31 The control of blood glucose and drug treatment of diabetes mellitus

OVERVIEW

In this chapter we describe the endocrine control of blood glucose by pancreatic hormones, especially *insulin* but also *glucagon* and *somatostatin*, and the gut hormones (*incretins*) *glucagon-like peptide-1* (GLP-1) and *gastric inhibitory peptide* (GIP, which is also known as glucose-dependent insulinotropic peptide). This underpins coverage of diabetes mellitus and its treatment with insulin preparations (including insulin analogues), and other hypoglycaemic agents – metformin, sulfonylureas, α-glucosidase inhibitors, glitazones, long-acting incretin mimetics such as exenatide, and gliptins, which potentiate incretins by blocking their degradation.

INTRODUCTION

Insulin is the main hormone controlling intermediary metabolism. Its most striking acute effect is to lower blood glucose. Reduced (or absent) secretion of insulin causes *diabetes mellitus*. It is often coupled with reduced sensitivity to its action, 'insulin resistance', which is closely related to obesity. Diabetes mellitus, recognised since ancient times, is named for the production of sugary urine in copious volumes (due to the osmotic diuretic action of the high urine glucose concentration). Diabetes is rapidly increasing to epidemic proportions (in step with obesity, Ch. 32), and its consequences are dire – especially accelerated atherosclerosis (myocardial and cerebral infarction, gangrene or limb amputation), kidney failure, neuropathy and blindness.

In this chapter, we first describe the control of blood sugar. The second part of the chapter is devoted to the different kinds of diabetes mellitus and the role of drugs in their treatment. Diabetes, along with obesity (Ch. 32), hypertension (Ch. 22) and dyslipidaemia (Ch. 23), comprise what is now termed 'metabolic syndrome', a common pathological cluster and a rapidly growing problem that is associated with many life-threatening conditions. New drugs, including several directed at controlling blood sugar, have been developed in recent years that act on some of the many mechanisms that become deranged in metabolic syndrome. Despite the effort and creativity that have gone into it, clinical success has so far been modest.

CONTROL OF BLOOD GLUCOSE

Glucose is the obligatory source of energy for the adult brain, and physiological control of blood glucose reflects the need to maintain adequate fuel supplies in the face of intermittent food intake and variable metabolic demands. More fuel is made available by feeding than is required immediately, and excess calories are stored as glycogen or fat. During fasting, these energy stores need to be mobilised in a regulated manner. The most important regulatory hormone is *insulin*, the actions of which are described below. Increased blood glucose stimulates insulin secretion (Fig. 31.1), whereas reduced blood glucose reduces insulin secretion. The effect of glucose on insulin secretion depends on whether the glucose load is administered intravenously or by mouth. Glucose administered by mouth is more effective in stimulating insulin secretion because it stimulates release from the gut of incretin hormones which promote insulin secretion (Fig. 31.1). Glucose is less effective in stimulating insulin secretion in patients with diabetes (Fig. 31.2). *Hypoglycaemia*, caused by excessive exogenous insulin, not only reduces endogenous insulin secretion but also elicits secretion of an array of 'counter-regulatory' hormones, including *glucagon*, *adrenaline* (Ch. 14), *glucocorticoids* (Ch. 33) and *growth hormone* (Ch. 33), all of which increase blood glucose. Their main effects on glucose uptake and carbohydrate metabolism are summarised and contrasted with those of insulin in Table 31.1.

PANCREATIC ISLET HORMONES

The islets of Langerhans, the endocrine part of the pancreas, contain four main types of peptide-secreting cells: B (or β) cells secrete *insulin*, A (or α) cells secrete *glucagon*, D cells secrete *somatostatin,* and PP cells secrete *pancreatic polypeptide* (PP).

▼ PP is a 36 amino acid peptide closely related to neuropeptide Y (Ch. 14) and peptide YY (Ch. 32). It is released by eating a meal and is implicated in control of food intake (Ch. 32): PP acts on G protein-coupled receptors and also inhibits secretion of exocrine pancreatic secretions and contraction of intestinal and biliary smooth muscle.

The core of each islet contains mainly the predominant B cells surrounded by a mantle of A cells interspersed with D cells or PP cells (see Fig. 31.1). In addition to insulin, B cells secrete a peptide known as *islet amyloid polypeptide* or *amylin* which delays gastric emptying and opposes insulin by stimulating glycogen breakdown in striated muscle, and C-peptide (see p. 381). Glucagon opposes insulin, increasing blood glucose and stimulating protein breakdown in muscle. Somatostatin inhibits secretion of insulin and of glucagon. It is widely distributed outside the pancreas and is also released from the hypothalamus, inhibiting the release of growth hormone from the pituitary gland (Ch. 33).

