATHOGENESIS OF ASTHMA

The classic immunologic model of asthma presents it as a disease mediated by reaginic immune globulin (IgE). Foreign materials that provoke IgE production are described as "allergens"; the most common are proteins from house dust mite, cockroach, animal danders, molds, and pollens. The tendency to produce IgE antibodies is genetically determined; asthma and other allergic diseases cluster in families. Once produced, IgE antibodies bind to mast cells in the airway mucosa (Figure 20–1). On reexposure to a specific allergen, antigen-antibody interaction on the surface of the mast cells triggers both the release of mediators stored in the cells' granules and the synthesis and release of other mediators. The histamine, tryptase, leukotrienes C_4 and D_4, and prostaglandin D_2 that are released diffuse through the airway mucosa, triggering the muscle contraction and vascular leakage responsible for the acute bronchoconstriction of the "early asthmatic response." This response is often followed in 3–6 hours by

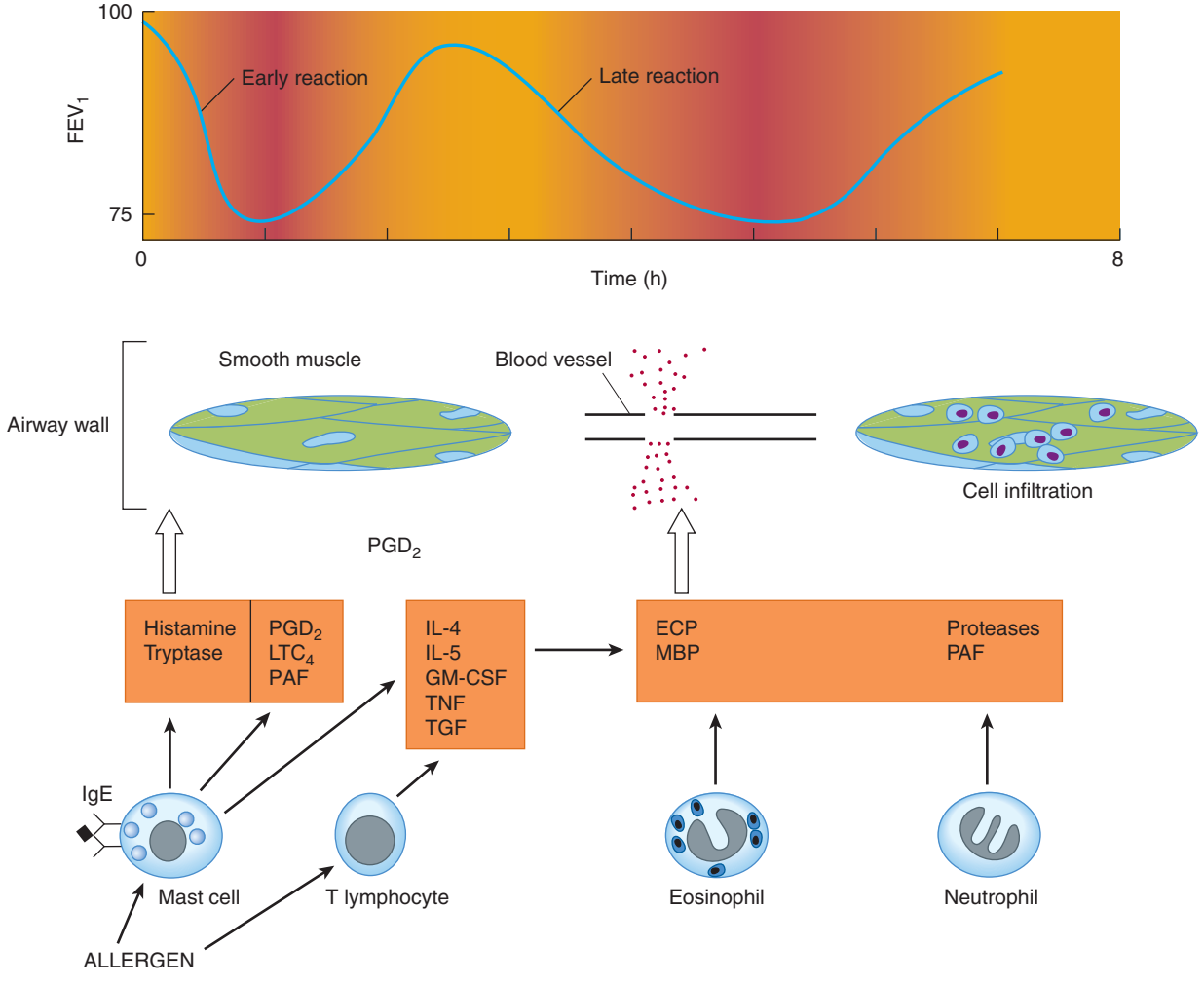


FIGURE 20–1 Conceptual model for the immunopathogenesis of asthma. Exposure to allergen causes synthesis of IgE, which binds to mast cells in the airway mucosa. On reexposure to allergen, antigen-antibody interaction on mast cell surfaces triggers release of mediators of anaphylaxis: histamine, tryptase, prostaglandin D_2 (PGD_2), leukotriene C_4, and platelet-activating factor (PAF). These agents provoke contraction of airway smooth muscle, causing the immediate fall in FEV_1. Reexposure to allergen also causes the synthesis and release of a variety of cytokines: interleukins 4 and 5, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF), and tissue growth factor (TGF) from T cells and mast cells. These cytokines in turn attract and activate eosinophils and neutrophils, whose products include eosinophil cationic protein (ECP), major basic protein (MBP), proteases, and platelet-activating factor. These mediators cause the edema, mucus hypersecretion, smooth muscle contraction, and increase in bronchial reactivity associated with the late asthmatic response, indicated by a second fall in FEV_1 3-6 hours after the exposure.

a second, more sustained phase of bronchoconstriction, the "late asthmatic response," which is associated with an influx of inflammatory cells into the bronchial mucosa and with an increase in bronchial reactivity that may last for several weeks after a single inhalation of allergen. The mediators responsible for this late response are thought to be cytokines characteristically produced by TH2 lymphocytes, especially interleukins 5, 9, and 13. These cytokines are thought to attract and activate eosinophils, stimulate IgE production by B lymphocytes, and stimulate mucus production by bronchial epithelial cells. It is not clear whether lymphocytes or mast cells in the airway mucosa are the primary source of the mediators responsible for the late inflammatory response, but the benefits of corticosteroid therapy are attributed to their inhibition of the production of pro-inflammatory cytokines in the airways.

The allergen challenge model does not reproduce all the features of asthma. Most asthma attacks are not triggered by inhalation of allergens. They are triggered by viral respiratory infection. Some adults with asthma have no evidence of allergic sensitivity to allergens, and even in people with allergic sensitivity, the severity of symptoms correlates poorly with levels of allergen in the atmosphere. Moreover, bronchospasm can be provoked by nonallergenic stimuli such as distilled water, exercise, cold air, sulfur dioxide, and rapid respiratory maneuvers.

This tendency to develop bronchospasm on encountering stimuli that do not affect healthy nonasthmatic airways is characteristic of asthma and is sometimes called "nonspecific bronchial hyperreactivity" to distinguish it from bronchial responsiveness to specific antigens. Bronchial reactivity is assessed by measuring the fall in forced expiratory volume in 1 second (FEV_1) provoked by inhaling serially increasing concentrations of aerosolized methacholine. The exaggerated reactivity of the airways appears to be fundamental to asthma's pathogenesis, because it is nearly ubiquitous in patients with asthma and its degree roughly correlates with the clinical severity of the disease.

The mechanisms underlying bronchial hyperreactivity are somehow related to inflammation of the airway mucosa. The agents that increase bronchial reactivity, such as ozone exposure, allergen inhalation, and infection with respiratory viruses, also cause airway inflammation. The increase in reactivity due to allergen inhalation is associated with an increase in both eosinophils and polymorphonuclear leukocytes in bronchial lavage fluid. The increase in reactivity that is associated with the late asthmatic response to allergen inhalation (Figure 20–1) is sustained and, because it is prevented by treatment with an inhaled corticosteroid, is thought to be caused by airway inflammation.

Whatever the mechanisms responsible for bronchial hyperreactivity, bronchoconstriction itself seems to result not simply from the direct effect of the released mediators but also from their activation of neural or humoral pathways. Evidence for the importance of neural pathways stems largely from studies of laboratory animals. The bronchospasm provoked in dogs by inhalation of histamine is reduced by pretreatment with an inhaled topical anesthetic agent, by transection of the vagus nerves, and by pretreatment with atropine. Studies of asthmatic humans, however, have shown that treatment with atropine causes only a reduction in—not abolition of—the bronchospastic responses to antigens and to nonantigenic stimuli. It is possible that activity in another neural pathway, such as the nonadrenergic, noncholinergic system, contributes to bronchomotor responses to stimuli (Figure 20–2).

The hypothesis suggested by these studies—that asthmatic bronchospasm results from a combination of release of mediators and an exaggeration of responsiveness to their effects—predicts that asthma may be effectively treated by drugs with different modes of action. Asthmatic bronchospasm might be reversed or prevented, for example, by drugs that reduce the amount of IgE bound to mast cells (anti-IgE antibody), prevent mast cell degranulation (cromolyn or nedocromil, sympathomimetic agents, calcium channel blockers), block the action of the products released (antihistamines and leukotriene receptor antagonists), inhibit the effect of acetylcholine released from vagal motor nerves (muscarinic antagonists), or directly relax airway smooth muscle (sympathomimetic agents, theophylline).

The second approach to the treatment of asthma is aimed not only at preventing or reversing acute bronchospasm but at reducing the level of bronchial responsiveness. Because increased responsiveness appears to be linked to airway inflammation and because airway inflammation is a feature of late asthmatic responses, this strategy is implemented both by reducing exposure to the allergens that provoke inflammation and by prolonged therapy with anti-inflammatory agents, especially inhaled corticosteroids.

BASIC PHARMACOLOGY OF AGENTS USED IN THE TREATMENT OF ASTHMA

The drugs most used for management of asthma are adrenoceptor agonists, or sympathomimetic agents (used as "relievers" or bronchodilators) and inhaled corticosteroids (used as "controllers" or anti-inflammatory agents). Their basic pharmacology is presented elsewhere (see Chapters 9 and 39). In this chapter, we review their pharmacology relevant to asthma.

SYMPATHOMIMETIC AGENTS

The adrenoceptor agonists have several pharmacologic actions that are important in the treatment of asthma. They relax airway smooth muscle and inhibit release of bronchoconstricting mediators from mast cells. They may also inhibit microvascular leakage and increase mucociliary transport by increasing ciliary activity. As in other tissues, the β agonists stimulate adenylyl cyclase and increase the formation of intracellular cAMP (Figure 20–3).