INSULIN

Insulin was the first protein for which the amino acid sequence was determined (by Sanger's group in

Table 31.1 The effect of hormones on blood glucose

Hormone	Main actions	Main stimuli for secretion	Main effect
Main regulatory hormone			
Insulin	↑ Glucose uptake ↑ Glycogen synthesis ↓ Glycogenolysis ↓ Gluconeogenesis	Acute rise in blood glucoseIncretins (GIP and GLP-1)	↓ Blood glucose
Main counter-regulatory hormones			
Glucagon	↑ Glycogenolysis ↑ Glyconeogenesis	Hypoglycaemia (i.e. blood glucose <3 mmol/l), (e.g. with exercise, stress, high protein meals), etc.	↑ Blood glucose
Adrenaline (epinephrine)	↑ Glycogenolysis		
Glucocorticoids	↓ Glucose uptake ↑ Gluconeogenesis ↓ Glucose uptake and utilisation		
Growth hormone	↓ Glucose uptake		

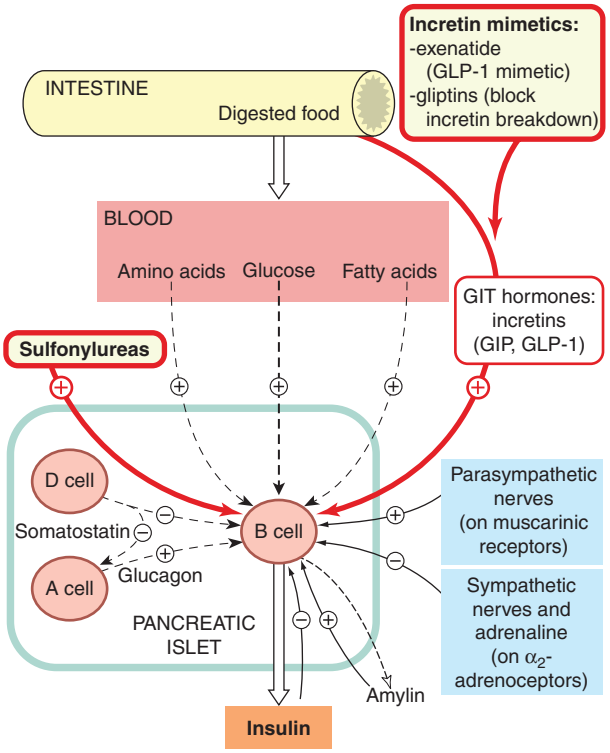

Fig. 31.1 Factors regulating insulin secretion. Blood glucose is the most important factor. Drugs used to stimulate insulin secretion are shown in red-bordered boxes. Glucagon potentiates insulin release but opposes some of its peripheral actions and increases blood glucose. GIP, gastric inhibitory peptide; GIT, gastrointestinal tract; GLP-1, glucagon-like peptide-1.

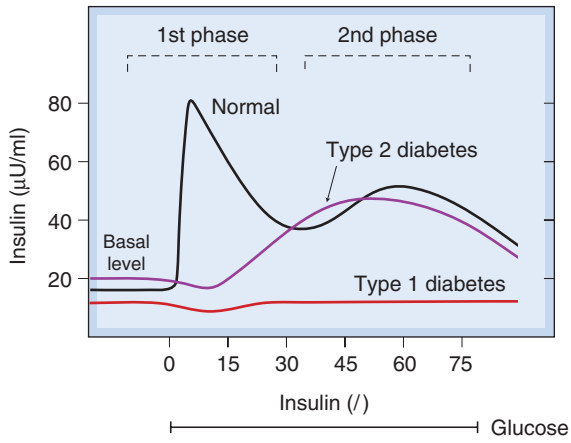

Fig. 31.2 Schematic diagram of the two-phase release of insulin in response to a constant glucose infusion. The first phase is missing in type 2 (non-insulin-dependent) diabetes mellitus, and both are missing in type 1 (insulin-dependent) diabetes mellitus. The first phase is also produced by amino acids, sulfonylureas, glucagon and gastrointestinal tract hormones. *(Data from Pfeifer MA, Halter JB, Porte D Jr 1981 Am J Med 70, 579–588.)*

Cambridge in 1955). It consists of two peptide chains (of 21 and 30 amino acid residues) linked by two disulfide bonds.