The best-characterized action of the adrenoceptor agonists in the airways is relaxation of airway smooth muscle. Although there is no evidence for direct sympathetic innervation of human airway smooth muscle, ample evidence exists for the presence of adrenoceptors on airway smooth muscle. In general, stimulation of β_2

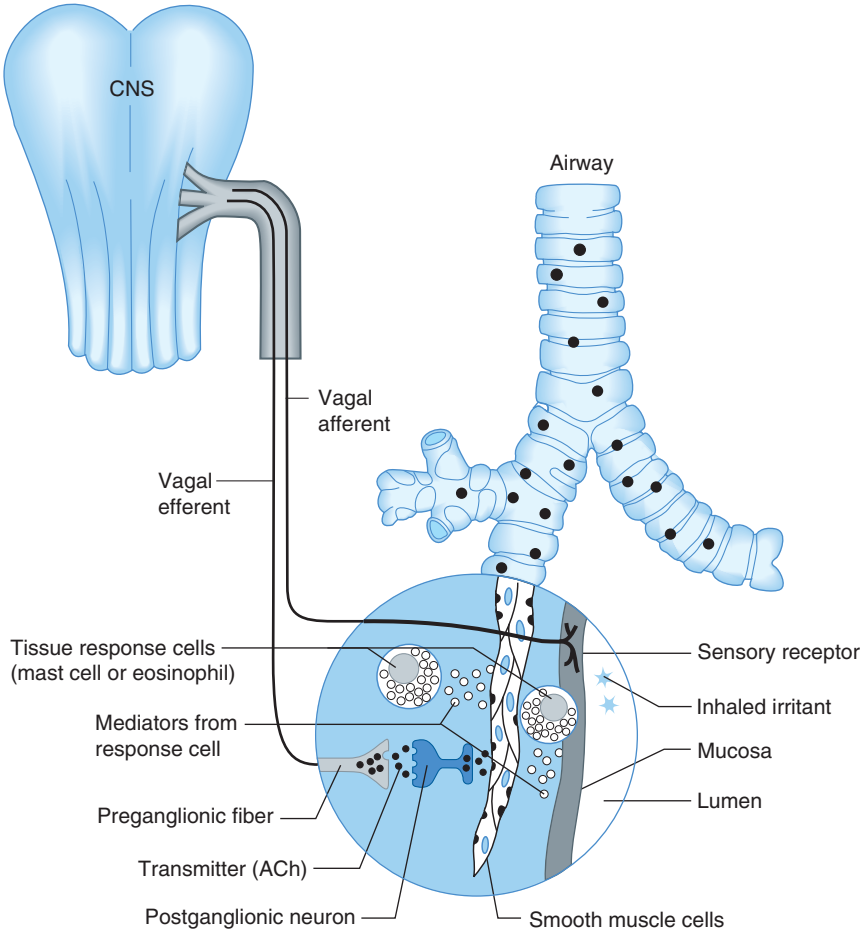


FIGURE 20–2 Mechanisms of response to inhaled irritants. The airway is represented microscopically by a cross-section of the wall with branching vagal sensory endings lying adjacent to the lumen. Afferent pathways in the vagus nerves travel to the central nervous system; efferent pathways from the central nervous system travel to efferent ganglia. Postganglionic fibers release acetylcholine (ACh), which binds to muscarinic receptors on airway smooth muscle. Inhaled materials may provoke bronchoconstriction by several possible mechanisms. First, they may trigger the release of chemical mediators from mast cells. Second, they may stimulate afferent receptors to initiate reflex bronchoconstriction or to release tachykinins (eg, substance P) that directly stimulate smooth muscle contraction.

receptors relaxes airway smooth muscle, inhibits mediator release, and causes tachycardia and skeletal muscle tremor as side effects.

The sympathomimetic agents that have been widely used in the treatment of asthma include epinephrine, ephedrine, isoproterenol, and albuterol and other β_2-selective agents (Figure 20–4). Because epinephrine and isoproterenol increase the rate and force of cardiac contraction (mediated mainly by β_1 receptors), they are reserved for special situations (see below).

In general, adrenoceptor agonists are best delivered by inhalation because this results in the greatest local effect on airway smooth muscle with the least systemic toxicity. Aerosol deposition depends on the particle size, the pattern of breathing, and the geometry of the airways. Even with particles in the optimal size range of 2–5 mm, 80–90% of the total dose of aerosol is deposited in the mouth or pharynx. Particles under 1–2 μm remain suspended and may be exhaled. Bronchial deposition of an aerosol is increased by slow inhalation of a nearly full breath and by more than 5 seconds of breath-holding at the end of inspiration.

Epinephrine is an effective, rapidly acting bronchodilator when injected subcutaneously (0.4 mL of 1:1000 solution) or inhaled as a microaerosol from a pressurized canister (320 mcg per puff). Maximal bronchodilation is achieved 15 minutes after inhalation and lasts 60–90 minutes. Because epinephrine stimulates α and β_1 as well as β_2 receptors, tachycardia, arrhythmias, and worsening of angina pectoris are troublesome adverse effects. The cardiovascular effects of epinephrine are of value for treating the acute vasodilation and shock as well as the bronchospasm of anaphylaxis, but its use in asthma has been displaced by other, more β_2-selective agents.

Ephedrine was used in China for more than 2000 years before its introduction into Western medicine in 1924. Compared with epinephrine, ephedrine has a longer duration, oral activity, more pronounced central effects, and much lower potency. Because of

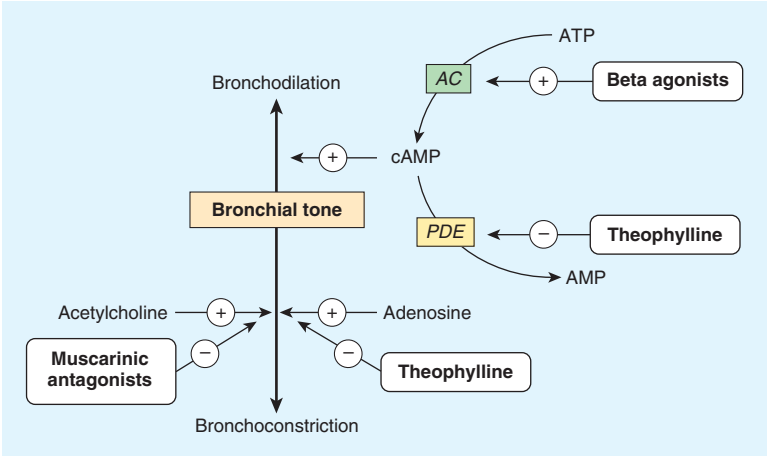


FIGURE 20–3 Bronchodilation is promoted by cAMP. Intracellular levels of cAMP can be increased by β-adrenoceptor agonists, which increase the rate of its synthesis by adenylyl cyclase (AC); or by phosphodiesterase (PDE) inhibitors such as theophylline, which slow the rate of its degradation. Bronchoconstriction can be inhibited by muscarinic antagonists and possibly by adenosine antagonists.

the development of more efficacious and β_2-selective agonists, ephedrine is now used infrequently in treating asthma.

Isoproterenol is a potent bronchodilator; when inhaled as a microaerosol from a pressurized canister, 80–120 mcg isoproterenol causes maximal bronchodilation within 5 minutes. Isoproterenol has a 60- to 90-minute duration of action. An increase in the asthma mortality rate that occurred in the United Kingdom in the mid-1960s was attributed to cardiac arrhythmias resulting from the use of high doses of inhaled isoproterenol. It is now rarely used for asthma.

Beta$_2$-Selective Drugs

The β_2-selective adrenoceptor agonist drugs, particularly albuterol, are the most widely used sympathomimetics for the treatment of the bronchoconstriction of asthma at present (Figure 20–4). These

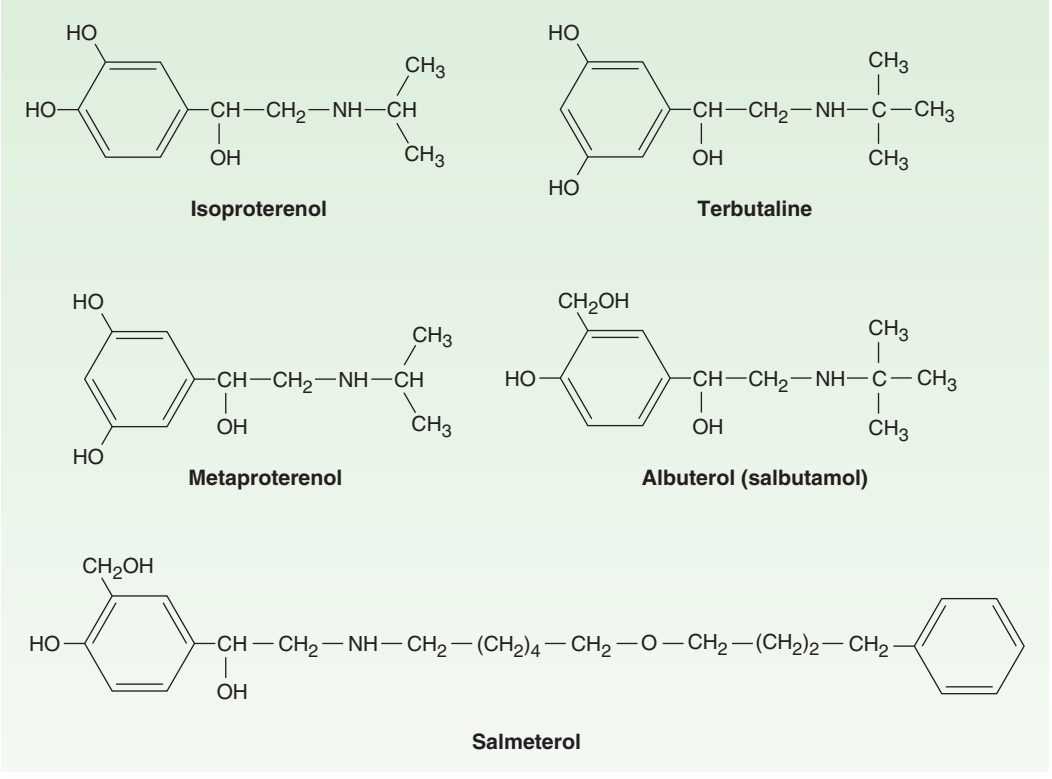


FIGURE 20–4 Structures of isoproterenol and several β_2-selective analogs.

agents differ structurally from epinephrine in having a larger substitution on the amino group and in the position of the hydroxyl groups on the aromatic ring. They are effective after inhaled or oral administration and have a long duration of action.

Albuterol, terbutaline, metaproterenol, and **pirbuterol** are available as metered-dose inhalers. Given by inhalation, these agents cause bronchodilation equivalent to that produced by isoproterenol. Bronchodilation is maximal within 15–30 minutes and persists for 3–4 hours. All can be diluted in saline for administration from a hand-held nebulizer. Because the particles generated by a nebulizer are much larger than those from a metered-dose inhaler, much higher doses must be given (2.5–5.0 mg vs 100–400 mcg) but are no more effective. Nebulized therapy should thus be reserved for patients unable to coordinate inhalation from a metered-dose inhaler.

Most preparations of β_2-selective drugs are a mixture of *R* and *S* isomers. Only the *R* isomer activates the β-agonist receptor. Reasoning that the *S* isomer may promote inflammation, a purified preparation of the *R* isomer of albuterol has been developed (levalbuterol). Whether this actually presents significant advantages in clinical use is unproven.

Albuterol and terbutaline are also available in tablet form. One tablet two or three times daily is the usual regimen; the principal adverse effects of skeletal muscle tremor, nervousness, and occasional weakness may be reduced by starting the patient on half-strength tablets for the first 2 weeks of therapy. This route of administration presents no advantage over inhaled treatment and is thus rarely prescribed.

Of these agents, only terbutaline is available for subcutaneous injection (0.25 mg). The indications for this route are similar to those for subcutaneous epinephrine—severe asthma requiring emergency treatment when aerosolized therapy is not available or has been ineffective—but it should be remembered that terbutaline's longer duration of action means that cumulative effects may be seen after repeated injections.

A new generation of long-acting β_2-selective agonists includes **salmeterol** (a partial agonist) and **formoterol** (a full agonist). Both drugs are potent selective β_2 agonists that achieve their long duration of action (12 hours or more) as a result of high lipid solubility. This permits them to dissolve in the smooth muscle cell membrane in high concentrations or, possibly, attach to "mooring" molecules in the vicinity of the adrenoceptor. These drugs appear to interact with inhaled corticosteroids to improve asthma control. Because they have no anti-inflammatory action, they are not recommended as monotherapy for asthma. An ultra-long acting β agonist, **indacaterol,** is currently approved in Europe. It needs to be taken only once a day but is used only for the treatment of chronic obstructive pulmonary disease (COPD). Data on its efficacy and safety in management of asthma are lacking.

Toxicities

The use of sympathomimetic agents by inhalation at first raised fears about possible cardiac arrhythmias and about hypoxemia acutely and tachyphylaxis or tolerance when given repeatedly. It is true that the vasodilating action of β_2-agonist treatment may increase perfusion of poorly ventilated lung units, transiently decreasing arterial oxygen tension (PaO_2). This effect is usually small, however, and may occur with any bronchodilator drug; the significance of such an effect depends on the initial PaO_2 of the patient. Administration of supplemental oxygen, routine in treatment of an acute severe attack of asthma, eliminates any concern over this effect. The other concern, that customary doses of β-agonist treatment may cause lethal cardiac arrhythmias, appears unsubstantiated. In patients presenting for emergency treatment of severe asthma, irregularities in cardiac rhythm *improve* with the improvements in gas exchange effected by bronchodilator treatment and oxygen administration.

The concept that β-agonist drugs worsen clinical asthma by inducing tachyphylaxis to their own action has not been established. Most studies have shown only a small change in the bronchodilator response to β stimulation after prolonged treatment with β-agonist drugs, but some studies have shown a loss in the ability of β-agonist treatment to inhibit the response to subsequent challenge with exercise, methacholine, or antigen challenge (referred to as a loss of bronchoprotective action).

Although it is true that β_2-adrenoceptor agonists appear to be safe and effective bronchodilators when taken on an "as needed" basis for relief of symptoms, there is some evidence of risk of adverse effects from chronic treatment with long-acting β agonists. These risks were suspected to be greater for individuals carrying a genetic variant for the β receptor, specifically at the B-16 locus of the β receptor. Retrospective analyses of studies of regular treatment with an inhaled β agonist suggested that asthma control deteriorated among patients homozygous for arginine at this locus, a genotype found in 16% of the Caucasian population and more commonly in African Americans in the USA. It was thus tempting to speculate that a genetic variant may underlie the report of an increase in asthma mortality from regular use of a long-acting β agonist in studies involving very large numbers of patients (see below), but it should be noted that only trivial differences were observed in multiple measures of asthma control in a study comparing patients with the Arg/Arg or Gly/Gly genotypes treated with salmeterol in combination with an inhaled corticosteroid. While studies so far have failed to prove that genetic variations at this particular site in the gene for the β receptor are related to adverse responses to prolonged β-agonist treatment, pharmacogenetic studies of asthma treatment will continue to be an active focus of research, as an approach to the development of "personalized therapy" for asthma and other diseases.

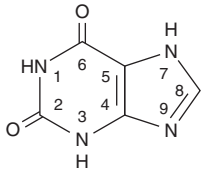
METHYLXANTHINE DRUGS

The three important methylxanthines are **theophylline, theobromine,** and **caffeine.** Their major source is beverages (tea, cocoa, and coffee, respectively). The importance of theophylline as a therapeutic agent in the treatment of asthma has waned as the greater effectiveness of inhaled adrenoceptor agents for acute asthma and of inhaled anti-inflammatory agents for chronic

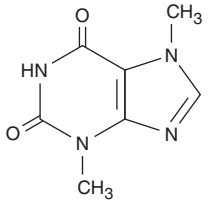
asthma has been established, but theophylline's very low cost is an important advantage for economically disadvantaged patients in societies in which health care resources are limited.

Chemistry

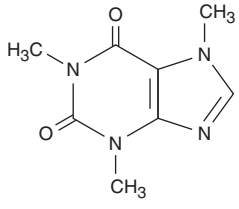
As shown below, theophylline is 1,3-dimethylxanthine; theobromine is 3,7-dimethylxanthine; and caffeine is 1,3,7-trimethylxanthine. A theophylline preparation commonly used for therapeutic purposes is **aminophylline,** a theophylline-ethylenediamine complex. The pharmacokinetics of theophylline are discussed below (see Clinical Uses of Methylxanthines). The metabolic products, partially demethylated xanthines (not uric acid), are excreted in the urine.



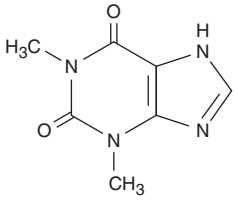
Xanthine



Theobromine



Caffeine



Theophylline

Mechanism of Action

Several mechanisms have been proposed for the actions of methylxanthines, but none has been firmly established. At high concentrations, they can be shown in vitro to inhibit several members of the phosphodiesterase (PDE) enzyme family (Figure 20–3). Because the phosphodiesterases hydrolyze cyclic nucleotides, this inhibition results in higher concentrations of intracellular cAMP and, in some tissues, cGMP. Cyclic AMP is responsible for a myriad of cellular functions including, but not limited to, stimulation of cardiac function, relaxation of smooth muscle, and reduction in the immune and inflammatory activity of specific cells.

Of the various isoforms of phosphodiesterase that have been identified, PDE4 appears to be the most directly involved in actions of methylxanthines on airway smooth muscle and on inflammatory cells. The inhibition of PDE4 in inflammatory cells reduces their release of cytokines and chemokines, which in turn results in a decrease in immune cell migration and activation.

In an effort to reduce toxicity while maintaining therapeutic efficacy, selective inhibitors of different isoforms of PDE4 were developed. Many were abandoned after clinical trials showed that their toxicities of nausea, headache, and diarrhea restricted dosing to subtherapeutic levels, but one, **roflumilast,** has recently been approved by the Food and Drug Administration (FDA) as a treatment for COPD, though not for asthma.

Another proposed mechanism is inhibition of cell surface receptors for adenosine. These receptors modulate adenylyl cyclase activity, and adenosine has been shown to provoke contraction of isolated airway smooth muscle and histamine release from airway mast cells. It has been shown, however, that xanthine derivatives devoid of adenosine antagonism (eg, enprofylline) may be potent in inhibiting bronchoconstriction in asthmatic subjects.

Some research suggests that the efficacy of theophyllines may be due to a third mechanism of action: enhancement of histone deacetylation. Acetylation of core histones is necessary for activation of inflammatory gene transcription. Corticosteroids act, at least in part, by recruiting histone deacetylactylases to the site of inflammatory gene transcription, an action enhanced by low-dose theophylline. This interaction should predict that low-dose theophylline treatment would enhance the effectiveness of corticosteroid treatment, and some clinical trials indeed support the idea that theophylline treatment is effective in restoring corticosteroid responsiveness in asthmatics who smoke and in patients with some forms of COPD.

Pharmacodynamics

The methylxanthines have effects on the central nervous system, kidney, and cardiac and skeletal muscle as well as smooth muscle. Of the three agents, theophylline is most selective in its smooth muscle effects, whereas caffeine has the most marked central nervous system effects.

A. Central Nervous System Effects

In low and moderate doses, the methylxanthines—especially caffeine—cause mild cortical arousal with increased alertness and deferral of fatigue. The caffeine contained in beverages—eg, 100 mg in a cup of coffee—is sufficient to cause nervousness and insomnia in sensitive individuals and slight bronchodilation in patients with asthma. The larger doses necessary for more effective bronchodilation commonly cause nervousness and tremor in some patients. Very high doses, from accidental or suicidal overdose, cause medullary stimulation and convulsions and may lead to death.

B. Cardiovascular Effects

The methylxanthines have positive chronotropic and inotropic effects. At low concentrations, these effects appear to result from inhibition of presynaptic adenosine receptors in sympathetic nerves increasing catecholamine release at nerve endings. The higher concentrations (> 10 µmol/L, 2 mg/L) associated with inhibition of phosphodiesterase and increases in cAMP may result in increased influx of calcium. At much higher concentrations (> 100 µmol/L), sequestration of calcium by the sarcoplasmic reticulum is impaired.

The clinical expression of these effects on cardiovascular function varies among individuals. Ordinary consumption of coffee and other methylxanthine-containing beverages usually produces slight tachycardia, an increase in cardiac output, and an increase in peripheral resistance, raising blood pressure slightly. In sensitive individuals, consumption of a few cups of coffee may result in arrhythmias.

In large doses, these agents also relax vascular smooth muscle except in cerebral blood vessels, where they cause contraction.

Methylxanthines decrease blood viscosity and may improve blood flow under certain conditions. The mechanism of this action is not well defined, but the effect is exploited in the treatment of intermittent claudication with **pentoxifylline,** a dimethylxanthine agent. However, no evidence suggests that this therapy is superior to other approaches.

C. Effects on Gastrointestinal Tract

The methylxanthines stimulate secretion of both gastric acid and digestive enzymes. However, even decaffeinated coffee has a potent stimulant effect on secretion, which means that the primary secretagogue in coffee is not caffeine.