SYNTHESIS AND SECRETION

Like other peptide hormones (see Ch. 19), insulin is synthesised as a precursor (preproinsulin) in the rough endoplasmic reticulum. Preproinsulin is transported to the Golgi apparatus, where it undergoes proteolytic cleavage to proinsulin and then to insulin plus a fragment of uncertain function called C-peptide.[1] Insulin and C-peptide are stored in granules in B cells, and are normally co-secreted by exocytosis in equimolar amounts together with smaller and variable amounts of proinsulin.

The main factor controlling the synthesis and secretion of insulin is the blood glucose concentration (Fig. 31.1). B cells respond both to the absolute glucose concentration and to the rate of change of blood glucose. Other

[1]Not to be confused with C-reactive peptide, which is an acute-phase reactant used clinically as a marker of inflammation (Ch. 6).

Table 31.2 Effects of insulin on carbohydrate, fat and protein metabolism

Type of metabolism	Liver cells	Fat cells	Muscle
Carbohydrate metabolism	↓ Gluconeogenesis ↓ Glycogenolysis ↑ Glycolysis ↑ Glycogenesis	↑ Glucose uptake ↑ Glycerol synthesis	↑ Glucose uptake ↑ Glycolysis ↑ Glycogenesis
Fat metabolism	↑ Lipogenesis ↓ Lipolysis	↑ Synthesis of triglycerides ↑ Fatty acid synthesis ↓ Lipolysis	
Protein metabolism	↓ Protein breakdown	–	↑ Amino acid uptake ↑ Protein synthesis

physiological stimuli to insulin release include amino acids (particularly arginine and leucine), fatty acids, the parasympathetic nervous system and *incretins* (especially *GLP-1* and *GIP*, see p. 385). Pharmacologically, sulfonylurea drugs (see p. 388-389) act by releasing insulin.

There is a steady basal release of insulin and an increase in blood glucose stimulates an additional response. This response has two phases: an initial rapid phase reflecting release of stored hormone, and a slower, delayed phase reflecting continued release of stored hormone and new synthesis (Fig. 31.2). The response is abnormal in diabetes mellitus, as discussed later.

ATP-sensitive potassium channels (K_{ATP}; Ch. 4) determine the resting membrane potential in B cells. Glucose enters B cells via a surface membrane transporter called Glut-2, and its subsequent metabolism via glucokinase (which is the rate-limiting glycolytic enzyme in B cells) links insulin secretion to extracellular glucose. The consequent rise in ATP within B cells blocks K_{ATP} channels, causing membrane depolarisation. Depolarisation opens voltage-dependent calcium channels, leading to Ca^{2+} influx. This triggers insulin secretion in the presence of amplifying messengers including diacylglycerol, non-esterified arachidonic acid (which facilitates further Ca^{2+} entry), and 12-lipoxygenase products of arachidonic acid (mainly *12-S-hydroxyeicosatetraenoic acid* or 12-*S*-HETE; see Ch. 17). Phospholipases are commonly activated by Ca^{2+}, but free arachidonic acid is liberated in B cells by an ATP-sensitive Ca^{2+}-insensitive (ASCI) phospholipase A_2. Consequently, in B cells, Ca^{2+} entry and arachidonic acid production are both driven by ATP, linking cellular energy status to insulin secretion.

Insulin release is inhibited by the sympathetic nervous system (Fig. 31.1). Adrenaline (epinephrine) increases blood glucose by inhibiting insulin release (via α_2 adrenoceptors) and by promoting glycogenolysis via β_2 adrenoceptors in striated muscle and liver. Several peptides, including somatostatin, galanin (an endogenous K_{ATP} activator) and amylin, also inhibit insulin release.

About one-fifth of the insulin stored in the pancreas of the human adult is secreted daily. The plasma insulin concentration after an overnight fast is 20–50 pmol/l. Plasma insulin concentration is reduced in patients with type 1 (insulin-dependent) diabetes mellitus (see p. 386), and markedly increased in patients with *insulinomas* (uncommon functioning tumours of B cells), as is C-peptide, with which it is co-released.[2] It is also raised in obesity and other normoglycaemic insulin-resistant states.