D. Effects on Kidney

The methylxanthines—especially theophylline—are weak diuretics. This effect may involve both increased glomerular filtration and reduced tubular sodium reabsorption. The diuresis is not of sufficient magnitude to be therapeutically useful.

E. Effects on Smooth Muscle

The bronchodilation produced by the methylxanthines is the major therapeutic action in asthma. Tolerance does not develop, but adverse effects, especially in the central nervous system, may limit the dose (see below). In addition to their effect on airway smooth muscle, these agents—in sufficient concentration—inhibit antigen-induced release of histamine from lung tissue; their effect on mucociliary transport is unknown.

F. Effects on Skeletal Muscle

The respiratory actions of the methylxanthines may not be confined to the airways, for they also strengthen the contractions of isolated skeletal muscle in vitro and improve contractility and reverse fatigue of the diaphragm in patients with COPD. This effect on diaphragmatic performance—rather than an effect on the respiratory center—may account for theophylline's ability to improve the ventilatory response to hypoxia and to diminish dyspnea even in patients with irreversible airflow obstruction.

Clinical Uses

Of the xanthines, theophylline is the most effective bronchodilator, and it has been shown repeatedly both to relieve airflow obstruction in acute asthma and to reduce the severity of symptoms and time lost from work or school in patients with chronic asthma. Theophylline base is only slightly soluble in water, so it has been administered as several salts containing varying amounts of theophylline base. Most preparations are well absorbed from the gastrointestinal tract, but absorption of rectal suppositories is unreliable.

Improvements in theophylline preparations have come from alterations in the physical state of the drugs rather than from new chemical formulations. For example, the increased surface area of anhydrous theophylline in a microcrystalline form facilitates solubilization for complete and rapid absorption after oral administration. Numerous sustained-release preparations (see Preparations Available) are available and can produce therapeutic blood levels for 12 hours or more. These preparations offer the advantages of less frequent drug administration, less fluctuation of theophylline blood levels, and, in many cases, more effective treatment of nocturnal bronchospasm.

Theophylline should be used only where methods to measure theophylline blood levels are available because it has a narrow therapeutic window, and its therapeutic and toxic effects are related to its blood level. Improvement in pulmonary function is correlated with plasma concentration in the range of 5–20 mg/L. Anorexia, nausea, vomiting, abdominal discomfort, headache, and anxiety occur at concentrations of 15 mg/L in some patients and become common at concentrations greater than 20 mg/L. Higher levels (> 40 mg/L) may cause seizures or arrhythmias; these may not be preceded by gastrointestinal or neurologic warning symptoms.

The plasma clearance of theophylline varies widely. Theophylline is metabolized by the liver, so usual doses may lead to toxic concentrations of the drug in patients with liver disease. Conversely, clearance may be increased through the induction of hepatic enzymes by cigarette smoking or by changes in diet. In normal adults, the mean plasma clearance is 0.69 mL/kg/min. Children clear theophylline faster than adults (1–1.5 mL/kg/min). Neonates and young infants have the slowest clearance (see Chapter 60). Even when maintenance doses are altered to correct for the above factors, plasma concentrations vary widely.

Theophylline improves long-term control of asthma when taken as the sole maintenance treatment or when added to inhaled corticosteroids. It is inexpensive, and it can be taken orally. Its use, however, also requires occasional measurement of plasma levels; it often causes unpleasant minor side effects (especially insomnia); and accidental or intentional overdose can result in severe toxicity or death. For oral therapy with the prompt-release formulation, the usual dose is 3–4 mg/kg of theophylline every 6 hours. Changes in dosage result in a new steady-state concentration of theophylline in 1–2 days, so the dosage may be increased at intervals of 2–3 days until therapeutic plasma concentrations are achieved (10–20 mg/L) or until adverse effects develop.

ANTIMUSCARINIC AGENTS

Observation of the use of leaves from *Datura stramonium* for asthma treatment in India led to the discovery of atropine, a potent competitive inhibitor of acetylcholine at postganglionic muscarinic receptors, as a bronchodilator. Interest in the potential value of antimuscarinic agents increased with demonstration of the importance of the vagus nerves in bronchospastic responses of laboratory animals and with the development of a potent atropine analog that is poorly absorbed after aerosol administration and is therefore relatively free of systemic atropine-like effects.

Mechanism of Action

Muscarinic antagonists competitively inhibit the effect of acetylcholine at muscarinic receptors (see Chapter 8). In the airways,

acetylcholine is released from efferent endings of the vagus nerves, and muscarinic antagonists block the contraction of airway smooth muscle and the increase in secretion of mucus that occurs in response to vagal activity (Figure 20–2). Very high concentrations—well above those achieved even with maximal therapy—are required to inhibit the response of airway smooth muscle to nonmuscarinic stimulation. This selectivity of muscarinic antagonists accounts for their usefulness as investigative tools in examining the role of parasympathetic pathways in bronchomotor responses but limits their usefulness in preventing bronchospasm. In the doses given, antimuscarinic agents inhibit only that portion of the response mediated by muscarinic receptors, which varies by stimulus, and which further appears to vary among individual responses to the same stimulus.

Clinical Uses

Antimuscarinic agents are effective bronchodilators. When given intravenously, atropine, the prototypical muscarinic antagonist, causes bronchodilation at a lower dose than that needed to cause an increase in heart rate. The selectivity of atropine's effect can be increased further by administering the drug by inhalation or by use of a more selective quaternary ammonium derivative of atropine, **ipratropium bromide.** Ipratropium can be delivered in high doses by this route because it is poorly absorbed into the circulation and does not readily enter the central nervous system. Studies with this agent have shown that the degree of involvement of parasympathetic pathways in bronchomotor responses varies among subjects. In some, bronchoconstriction is inhibited effectively; in others, only modestly. The failure of higher doses of the muscarinic antagonist to further inhibit the response in these individuals indicates that mechanisms other than parasympathetic reflex pathways must be involved.

Even in the subjects least protected by this antimuscarinic agent, however, the bronchodilation and partial inhibition of provoked bronchoconstriction are of potential clinical value, and antimuscarinic agents are valuable for patients intolerant of inhaled β-agonist agents. Although antimuscarinic drugs appear to be slightly less effective than β-agonist agents in reversing asthmatic bronchospasm, the addition of ipratropium enhances the bronchodilation produced by nebulized albuterol in acute severe asthma.

Ipratropium appears to be at least as effective in patients with COPD that includes a partially reversible component. A longer-acting, selective antimuscarinic agent, **tiotropium,** is approved as a treatment for COPD. It binds to M_1, M_2, and M_3 receptors with equal affinity, but dissociates most rapidly from M_2 receptors, expressed on the efferent nerve ending. This means that tiotropium does not inhibit the M_2-receptor–mediated auto–down-regulation of acetylcholine release, and thus confers a degree of receptor selectivity. Tiotropium is also taken by inhalation, and a single dose of 18 mcg has 24-hour duration of action. Daily inhalation of tiotropium has been shown not only to improve functional capacity of patients with COPD, but also to reduce the frequency of exacerbations of their condition, and tiotropium is approved by the FDA as a treatment for COPD. It has not been approved as a treatment for asthma, but the addition of tiotropium has recently been shown to be as effective as the addition of a long-acting β-agonist in asthmatic patients insufficiently controlled by inhaled corticosteroid therapy alone.

CORTICOSTEROIDS

Mechanism of Action

Corticosteroids have been used to treat asthma since 1950 and are presumed to act by their broad anti-inflammatory efficacy, mediated in part by inhibition of production of inflammatory cytokines (see Chapter 39). They do not relax airway smooth muscle directly but reduce bronchial reactivity and reduce the frequency of asthma exacerbations if taken regularly. Their effect on airway obstruction may be due in part to their contraction of engorged vessels in the bronchial mucosa and their potentiation of the effects of β-receptor agonists, but their most important action is inhibition of the infiltration of asthmatic airways by lymphocytes, eosinophils, and mast cells.

Clinical Uses

Clinical studies of corticosteroids consistently show them to be effective in improving all indices of asthma control—severity of symptoms, tests of airway caliber and bronchial reactivity, frequency of exacerbations, and quality of life. Because of severe adverse effects when given chronically, oral and parenteral corticosteroids are reserved for patients who require urgent treatment, ie, those who have not improved adequately with bronchodilators or who experience worsening symptoms despite maintenance therapy. Regular or "controller" therapy is maintained with aerosol corticosteroids.

Urgent treatment is often begun with an oral dose of 30–60 mg prednisone per day or an intravenous dose of 1 mg/kg methylprednisolone every 6–12 hours; the daily dose is decreased after airway obstruction has improved. In most patients, systemic corticosteroid therapy can be discontinued in 7–10 days, but in other patients symptoms may worsen as the dose is decreased to lower levels. Because adrenal suppression by corticosteroids is related to dose and because secretion of endogenous corticosteroids has a diurnal variation, it is customary to administer corticosteroids early in the morning after endogenous adrenocorticotropic hormone secretion has peaked. For prevention of nocturnal asthma, however, oral or inhaled corticosteroids are most effective when given in the late afternoon.

Aerosol treatment is the most effective way to avoid the systemic adverse effects of corticosteroid therapy. The introduction of corticosteroids such as **beclomethasone, budesonide, ciclesonide, flunisolide, fluticasone, mometasone,** and **triamcinolone** has made it possible to deliver corticosteroids to the airways with minimal systemic absorption. An average daily dose of four puffs twice daily of beclomethasone (400 mcg/d) is equivalent to about 10–15 mg/d of oral prednisone for the control of asthma, with far fewer systemic effects. Indeed, one of the cautions in

switching patients from oral to inhaled corticosteroid therapy is to taper oral therapy slowly to avoid precipitation of adrenal insufficiency. In patients requiring continued prednisone treatment despite inhalation of standard doses of an aerosol corticosteroid, higher doses appear to be more effective; inhalation of high doses of both fluticasone and ciclesonide, for example, have been shown to be effective in weaning patients from chronic prednisone therapy. Although these high doses of inhaled steroids may cause adrenal suppression, the risks of systemic toxicity from chronic use appear negligible compared with those of the oral corticosteroid therapy they replace.

A special problem caused by inhaled topical corticosteroids is the occurrence of oropharyngeal candidiasis. The risk of this complication can be reduced by having patients gargle water and spit after each inhaled treatment. Hoarseness can also result from a direct local effect of inhaled corticosteroids on the vocal cords. These agents are remarkably free of other short-term complications in adults but may increase the risks of osteoporosis and cataracts over the long term. In children, inhaled corticosteroid therapy has been shown to slow the rate of growth by about 1 cm over the first year of treatment, but not the rate of growth thereafter, so that the effect on adult height is minimal.