ACTIONS

Insulin is the main hormone controlling intermediary metabolism, having its main actions on liver, fat and muscle (Table 31.2). It is an *anabolic hormone*: its overall effect is to conserve fuel by facilitating the uptake and storage of glucose, amino acids and fats after a meal. Acutely, it reduces blood glucose. Consequently, a fall in plasma insulin increases blood glucose. The biochemical pathways through which insulin exerts its effects are summarised in Figure 31.3, and molecular aspects of its mechanism are discussed below.

Insulin influences glucose metabolism in most tissues, especially the liver, where it inhibits glycogenolysis (glycogen breakdown) and gluconeogenesis (synthesis of glucose from non-carbohydrate sources) while stimulating glycogen synthesis. It also increases glucose utilisation by glycolysis, but the overall effect is to increase hepatic glycogen stores.

In muscle, unlike liver, uptake of glucose is slow and is the rate-limiting step in carbohydrate metabolism. Insulin causes a glucose transporter called Glut-4 which is sequestered in vesicles to be expressed within minutes on the surface membrane. This facilitates glucose uptake, and stimulates glycogen synthesis and glycolysis.

Insulin increases glucose uptake by Glut-4 in adipose tissue as well as in muscle. One of the main products of glucose metabolism in adipose tissue is glycerol, which is esterified with fatty acids to form triglycerides, thereby affecting fat metabolism (see Table 31.2).

Insulin increases synthesis of fatty acid and triglyceride in adipose tissue and in liver. It inhibits lipolysis, partly via dephosphorylation – and hence inactivation – of lipases (Table 31.2). It also inhibits the lipolytic actions of adrenaline, growth hormone and glucagon by opposing their actions on adenylyl cyclase.

[2]Insulin for injection does not contain C-peptide, which therefore provides a means of distinguishing endogenous from exogenous insulin. This is used to differentiate insulinoma (an insulin-secreting tumour causing high circulating insulin with high C-peptide) from surreptitious injection of insulin (high insulin with low C-peptide). Deliberate induction of hypoglycaemia by self-injection with insulin is a well-recognised, if unusual, manifestation of psychiatric disorder, especially in health professionals – it has also been used in murder.

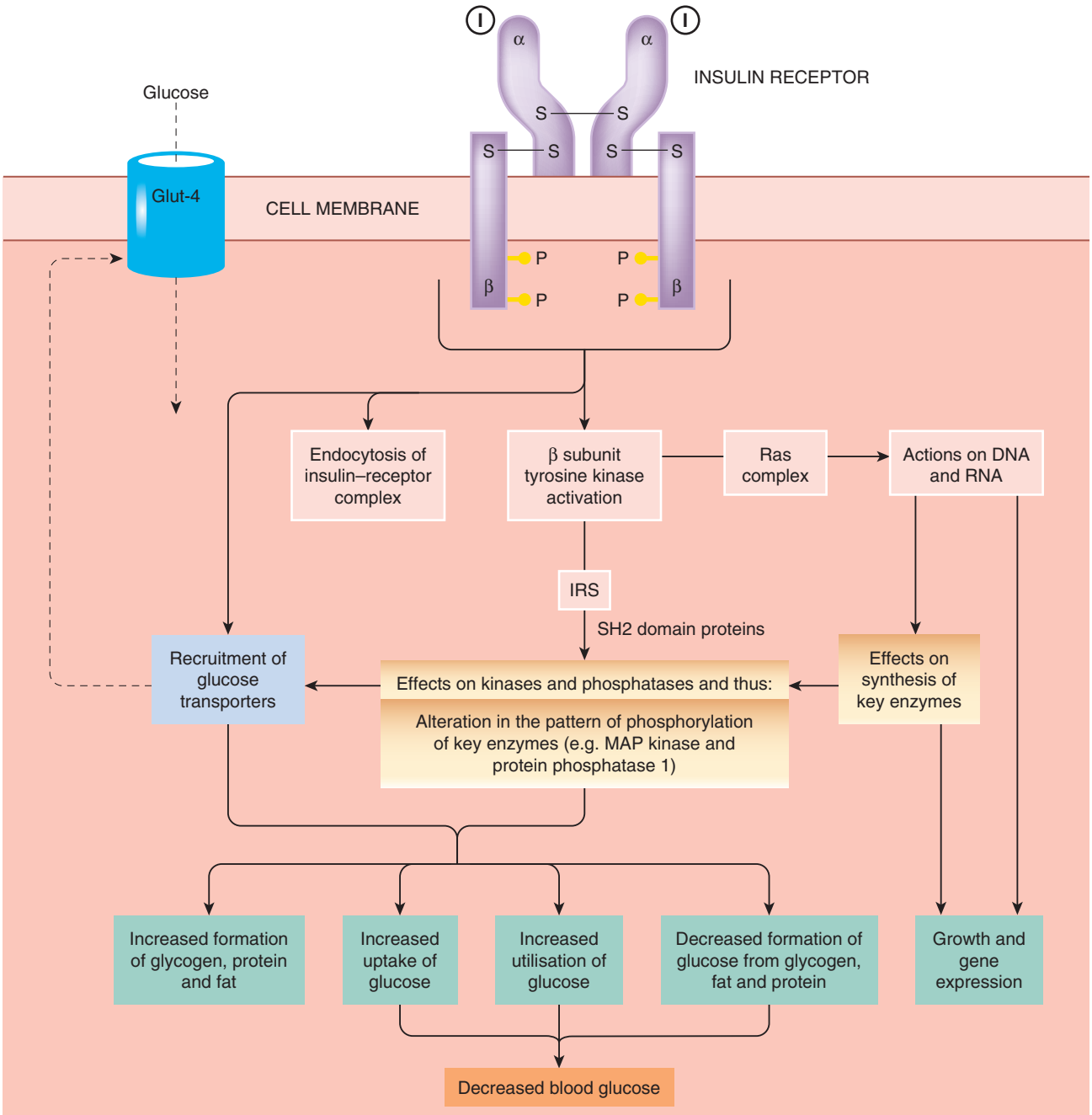

Fig. 31.3 Insulin signalling pathways. I, insulin; Glut-4, an insulin-sensitive glucose transporter present in muscle and fat cells; IRS, insulin receptor substrate (several forms: 1–4).

Insulin stimulates uptake of amino acids into muscle and increases protein synthesis. It also decreases protein catabolism and inhibits oxidation of amino acids in the liver.

Other metabolic effects of insulin include transport into cells of K^+, Ca^{2+}, nucleosides and inorganic phosphate.[3]

[3]The action on K^+ is exploited in the emergency treatment of hyperkalaemia by intravenous glucose with insulin (see Ch. 29).

Long-term effects of insulin

In addition to rapid effects on metabolism, exerted via altered activity of enzymes and transport proteins, insulin has long-term actions via altered enzyme synthesis. It is an important anabolic hormone during fetal development. It stimulates cell proliferation and is implicated in somatic and visceral growth and development.

Mitogenic actions of insulin are of great concern in the development of insulin analogues; **insulin glargine** (one widely used analogue; see p. 387) is six- to eight-fold more

mitogenic than human insulin, and cultured breast cancer cells proliferate in response to near-therapeutic concentrations of this analogue *in vitro*, but it is not known if there is any clinically significant parallel *in vivo*. Mammary tumours developed in rats given one long-acting insulin analogue.

Mechanism of action

Insulin binds to a specific receptor on the surface of its target cells. The receptor is a large transmembrane glycoprotein complex belonging to the tyrosine kinase-linked type 3 receptor superfamily (Ch. 3) and consisting of two α and two β subunits (Fig. 31.3). Occupied receptors aggregate into clusters, which are subsequently internalised in vesicles, resulting in downregulation. Internalised insulin is degraded in lysosomes, but the receptors are recycled to the plasma membrane.

▼ The signal transduction mechanisms that link receptor binding to the biological effects of insulin are complex. Receptor autophosphorylation – the first step in signal transduction – is a consequence of dimerisation, allowing each receptor to phosphorylate the other, as explained in Chapter 3.

Insulin receptor substrate (IRS) proteins undergo rapid tyrosine phosphorylation specifically in response to insulin and insulin-like growth factor-1 but not to other growth factors. The best-characterised substrate is IRS-1, which contains 22 tyrosine residues that are potential phosphorylation sites. It interacts with proteins that contain a so-called SH2 domain (see Ch. 3, Fig. 3.15), thereby passing on the insulin signal. Knockout mice lacking IRS-1 are hyporesponsive to insulin (insulin-resistant) but do not become diabetic, because of robust B-cell compensation with increased insulin secretion. By contrast, mice lacking IRS-2 fail to compensate and develop overt diabetes, implicating the IRS-2 gene as a candidate for human type 2 diabetes (IRS proteins are reviewed by Lee & White, 2004). Activation of phosphatidylinositol 3-kinase by interaction of its SH2 domain with phosphorylated IRS has several important effects, including recruitment of insulin-sensitive glucose transporters (Glut-4) from the Golgi apparatus to the plasma membrane in muscle and fat cells.