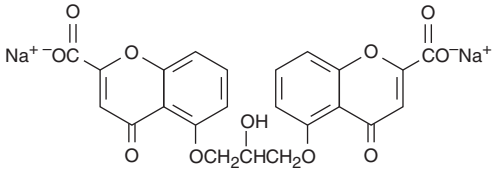
A novel approach to minimizing the risk of toxicity from systemic absorption of an inhaled corticosteroid underlay the development of **ciclesonide.** This recently approved corticosteroid is inhaled as a prodrug activated by cleavage by esterases in bronchial epithelial cells. When absorbed into the circulation, the active product is tightly bound to serum proteins, and so has little access to glucocorticoid receptors in skin, eye, and bone, minimizing its risk of causing cutaneous thinning, cataracts, osteoporosis, or temporary slowing of growth. Ciclesonide has been shown to be effective in improving asthma control in clinical trials, but studies have not yet proven that its use is associated with the significant reduction in systemic toxicity predicted from its design as a prodrug with low corticosteroid activity, activated to a much more potent corticosteroid agonist by esterases at its site of deposition in the airways.

Chronic use of inhaled corticosteroids effectively reduces symptoms and improves pulmonary function in patients with mild asthma. Such use also reduces or eliminates the need for oral corticosteroids in patients with more severe disease. In contrast to β-stimulant agents and theophylline, chronic use of inhaled corticosteroids reduces bronchial reactivity. Because of the efficacy and safety of inhaled corticosteroids, national and international guidelines for asthma management recommend their prescription for patients who require more than occasional inhalations of a β agonist for relief of symptoms. This therapy is continued for 10–12 weeks and then withdrawn to determine whether more prolonged therapy is needed. Inhaled corticosteroids are not curative. In most patients, the manifestations of asthma return within a few weeks after stopping therapy even if they have been taken in high doses for 2 years or longer. A prospective, placebo-controlled study of the early, sustained use of an inhaled corticosteroids in young children with asthma showed significantly greater improvement in asthma symptoms, pulmonary function, and frequency of asthma exacerbations over the 2 years of treatment, but no difference in overall asthma control 3 months after the end of the trial. Inhaled corticosteroids are thus properly labeled as "controllers." They are effective only so long as they are taken.

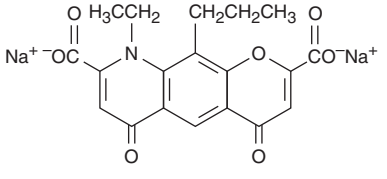
Another approach to reducing the risk of long-term, twice-daily use of inhaled corticosteroids is to administer them only intermittently, when symptoms of asthma flare. Taking a single inhalation of an inhaled corticosteroid with each inhalation of a short-acting β-agonist reliever (eg, an inhalation of beclomethasone for each inhalation of albuterol) or taking a 5–10 day course of twice-daily high-dose budesonide or beclomethasone when asthma symptoms worsen has been found to be as effective as regular daily therapy in adults and children with mild to moderate asthma, although these approaches to treatment are neither endorsed by guidelines for asthma management nor approved by the FDA.

CROMOLYN & NEDOCROMIL

Cromolyn sodium (disodium cromoglycate) and nedocromil sodium were once widely used for asthma management, especially in children, but have now been supplanted so completely by other therapies that they are mostly of historic interest. Both have low solubility, are poorly absorbed from the gastrointestinal tract, and must be inhaled as a microfine powder or microfine suspension. When taken by inhalation, they effectively inhibit both antigen- and exercise-induced asthma, and chronic use (four times daily) slightly reduces the overall level of bronchial reactivity. However, these drugs have no effect on airway smooth muscle tone and are ineffective in reversing asthmatic bronchospasm; they are only of value when taken prophylactically.



Cromolyn sodium



Nedocromil sodium

Mechanism of Action

Cromolyn and nedocromil are thought to alter the function of delayed chloride channels in the cell membranes, inhibiting cell activation. This action on airway nerves is thought to mediate nedocromil's inhibition of cough; on mast cells, the same

mechanism is responsible for inhibition of the early response to antigen challenge and on eosinophils, for inhibition of the inflammatory response to inhalation of allergens. The inhibitory effect on mast cells appears to be specific for cell type, since cromolyn has little inhibitory effect on mediator release from human basophils. It may also be specific for different organs, since cromolyn inhibits mast cell degranulation in human and primate lung but not in skin. This in turn may reflect known differences in mast cells found in different sites, as in their neutral protease content.

At one time, the idea that cromolyn inhibits mast cell degranulation was so well accepted that the inhibition of a response by cromolyn was thought to indicate the involvement of mast cells in the response. This simplistic idea was overturned in part by the finding that cromolyn and nedocromil inhibit the function of cells other than mast cells and in part by the finding that nedocromil inhibits appearance of the late response even when given after the early response to antigen challenge, ie, after mast cell degranulation has occurred.

Clinical Uses

In short-term clinical trials, pretreatment with cromolyn or nedocromil blocks the bronchoconstriction caused by allergen inhalation, by exercise, by sulfur dioxide, and by a variety of causes of occupational asthma. This acute protective effect of a single treatment makes cromolyn useful for administration shortly before exercise or before unavoidable exposure to an allergen.

When taken regularly (two to four puffs two to four times daily) by patients with perennial (nonseasonal) asthma, both agents modestly but significantly reduce symptomatic severity and the need for bronchodilator medications, particularly in young patients with extrinsic asthma. These drugs are not as potent nor as predictably effective as inhaled corticosteroids, and the only way of determining whether a patient will respond is by a therapeutic trial of 4 weeks' duration.

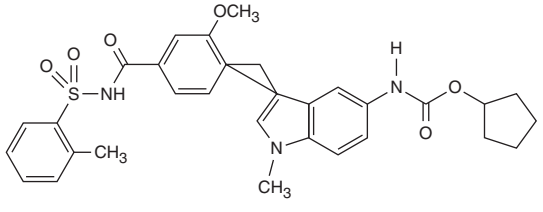
Cromolyn and nedocromil solutions are also useful in reducing symptoms of **allergic rhinoconjunctivitis.** Applying the solution by nasal spray or eye drops several times a day is effective in about 75% of patients, even during the peak pollen season.

Because the drugs are so poorly absorbed, adverse effects of cromolyn and nedocromil are minor and are localized to the sites of deposition. These include such minor symptoms as throat irritation, cough, and mouth dryness, and, rarely, chest tightness, and wheezing. Some of these symptoms can be prevented by inhaling a β_2-adrenoceptor agonist before cromolyn or nedocromil treatment. Serious adverse effects are rare. Reversible dermatitis, myositis, or gastroenteritis occurs in less than 2% of patients, and a very few cases of pulmonary infiltration with eosinophilia and anaphylaxis have been reported. This lack of toxicity accounts for cromolyn's formerly widespread use in children, especially those at ages of rapid growth. Its place in treatment of childhood asthma has lately diminished, however, because of the significantly greater efficacy of even low-dose corticosteroid treatment and because of the availability of an alternate nonsteroidal controller class of medication, the leukotriene pathway inhibitors (see below).

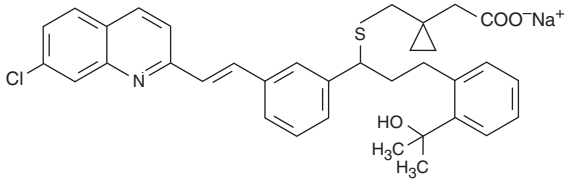
LEUKOTRIENE PATHWAY INHIBITORS

Because of the evidence of leukotriene involvement in many inflammatory diseases (see Chapter 18) and in anaphylaxis, considerable effort has been expended on the development of drugs that block the synthesis of these arachidonic acid derivatives or their receptors. Leukotrienes result from the action of 5-lipoxygenase on arachidonic acid and are synthesized by a variety of inflammatory cells in the airways, including eosinophils, mast cells, macrophages, and basophils. Leukotriene B_4 (LTB_4) is a potent neutrophil chemoattractant, and LTC_4 and LTD_4 exert many effects known to occur in asthma, including bronchoconstriction, increased bronchial reactivity, mucosal edema, and mucus hypersecretion. Early studies established that antigen challenge of sensitized human lung tissue results in the generation of leukotrienes, whereas other studies of human subjects have shown that inhalation of leukotrienes causes not only bronchoconstriction but also an increase in bronchial reactivity to histamine that persists for several days.

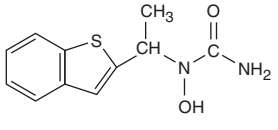
Two approaches to interrupting the leukotriene pathway have been pursued: inhibition of 5-lipoxygenase, thereby preventing leukotriene synthesis; and inhibition of the binding of LTD_4 to its receptor on target tissues, thereby preventing its action. Efficacy in blocking airway responses to exercise and to antigen challenge has been shown for drugs in both categories: **zileuton,** a 5-lipoxygenase inhibitor, and **zafirlukast** and **montelukast,** LTD_4-receptor antagonists. All have been shown to improve asthma control and to reduce the frequency of asthma exacerbations in outpatient clinical trials. Their effects on symptoms, airway caliber, bronchial reactivity, and airway inflammation are less marked than the effects of inhaled corticosteroids, but they are more nearly equal in reducing the frequency of exacerbations. Their principal advantage is that they are taken orally; some patients—especially children—comply poorly with inhaled therapies. Montelukast is approved for children as young as 6 years of age.



Zafirlukast



Montelukast



Zileuton

Some patients appear to have particularly favorable responses, but no clinical features allow identification of "responders" before a trial of therapy. In the USA, zileuton is approved for use in an oral dosage of 1200 mg of the sustained-release form twice daily; zafirlukast, 20 mg twice daily; and montelukast, 10 mg (for adults) or 4 mg (for children) once daily.

Trials with leukotriene inhibitors have demonstrated an important role for leukotrienes in aspirin-induced asthma. It has long been known that 5–10% of asthmatics are exquisitely sensitive to aspirin, so that ingestion of even a very small dose causes profound bronchoconstriction and symptoms of systemic release of histamine, such as flushing and abdominal cramping. Because this reaction to aspirin is not associated with any evidence of allergic sensitization to aspirin or its metabolites and because it is produced by any of the nonsteroidal anti-inflammatory agents, it is thought to result from inhibition of prostaglandin synthetase (cyclooxygenase), shifting arachidonic acid metabolism from the prostaglandin to the leukotriene pathway. Support for this idea was provided by the demonstration that leukotriene pathway inhibitors impressively reduce the response to aspirin challenge and improve overall control of asthma on a day-to-day basis.

Of these agents, zileuton is the least prescribed because of reports of liver toxicity. The receptor antagonists appear to have little toxicity. Reports of Churg-Strauss syndrome (a systemic vasculitis accompanied by worsening asthma, pulmonary infiltrates, and eosinophilia) appear to have been coincidental, with the syndrome unmasked by the reduction in prednisone dosage made possible by the addition of zafirlukast or montelukast. Of these two, montelukast is the most prescribed, probably because it can be taken without regard to meals, and because of the convenience of once-daily treatment.