The longer-term actions of insulin entail effects on DNA and RNA, mediated partly at least by the Ras signalling complex. Ras is a protein that regulates cell growth and cycles between an active GTP-bound form and an inactive GDP-bound form (see Chs 3 and 56). Insulin shifts the equilibrium in favour of the active form, and initiates a phosphorylation cascade that results in activation of mitogen-activated protein kinase (MAP-kinase), which in turn activates several nuclear transcription factors, leading to the expression of genes that are involved with cell growth and with intermediary metabolism.

Insulin for treatment of diabetes mellitus is considered below.

GLUCAGON

SYNTHESIS AND SECRETION

Glucagon is a single-chain polypeptide of 21 amino acid residues synthesised mainly in the A cell of the islets, but also in the upper gastrointestinal tract. It has considerable structural homology with other gastrointestinal tract hormones, including secretin, vasoactive intestinal peptide and GIP (see Ch. 30).

Amino acids (especially L-arginine) stimulate glucagon secretion and ingestion of a high-protein meal increases glucagon secretion, but diurnal variation in plasma glucagon concentrations is less than for insulin. Glucagon secretion is stimulated by low and inhibited by high concentrations of glucose and fatty acids in the plasma. Sympathetic nerve activity and circulating adrenaline stimulate glucagon release via β adrenoceptors. Parasympathetic nerve activity also increases secretion, whereas somatostatin, released from D cells adjacent to the glucagon-secreting A cells in the periphery of the islets, inhibits glucagon release.

Endocrine pancreas and blood glucose

- Islets of Langerhans secrete insulin from B (or β) cells, glucagon from A cells and somatostatin from D cells.
- Many factors stimulate insulin secretion, but the main one is blood glucose. Incretins, especially GIP and GLP-1 secreted, respectively, by K and L cells in the gut are also important.
- Insulin has essential metabolic actions as a fuel storage hormone and also affects cell growth and differentiation. It decreases blood glucose by:
  - increasing glucose uptake into muscle and fat via Glut-4
  - increasing glycogen synthesis
  - decreasing gluconeogenesis
  - decreasing glycogen breakdown.
- Glucagon is a fuel-mobilising hormone, stimulating gluconeogenesis and glycogenolysis, also lipolysis and proteolysis. It increases blood sugar and also increases the force of contraction of the heart.
- Diabetes mellitus is a chronic metabolic disorder in which there is hyperglycaemia. There are two main types:
  - type 1 (insulin-dependent) diabetes, with an absolute deficiency of insulin
  - type 2 (non-insulin-dependent) diabetes, with a relative deficiency of insulin associated with reduced sensitivity to its action (insulin resistance).

ACTIONS

Glucagon increases blood glucose and causes breakdown of fat and protein. It acts on specific G protein-coupled receptors to stimulate adenylyl cyclase, and its actions are somewhat similar to β-adrenoceptor-mediated actions of adrenaline. Unlike adrenaline, however, its metabolic effects are more pronounced than its cardiovascular actions. Glucagon is proportionately more active on liver, while the metabolic actions of adrenaline are more pronounced on muscle and fat. Glucagon stimulates glycogen breakdown and gluconeogenesis, and inhibits glycogen synthesis and glucose oxidation. Its metabolic actions on target tissues are thus the opposite of those of insulin. Glucagon increases the rate and force of contraction of the heart, although less markedly than adrenaline.

Clinical uses of glucagon are summarised in the clinical box.

SOMATOSTATIN

Somatostatin is secreted by the D cells of the islets. It is also generated in the hypothalamus, where it acts to inhibit the release of growth hormone (see Ch. 32). In the

Clinical uses of glucagon

- **Glucagon** can be given intramuscularly or subcutaneously as well as intravenously.
- Treatment of *hypoglycaemia* in unconscious patients (who cannot drink); unlike intravenous glucose, it can be administered by non-medical personnel (e.g. spouses or ambulance crew). It is useful if obtaining intravenous access is difficult.
- Treatment of *acute cardiac failure* precipitated by β-adrenoceptor antagonists.

islet, it inhibits release of insulin and of glucagon. **Octreotide** is a long-acting analogue of somatostatin. It inhibits release of a number of hormones, and is used clinically to relieve symptoms from several uncommon gastroenteropancreatic endocrine tumours, and for treatment of acromegaly[4] (the endocrine disorder caused by a functioning tumour of cells that secrete growth hormone from the anterior pituitary; see Ch. 33).