OTHER DRUGS IN THE TREATMENT OF ASTHMA

Anti-IgE Monoclonal Antibodies

An entirely new approach to the treatment of asthma exploits advances in molecular biology to target IgE antibody. From a collection of monoclonal antibodies raised in mice against IgE antibody itself, a monoclonal antibody was selected that is targeted against the portion of IgE that binds to its receptors (FCε-R1 and FCε-R2 receptors) on mast cells and other inflammatory cells. **Omalizumab** (an anti-IgE monoclonal antibody) inhibits the binding of IgE to mast cells but does not activate IgE already bound to these cells and thus does not provoke mast cell degranulation. The murine antibody has been genetically humanized by replacing all but a small fraction of its amino acids with those found in human proteins, and it does not appear to cause sensitization when given to human subjects.

Administration of omalizumab to asthmatic patients for 10 weeks lowers plasma IgE to undetectable levels and significantly reduces the magnitude of both early and late bronchospastic responses to antigen challenge. Repeated administration lessens asthma severity and reduces the corticosteroid requirement in patients with moderate to severe disease, especially those with a clear environmental antigen precipitating factor, and improves nasal and conjunctival symptoms in patients with perennial or seasonal allergic rhinitis. Omalizumab's most important effect is reduction of the frequency and severity of asthma exacerbations, even while enabling a reduction in corticosteroid requirements. Combined analysis of several clinical trials has shown that the patients most likely to respond are, fortunately, those with the greatest need: those with a history of repeated exacerbations, a high requirement for corticosteroid treatment, and poor pulmonary function. Similarly, the exacerbations most prevented are the ones most important to prevent: Omalizumab treatment reduced exacerbations requiring hospitalization by 88%. These benefits justify the high cost of this treatment in selected individuals with severe disease characterized by frequent exacerbations.

POSSIBLE FUTURE THERAPIES

The rapid advance in the scientific description of the immunopathogenesis of asthma has spurred the development of many new therapies targeting different sites in the immune cascade. These include monoclonal antibodies directed against cytokines (IL-4, IL-5, IL-13), antagonists of cell adhesion molecules, protease inhibitors, and immunomodulators aimed at shifting CD4 lymphocytes from the TH2 to the TH1 phenotype or at selective inhibition of the subset of TH2 lymphocytes directed against particular antigens. There is evidence that asthma may be aggravated—or even caused—by chronic airway infection with *Chlamydia pneumoniae* or *Mycoplasma pneumoniae.* This may explain the reports of benefit to some patients from treatment with macrolide antibiotics, but a recent trial of prolonged treatment with clarithromycin (500 mg twice daily for weeks) failed to improve asthma control in patients with moderately severe asthma.

CLINICAL PHARMACOLOGY OF DRUGS USED IN THE TREATMENT OF ASTHMA

Asthma is best thought of as a disease in two time domains. In the present domain, it is important for the distress it causes—cough, nocturnal awakenings, and shortness of breath that interferes with the ability to exercise or to pursue desired activities. For mild asthma, occasional inhalation of a bronchodilator may be all that is needed. For more severe asthma, treatment with a long-term controller, like an inhaled corticosteroid, is necessary to relieve symptoms and restore function. The second domain of asthma is the risk it presents of future events, such as exacerbations, or of progressive loss of pulmonary function. A patient's satisfaction with his or her ability to control symptoms and maintain function by frequent use of an inhaled β_2 agonist does not mean that the risk of future events is also controlled. In fact, use of two or more canisters of an inhaled β agonist per month is a marker of increased risk of asthma fatality.

The challenges of assessing severity and adjusting therapy for these two domains of asthma are different. For relief of distress in the present domain, the key information can be obtained by asking specific questions about the frequency and severity of symptoms, the frequency of use of an inhaled β_2 agonist for relief of symptoms, the frequency of nocturnal awakenings, and the ability to exercise. Estimating the risk for future exacerbations is more difficult. In general, patients with poorly controlled symptoms in the present have a heightened risk of exacerbations in the future, but some patients seem unaware of the severity of their underlying airflow obstruction (sometimes described as "poor perceivers") and can be identified only by measurement of pulmonary function, as by spirometry. Reductions in the FEV_1 correlate with heightened risk of attacks of asthma in the future. Other possible markers of heightened risk are unstable pulmonary function (large variations in FEV_1 from visit to visit, large change with bronchodilator treatment), extreme bronchial reactivity, or high numbers of eosinophils in sputum or of nitric oxide in exhaled air. Assessment of these features may identify patients who need increases in therapy for protection against exacerbations.

BRONCHODILATORS

Bronchodilators, such as inhaled albuterol, are rapidly effective, safe, and inexpensive. Patients with only occasional symptoms of asthma require no more than an inhaled β_2-receptor agonist taken on an as-needed basis. If symptoms require this "rescue" therapy more than twice a week, if nocturnal symptoms occur more than twice a month, or if the FEV_1 is less than 80% predicted, additional treatment is needed. The treatment first recommended is a low dose of an inhaled corticosteroid, although treatment with a leukotriene receptor antagonist or with cromolyn may be used. Theophylline is now largely reserved for patients in whom symptoms remain poorly controlled despite the combination of regular treatment with an inhaled anti-inflammatory agent and as-needed use of a β_2 agonist. If the addition of theophylline fails to improve symptoms or if adverse effects become bothersome, it is important to check the plasma level of theophylline to be sure it is in the therapeutic range (10–20 mg/L).

An important caveat for patients with mild asthma is that although the risk of a severe, life-threatening attack is lower than in patients with severe asthma, it is not zero. All patients with asthma should be instructed in a simple action plan for severe, frightening attacks: to take up to four puffs of albuterol every 20 minutes over 1 hour. If they do not note clear improvement after the first four puffs, they should take the additional treatments while on their way to an emergency department or some other higher level of care.

MUSCARINIC ANTAGONISTS

Inhaled muscarinic antagonists have so far earned a limited place in the treatment of asthma. The effects of short-acting agents (eg, ipratropium bromide) on baseline airway resistance is nearly as great as, but no greater than, those of the sympathomimetic drugs, so they are used largely as alternative therapies for patients intolerant of β_2-adrenoceptor agonists. The airway effects of antimuscarinic and sympathomimetic drugs given in full doses have been shown to be additive only in patients with severe airflow obstruction who present for emergency care.

The long-acting antimuscarinic agent tiotropium has not yet earned a place in the treatment for asthma, although it has been shown to be as effective as a long-acting β_2-agonist when used in addition to an inhaled corticosteroid treatment for that condition. As a treatment for COPD, tiotropium both improves functional capacity, presumably through its action as a bronchodilator, and reduces the frequency of exacerbations, through mechanisms not yet defined.

Although it was predicted that muscarinic antagonists would dry airway secretions and interfere with mucociliary clearance, direct measurements of fluid volume secretion from single airway submucosal glands in animals show that atropine decreases baseline secretory rates only slightly. The drugs do, however, inhibit the increase in mucus secretion caused by vagal stimulation. No cases of inspissation of mucus have been reported following administration of these drugs.

CORTICOSTEROIDS

If asthmatic symptoms occur frequently or if significant airflow obstruction persists despite bronchodilator therapy, inhaled corticosteroids should be started. For patients with severe symptoms or severe airflow obstruction (eg, FEV_1 < 50% predicted), initial treatment with a combination of inhaled and oral corticosteroid (eg, 30 mg/d of prednisone for 3 weeks) is appropriate. Once clinical improvement is noted, usually after 7–10 days, the oral dose should be discontinued or reduced to the minimum necessary to control symptoms.

An issue for inhaled corticosteroid treatment is patient adherence. Analysis of prescription renewals shows that corticosteroids are taken regularly by a minority of patients. This may be a function of a general "steroid phobia" fostered by emphasis in the lay press on the hazards of long-term oral corticosteroid therapy and by ignorance of the difference between corticosteroids and anabolic steroids, taken to enhance muscle strength by now-infamous athletes. This fear of corticosteroid toxicity makes it hard to persuade patients whose symptoms have improved after starting treatment that they should continue it for protection against attacks. This context accounts for the interest in reports that instructing patients with mild but persistent asthma to take inhaled corticosteroid therapy only when their symptoms worsen is as effective in maintaining pulmonary function and preventing attacks as is taking the inhaled corticosteroid twice each day (see above).

In patients with more severe asthma whose symptoms are inadequately controlled by a standard dose of an inhaled corticosteroid, two options may be considered: to double the dose of inhaled corticosteroid or to combine it with another drug. The addition of theophylline or a leukotriene-receptor antagonist modestly increases asthma control, but the most impressive benefits are noted from addition of a *long-acting* inhaled β_2-receptor agonist (salmeterol or formoterol). Many studies have shown this

combination therapy to be more effective than doubling the dose of the inhaled corticosteroid for reducing symptoms, reducing the "as-needed" use of albuterol, and preventing attacks of asthma. Combinations of an inhaled corticosteroid and a long-acting β agonist in a single inhaler are now commonly prescribed (eg, fluticasone and salmeterol [Advair]; budesonide and formoterol [Symbicort]). Offsetting the clear benefits is evidence of a statistically significant increase in the very low risk of fatal asthma attacks from use of a long-acting β agonist, perhaps even when taken in combination with an inhaled corticosteroid. This evidence prompted the FDA to issue a "black box" warning that the use of a long-acting β agonist is associated with a small but statistically significant increase in the risk of death or near-death from an asthma attack, especially in African Americans. The FDA did not withdraw approval of these drugs, for it recognizes that they are clinically effective. The major implications of the "black box" warning for the practitioner are: (1) that patients with mild to moderate asthma should be treated with a low-dose inhaled corticosteroid alone, and additional therapy considered only if their asthma is not well controlled; and, (2) that if their asthma is not well controlled, the possible increase in risk of a rare event, asthma fatality, should be discussed in presenting the options for treatment—an increase to a higher dose of the inhaled corticosteroid versus addition of a long-acting β agonist.

The FDA's warning has not so far had much effect on prescriptions for combinations of an inhaled corticosteroid with a long-acting β agonist, probably because their combination in a single inhaler offers several advantages. Combination inhalers are convenient; they ensure that the long-acting β agonist will not be taken as monotherapy (known not to protect against attacks); and they produce prompt, sustained improvements in clinical symptoms and pulmonary function and reduce the frequency of exacerbations requiring oral corticosteroid treatment. In patients prescribed such combination treatment, it is important to provide explicit instructions that a rapid-acting inhaled β_2 agonist, such as albuterol, should be used as needed for relief of acute symptoms.

An emerging approach to treatment takes advantage of the rapidity of onset of the bronchodilator action of the long-acting β agonist formoterol delivered in fixed-dose combination with the inhaled corticosteroid budesonide in Symbicort metered-dose inhalers. Several studies have confirmed that twice-daily and as-needed inhalation of this combination is as effective in preventing asthma exacerbations as a four-times-higher dose of budesonide twice daily with only albuterol for relief of symptoms. Use of this flexible dosing strategy is widespread in Europe, but is not approved in the USA.

LEUKOTRIENE ANTAGONISTS; CROMOLYN & NEDOCROMIL

A leukotriene receptor antagonist taken as an oral tablet may be considered as an alternative to inhaled corticosteroid treatment in patients with symptoms occurring more than twice a week or those who are awakened from sleep by asthma more than twice a month. This place in asthma therapy was once held by cromolyn and nedocromil, but neither is now available in the USA. Although these treatments are not as effective as even a low dose of an inhaled corticosteroid, both avoid the issue of "steroid phobia" described above and are commonly used in the treatment of children.

The leukotriene receptor antagonist montelukast (Singulair) is widely prescribed, especially by primary care providers. This drug, taken orally, is easy to administer and appears to be used more regularly than inhaled corticosteroids. Leukotriene receptor antagonists are rarely associated with troublesome side effects. Because of concerns over the possible long-term toxicity of systemic absorption of inhaled corticosteroids, this maintenance therapy is widely used for treating children in the USA.

ANTI-IGE MONOCLONAL ANTIBODY

Treatment with omalizumab, the monoclonal humanized anti-IgE antibody, is reserved for patients with chronic severe asthma inadequately controlled by high-dose inhaled corticosteroid plus long-acting β-agonist combination treatment (eg, fluticasone, 500 mcg, plus salmeterol, 50 mcg, inhaled twice daily). Omalizumab reduces lymphocytic, eosinophilic bronchial inflammation and effectively reduces the frequency and severity of exacerbations. It is reserved for patients with demonstrated IgE-mediated sensitivity (by positive skin test or radioallergosorbent test [RAST] to common allergens) and an IgE level within a range that can be reduced sufficiently by twice-weekly subcutaneous injection.

OTHER ANTI-INFLAMMATORY THERAPIES

Some reports suggest that agents commonly used to treat rheumatoid arthritis may also be used to treat patients with chronic steroid-dependent asthma. The development of an alternative treatment is important, because chronic treatment with oral corticosteroids may cause osteoporosis, cataracts, glucose intolerance, worsening of hypertension, and cushingoid changes in appearance. Initial studies suggested that oral methotrexate or gold salt injections were beneficial in prednisone-dependent asthmatic patients, but subsequent studies did not confirm this promise. In contrast, the benefit from treatment with cyclosporine seems real. However, this drug's great toxicity makes this finding only a source of hope that other immunomodulatory therapies will ultimately be developed for the small proportion of patients whose asthma can be managed only with high oral doses of prednisone. An immunomodulatory therapy recently reported to improve asthma is injection of etanercept, a TNF-α antagonist used in the treatment of ankylosing spondylitis and severe rheumatoid arthritis.

MANAGEMENT OF ACUTE ASTHMA

The treatment of acute attacks of asthma in patients reporting to the hospital requires close, continuous clinical assessment and repeated objective measurement of lung function. For patients with mild attacks, inhalation of a β_2-receptor agonist is as effective

as subcutaneous injection of epinephrine. Both of these treatments are more effective than intravenous administration of aminophylline (a soluble salt of theophylline). Severe attacks require treatment with oxygen, frequent or continuous administration of aerosolized albuterol, and systemic treatment with prednisone or methylprednisolone (0.5 mg/kg every 6–12 hours). Even this aggressive treatment is not invariably effective, and patients must be watched closely for signs of deterioration. General anesthesia, intubation, and mechanical ventilation of asthmatic patients cannot be undertaken lightly but may be lifesaving if respiratory failure supervenes.

PROSPECTS FOR PREVENTION

The high prevalence of asthma in the developed world and its rapid increases in the developing world call for a strategy for primary prevention. Strict antigen avoidance during infancy, once thought to be sensible, has now been shown to be ineffective. In fact, growing up from birth on a farm with domestic animals or in a household where cats or dogs are kept as pets appears to *protect* against developing asthma. The best hope seems to lie in understanding the mechanisms by which microbial exposures during infancy foster development of a balanced immune response and then mimicking the effects of natural environmental exposures through administration of harmless microbial commensals (probiotics) or of nutrients that foster their growth (prebiotics) in the intestinal tract during the critical period of immune development in early infancy.

TREATMENT OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

COPD is characterized by airflow limitation that is not fully reversible with bronchodilator treatment. The airflow limitation is usually progressive and is believed to reflect an abnormal inflammatory response of the lung to noxious particles or gases. The condition is most often a consequence of prolonged habitual cigarette smoking, but approximately 15% of cases occur in nonsmokers. Although COPD is different from asthma, some of the same drugs are used in its treatment. This section discusses the drugs that are useful in both conditions.

Although asthma and COPD are both characterized by airway inflammation, reduction in maximum expiratory flow, and episodic exacerbations of airflow obstruction, most often triggered by viral respiratory infection, they differ in many important respects. Most important among them are differences in the populations affected, characteristics of airway inflammation, reversibility of airflow obstruction, responsiveness to corticosteroid treatment, and course and prognosis. Compared to asthma, COPD occurs in older patients, is associated with neutrophilic rather than eosinophilic inflammation, is poorly responsive even to high-dose inhaled corticosteroid therapy, and is associated with progressive, inexorable loss of pulmonary function over time, especially with continued cigarette smoking.

Despite these differences, the approaches to treatment are similar, although the benefits expected (and achieved) are less for COPD than for asthma. For relief of acute symptoms, inhalation of a short-acting β agonist (eg, albuterol), of an anticholinergic drug (eg, ipratropium bromide), or of the two in combination is usually effective. For patients with persistent symptoms of exertional dyspnea and limitation of activities, regular use of a long-acting bronchodilator, whether a long-acting β agonist (eg, salmeterol) or a long-acting anticholinergic (eg, tiotropium) is indicated. For patients with severe airflow obstruction or with a history of prior exacerbations, regular use of an inhaled corticosteroid reduces the frequency of future exacerbations. Theophylline may have a particular place in the treatment of COPD, as it may improve contractile function of the diaphragm, thus improving ventilatory capacity. The major difference in treatment of these conditions centers on management of exacerbations. The use of antibiotics in this context is routine in COPD, because such exacerbations involve bacterial infection of the lower airways far more often in COPD than in asthma.

SUMMARY Drugs Used in Asthma

Subclass	Mechanism of Action	Effects	Clinical Applications	Pharmacokinetics, Toxicities
BETA AGONISTS				
• Albuterol	Selective β_2 agonist	Prompt, efficacious bronchodilation	Asthma, chronic obstructive pulmonary disease (COPD) • drug of choice in acute asthmatic bronchospasm	Aerosol inhalation • duration several hours • also available for nebulizer and parenteral use • *Toxicity:* Tremor, tachycardia • overdose: arrhythmias
• Salmeterol	Selective β_2 agonist	Slow onset, primarily preventive action; potentiates corticosteroid effects	Asthma prophylaxis	Aerosol inhalation • duration 12–24 h • *Toxicity:* Tremor, tachycardia • overdose: arrhythmias
• *Metaproterenol, terbutaline: Similar to albuterol; terbutaline available as an oral drug*				
• *Formoterol: Similar to salmeterol*				
• Epinephrine	Nonselective α and β agonist	Bronchodilation plus all other sympathomimetic effects on cardiovascular and other organ systems (see Chapter 9)	Anaphylaxis, asthma, others (see Chapter 9) • rarely used for asthma (β_2-selective agents preferred)	Aerosol, nebulizer, or parenteral • see Chapter 9
• Isoproterenol	β_1 and β_2 agonist	Bronchodilation plus powerful cardiovascular effects	Asthma, but β_2-selective agents preferred	Aerosol, nebulizer, or parenteral • see Chapter 9
CORTICOSTEROIDS, INHALED				
• Fluticasone	Alters gene expression	Reduces mediators of inflammation • powerful prophylaxis of exacerbations	Asthma • adjunct in COPD • hay fever (nasal)	Aerosol • duration hours • *Toxicity:* Limited by aerosol application • candidal infection, vocal cord changes
• *Beclomethasone, budesonide, flunisolide, others: Similar to fluticasone*				
CORTICOSTEROIDS, SYSTEMIC				
• Prednisone	Like fluticasone	Like fluticasone	Asthma • adjunct in COPD	Oral • duration 12–24 hours • *Toxicity:* Multiple • see Chapter 39
• *Methylprednisolone: Parenteral agent like prednisone*				
STABILIZERS OF MAST AND OTHER CELLS				
• Cromolyn, nedocromil (no longer available in the USA)	Alter function of delayed chloride channels • inhibits inflammatory cell activation	Prevents acute bronchospasm	Asthma (other routes used for ocular, nasal, and gastrointestinal allergy)	Aerosol • duration 6–8 h • *Toxicity:* Cough • not absorbed so other toxicities are minimal
METHYLXANTHINES				
• Theophylline	Uncertain • phosphodiesterase inhibition • adenosine receptor antagonist	Bronchodilation, cardiac stimulation, increased skeletal muscle strength (diaphragm)	Asthma, COPD	Oral • duration 8–12 h but extended-release preparations often used • *Toxicity:* Multiple (see text)
LEUKOTRIENE ANTAGONISTS				
• Montelukast, zafirlukast	Block leukotriene D_4 receptors	Block airway response to exercise and antigen challenge	Prophylaxis of asthma, especially in children and in aspirin-induced asthma	Oral • duration hours • *Toxicity:* Minimal
• *Zileuton: Inhibits lipoxygenase, reduces synthesis of leukotrienes*				
IGE ANTIBODY				
• Omalizumab	Humanized IgE antibody reduces circulating IgE	Reduces frequency of asthma exacerbations	Severe asthma inadequately controlled by above agents	Parenteral • duration 2–4 d • *Toxicity:* Injection site reactions (anaphylaxis extremely rare)

PREPARATIONS AVAILABLE

SYMPATHOMIMETICS USED IN ASTHMA

Albuterol (generic, Proventil, Ventolin)
Inhalant: 90 mcg/puff aerosol; 0.021, 0.042, 0.083, 0.5, 0.63% solution for nebulization
Oral: 2, 4 mg tablets; 2 mg/5 mL syrup
Oral sustained-release: 4, 8 mg tablets

Albuterol/Ipratropium (Combivent, DuoNeb)
Inhalant: 103 mcg albuterol + 18 mcg ipratropium/puff; 3 mg albuterol + 0.5 mg ipratropium/3 mL solution for nebulization