AMYLIN (ISLET AMYLOID POLYPEPTIDE)

▼ The term *amyloid* refers to amorphous protein deposits in different tissues that occur in a variety of diseases, including several neurodegenerative conditions (see Ch. 40). Amyloid deposits occur in the pancreas of patients with diabetes mellitus, although it is not known if this is functionally important. The major component of pancreatic amyloid is a 37-amino acid residue peptide known as islet amyloid polypeptide or amylin. This is stored with insulin in secretory granules in B cells and is co-secreted with insulin. Amylin delays gastric emptying. Supraphysiological concentrations stimulate the breakdown of glycogen to lactate in striated muscle. Amylin also inhibits insulin secretion (Fig. 31.1). It is structurally related to calcitonin (see Ch. 36) and has weak calcitonin-like actions on calcium metabolism and osteoclast activity. It is also about 50% identical with calcitonin gene-related peptide (CGRP; see Ch. 18), and large intravenous doses cause vasodilatation, presumably by an action on CGRP receptors.

Pramlintide, an amylin analogue with three proline substitutions that reduce its tendency to aggregate into insoluble fibrils, has been licensed since 2005 by the FDA for patients with type 1 diabetes and for type 2 diabetics who use insulin. It is injected subcutaneously before meals as an adjunct to insulin, and reduces insulin requirements. Unwanted effects include hypoglycaemia and nausea – it is contraindicated in patients with loss of gastric motility (gastroparesis), a complication of diabetic autonomic neuropathy (Younk et al., 2011).

INCRETINS

La Barre suggested in the 1930s that crude secretin contained two active principles: 'excretin', which stimulates the exocrine pancreas, and 'incretin', which stimulates insulin release. He proposed that incretin presented possibilities for the treatment of diabetes. 'Excretin' did not catch on (perhaps not helped by an unfortunate association with other bodily functions – at least to an Anglo-Saxon ear), but 'incretin' has gone from strength to strength, and some 80 years later several incretin-based drugs are now licensed for clinical use (see below). Incretin action proved to be due to peptide hormones released from the gut, mainly *glucagon-like insulinotropic peptide* (GIP) and *glucagon-like peptide-1* (GLP-1). These are both members of the glucagon peptide superfamily (Ch. 18). GIP is a 42-amino acid peptide stored in and secreted by enteroendocrine K cells in the duodenum and proximal jejunum. GLP-1 is secreted by L cells which are more widely distributed in the gut, including in the ileum and colon as well as more proximally. Two forms of GLP-1 are secreted after a meal: GLP-1(7-37) and GLP-1(7-36) amide; these are similarly potent. Most of the circulating activity is due to GLP-1(7-36) amide. Release of GIP and GLP-1 by ingested food provides an early stimulus to insulin secretion before absorbed glucose or other products of digestion reach the islet cells in the portal blood (Fig. 31.1). As well as stimulating insulin secretion, both these hormones inhibit pancreatic glucagon secretion and slow the rate of absorption of digested food by reducing gastric emptying. They are also implicated in control of food intake via appetite and satiety (see Ch. 32). The actions of GIP and GLP-1 are terminated rapidly by dipeptidyl peptidase-4 (DPP-4). This enzyme is a membrane glycoprotein with rather wide substrate specificity – it has been implicated in suppression of malignancy (e.g. Yu et al., 2010).

DIABETES MELLITUS

Diabetes mellitus is a chronic metabolic disorder characterised by a high blood glucose concentration – hyperglycaemia (fasting plasma glucose >7.0 mmol/l, or plasma glucose >11.1 mmol/l, 2 h after a meal) – caused by insulin deficiency, often combined with insulin resistance. Hyperglycaemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis. When the renal threshold for glucose reabsorption is exceeded, glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria) which, in turn, results in dehydration, thirst and increased drinking (polydipsia). Insulin deficiency causes muscle wasting through increased breakdown and reduced synthesis of proteins. Diabetic ketoacidosis is an acute emergency. It develops in the absence of insulin because of accelerated breakdown of fat to acetyl-CoA, which, in the absence of aerobic carbohydrate metabolism, is converted to acetoacetate and β-hydroxybutyrate (which cause acidosis) and acetone (a ketone).