Arformoterol (Brovana)
Inhalant: 15 mcg/2 mL solution for nebulization

Bitolterol (Tornalate)
Inhalant: 0.2% solution for nebulization

Ephedrine (generic)
Oral: 25 mg capsules
Parenteral: 50 mg/mL for injection

Epinephrine (generic, Adrenalin)
Inhalant: 1, 10 mg/mL for nebulization; 0.22 mg/spray epinephrine base aerosol
Parenteral: 1:10,000 (0.1 mg/mL), 1:1000 (1 mg/mL)

Formoterol (Foradil)
Inhalant: 12 mcg/unit inhalant powder; 1% solution for nebulization

Formoterol/Budesonide (Symbicort)
Inhalant: 4.5 mcg/80 mcg, 4.5 mcg/160 mcg /puff

Isoetharine (generic)
Inhalant: 1% solution for nebulization

Isoproterenol (generic, Isuprel)
Inhalant: 0.5, 1% for nebulization; 80, 131 mcg/puff aerosols
Parenteral: 0.02, 0.2 mg/mL for injection

Levalbuterol (Xenopex)
Inhalant: 0.31, 0.63, 1.25 mg/3 mL solution

Metaproterenol (Alupent, generic)
Inhalant: 0.65 mg/puff aerosol in 7, 14 g containers; 0.4, 0.6, 5% for nebulization

Pirbuterol (Maxair)
Inhalant: 0.2 mg/puff aerosol in 80 and 300 dose containers

Salmeterol (Serevent)
Inhalant powder: 50 mcg/unit

Salmeterol/Fluticasone (Advair Diskus)
Inhalant: 100, 250, 500 mcg fluticasone + 50 mcg salmeterol/unit

Terbutaline (generic, Brethine)
Oral: 2.5, 5 mg tablets
Parenteral: 1 mg/mL for injection

AEROSOL CORTICOSTEROIDS (SEE ALSO CHAPTER 39)

Beclomethasone (QVAR)
Aerosol: 40, 80 mcg/puff in 100 dose containers

Budesonide (Pulmicort)
Aerosol powder (Turbuhaler): 160 mcg/activation
Inhalation suspension (Respules): 0.25, 0.5 mg/2 mL

Flunisolide (AeroBid, Aerospan)
Aerosol: 80, 250 mcg/puff in 80, 100, and 120 dose containers

Fluticasone (Flovent)
Aerosol: 44, 110, and 220 mcg/puff in 120 dose container; powder, 50, 100, 250 mcg/activation

Mometasone (Asmanex Twisthaler)
Inhalant: 110, 220 mcg/actuation in 14, 30, 60, 120 dose units

Triamcinolone (Azmacort)
Aerosol: 75 mcg/puff in 240 dose container

LEUKOTRIENE INHIBITORS

Montelukast (Singulair)
Oral: 10 mg tablets; 4, 5 mg chewable tablets; 4 mg/packet granules

Zafirlukast (Accolate)
Oral: 10, 20 mg tablets

Zileuton (Zyflo)
Oral: 600 mg tablets; 600 mg extended-release tablets

PHOSPHODIESTERASE INHIBITORS

Aminophylline (theophylline ethylenediamine, 79% theophylline) (generic)
Oral: 100, 200 mg tablets
Parenteral: 250 mg/10 mL for injection

Roflumilast (Daliresp)
Oral: 500 mcg tablets

Theophylline (generic, Elixophyllin, Slo-Phyllin, Uniphyl, Theo-Dur, Theo-24, others)
Oral: 50 mg/5 mL elixirs
Oral extended-release, 12 hours: 100, 200, 300, 450 mg tablets
Oral extended-release, 24 hours: 100, 200, 300 mg tablets and capsules; 400, 600 mg tablets
Parenteral: 0.08, 1.6, 2.0, 3.2, 4 mg/mL, theophylline and 5% dextrose for injection

OTHER METHYLXANTHINES

Dyphylline (Dylix, Lufyllin)
Oral: 200, 400 mg tablets; 100 mg/15 mL elixir

Pentoxifylline (generic, Trental)
Oral: 400 mg tablets and controlled-release tablets
Note: Pentoxifylline is labeled for use in intermittent claudication only.

ANTIMUSCARINIC DRUGS USED IN ASTHMA

Ipratropium (generic, Atrovent)
Aerosol: 17 (freon-free), 18 mcg/puff in 200 metered-dose inhaler; 0.02% (500 mcg/vial) for nebulization
Nasal spray: 21, 42 mcg/spray

Tiotropium (Spiriva)
Aerosol: 18 mcg/puff in 6 packs

ANTIBODY

Omalizumab (Xolair)
Powder for SC injection: 202.5 mg

REFERENCES

Pathophysiology of Airway Disease

Boushey H et al: Asthma. In Mason R et al (editors): *Textbook of Respiratory Medicine.* Elsevier Saunders, 2005:1168.

Locksley RM: Asthma and allergic inflammation. Cell 2010;140:777.

Asthma Treatment

Bateman ED et al: Overall asthma control: The relationship between current control and future risk. J Allergy Clin Immunol 2010;125:600.

Fanta CH: Drug therapy: Asthma. N Engl J Med 2009;360:1002.

Beta Agonists

Ducharme FM et al: Addition of long-acting beta2-agonists to inhaled corticosteroids versus same dose inhaled corticosteroids for chronic asthma in adults and children. Cochrane Database Syst Rev 2010;(5):CD005535.

Kelly HW, Harkins MS, Boushey H: The role of inhaled long-acting beta-2 agonists in the management of asthma. J Natl Med Assoc 2006;98:8.

Walters E, Walters J, Gibson P: Regular treatment with long acting beta agonists versus daily regular treatment with short acting beta agonists in adults and children with stable asthma. Cochrane Database Syst Rev 2002;(4): CD003901.

Methylxanthines & Roflumilast

Ito K et al: A molecular mechanism of action of theophylline: Induction of histone deacetylase activity to decrease inflammatory gene expression. Proc Natl Acad Sci USA 2002;99:8921.

Rabe KF: Roflumilast for the treatment of chronic obstructive pulmonary disease. Expert Rev Respir Med 2010;4:543.

Spina D: PDE4 inhibitors: Current status. Br J Pharmacol 2008;155:308.

Cromolyn & Nedocromil

Guevara J et al: Inhaled corticosteroids versus sodium cromoglycate in children and adults with asthma. Cochrane Database Syst Rev 2006;(2):CD003558.

Yoshihara S et al: Effects of early intervention with inhaled sodium cromoglycate in childhood asthma. Lung 2006;184:63.

Corticosteroids

Barnes P: How corticosteroids control inflammation: Quintiles Prize Lecture 2005. Br J Pharmacol 2006;148:245.

Boushey HA et al: Daily versus as-needed corticosteroids for mild persistent asthma. N Engl J Med 2005;352:1519.

O'Byrne PM et al: Budesonide/formoterol combination therapy as both maintenance and reliever medication in asthma. Am J Respir Crit Care Med 2005;171:129.

Suissa S et al: Low-dose inhaled corticosteroids and the prevention of death from asthma. N Engl J Med 2000;343:332.

Antimuscarinic Drugs

Gross N: Anticholinergic agents in asthma and COPD. Eur J Pharmacol 2006;8:533

Lee AM, Jacoby DB, Fryer AD: Selective muscarinic receptor antagonists for airway diseases. Curr Opin Pharmacol 2001;1:223.

Peters SP et al: Tiotropium bromide step-up therapy for adults with uncontrolled asthma. N Engl J Med 2010;363:1715.

Leukotriene Pathway Inhibitors

Calhoun WJ: Anti-leukotrienes for asthma. Curr Opin Pharmacol 2001;1:230.

Krawiec ME, Wenzel SE: Leukotriene inhibitors and non-steroidal therapies in the treatment of asthma. Exp Opin Invest Drugs 2001;2:47.

Wang L et al: Cost-effectiveness analysis of fluticasone versus montelukast in children with mild-to-moderate persistent asthma in the Pediatric Asthma Controller Trial. J Allergy Clin Immunol 2011;127:161.

Anti-IgE Therapy

Walker S et al: Anti-IgE for chronic asthma in adults and children. Cochrane Database Syst Rev 2006;(2):CD003559.

Clinical Management of Airway Disease

Barnes PJ: Drugs for asthma. Br J Pharmacol 2006;147(Suppl 1):S297.

Expert Panel Report 3 (EPR3): Guidelines for the diagnosis and management of asthma. National Institutes of Health, 2008; http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm.

Tattersfield AE et al: Asthma. Lancet 2002;360:1313.

Treatment of COPD

Buist S for the Executive Committee, Global Initiative for Chronic Obstructive Lung Disease: Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Medical Communications Resources, 2007; http://www.goldcopd.com.

Matera MG, Page CP, Cazzola M: Novel bronchodilators for the treatment of chronic obstructive pulmonary disease. Trends Pharmacol Sci 2011;32:495.

Vogelmeier C et al: Tiotropium versus salmeterol for the prevention of exacerbations of COPD. N Engl J Med 2011;364;1093.

CASE STUDY ANSWER

This patient demonstrates the destabilizing effects of a respiratory infection on asthma, and the parents demonstrate the common (and dangerous) phobia about "overuse" of bronchodilator or steroid inhalers. The patient has signs of imminent respiratory failure, including her refusal to lie down, her fear, and her tachycardia—which cannot be attributed to her minimal treatment with albuterol. Critically important immediate steps are to administer high-flow oxygen and to start albuterol by nebulization. Adding ipratropium (Atrovent) to the nebulized solution is recommended. A corticosteroid (0.5–1.0 mg/kg of methylprednisolone) should be administered intravenously. It is also advisable to alert the intensive care unit, because a patient with severe bronchospasm who tires can slip into respiratory failure quickly, and intubation can be difficult.

Fortunately, most patients treated in hospital emergency departments do well. Asthma mortality is rare (fewer than 5000 deaths per year among a population of 20 million asthmatics in the USA), but when it occurs, it is often out of hospital. Presuming this patient recovers, she needs adjustments to her therapy before discharge. The strongest predictor of severe attacks of asthma is their occurrence in the past. Thus, this patient needs to be started on a long-term controller, especially an inhaled corticosteroid, and needs instruction in an action plan for managing severe symptoms. This can be as simple as advising her and her parents that if she has a severe attack that frightens her, she can take up to four puffs of albuterol every 15 minutes, but if the first treatment does not bring significant relief, she should take the next four puffs while on her way to an emergency department or urgent care clinic. She should also be given a prescription for prednisone, with instructions to take 40–60 mg orally for severe attacks, but not to wait for it to take effect if she remains severely short of breath even after albuterol inhalations. Asthma is a chronic disease, and good care requires close follow-up and creation of a provider-patient partnership for optimal management.