Various complications develop as a consequence of the metabolic derangements in diabetes, often over several years. Many of these are the result of disease of blood vessels, either large (macrovascular disease) or small (microangiopathy). Dysfunction of vascular endothelium (see Ch. 22) is an early and critical event in the development of vascular complications. Oxygen-derived free radicals, protein kinase C and non-enzymic products of glucose and albumin called *advanced glycation end products* (AGE) have been implicated. Macrovascular disease consists of accelerated atheroma (Ch. 23) and its

[4]Octreotide is used either short term before surgery on the pituitary tumour, or while waiting for radiotherapy of the tumour to take effect, or if other treatments have been ineffective.

thrombotic complications (Ch. 24), which are commoner and more severe in diabetic patients. Microangiopathy is a distinctive feature of diabetes mellitus and particularly affects the retina, kidney and peripheral nerves. Diabetes mellitus is the commonest cause of chronic renal failure, a huge and rapidly increasing problem, and a major burden to society as well as to individual patients. Coexistent hypertension promotes progressive renal damage, and treatment of hypertension slows the progression of diabetic nephropathy and reduces the risk of myocardial infarction. Angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists (Ch. 22) are more effective in preventing diabetic nephropathy than other antihypertensive drugs, perhaps because they prevent fibroproliferative actions of angiotensin II and aldosterone.

Diabetic neuropathy[5] is associated with accumulation of osmotically active metabolites of glucose, produced by the action of aldose reductase, but *aldose reductase inhibitors* have been disappointing as therapeutic drugs (see Farmer et al., 2012, for a review).

There are two main types of diabetes mellitus:

1. **Type 1 diabetes** (previously known as insulin-dependent diabetes mellitus – IDDM – or juvenile-onset diabetes).
2. **Type 2 diabetes** (previously known as non-insulin-dependent diabetes mellitus – NIDDM – or maturity-onset diabetes).

In type 1 diabetes, there is an absolute deficiency of insulin resulting from autoimmune destruction of pancreatic B cells. Without insulin treatment, such patients will sooner or later die with diabetic ketoacidosis.

▼ Type 1 diabetes can occur at any age, but patients are usually young (children or adolescents) and not obese when they first develop symptoms. There is an inherited predisposition, with a 10–15-fold increased incidence in first-degree relatives of an index case, and strong associations with particular histocompatibility antigens (HLA types). Identical twins are less than fully concordant, so environmental factors such as viral infection (e.g. with coxsackievirus or echovirus) are believed to be necessary for genetically predisposed individuals to express the disease. Viral infection may damage pancreatic B cells and expose antigens that initiate a self-perpetuating autoimmune process. The patient becomes overtly diabetic only when more than 90% of the B cells have been destroyed. This natural history provides a tantalising prospect of intervening in the prediabetic stage, and a variety of strategies have been mooted, including immunosuppression, early insulin therapy, antioxidants, nicotinamide and many others; so far these have disappointed, but this remains a very active field.

Type 2 diabetes is accompanied both by insulin resistance (which precedes overt disease) and by impaired insulin secretion, each of which are important in its pathogenesis. Such patients are often obese and usually present in adult life, the incidence rising progressively with age as B-cell function declines. Treatment is initially dietary, although oral hypoglycaemic drugs usually become necessary, and most patients ultimately benefit from exogenous insulin. Prospective studies have demonstrated a relentless deterioration in diabetic control[6] over the years.

Insulin secretion (basal, and in response to a meal) in a type 1 and a type 2 diabetic patient is contrasted schematically with that in a healthy control in Figure 31.2.

There are many other less common forms of diabetes mellitus in addition to the two main ones described above (for example, syndromes associated with autoantibodies directed against insulin receptors which cause severe insulin resistance, functional A-cell tumours, 'glucagonomas', and many other rarities), and hyperglycaemia can also be a clinically important adverse effect of several drugs, including glucocorticoids (Ch. 33), high doses of thiazide diuretics (Ch. 29) and several of the protease inhibitors used to treat HIV infection (Ch. 52).

TREATMENT OF DIABETES MELLITUS

Insulin is essential for the treatment of type 1 diabetes, and a valuable component of the treatment of many patients with type 2 disease.

▼ For many years it was assumed, as an act of faith, that normalising plasma glucose would prevent diabetic complications. The Diabetes Control and Complications Trial (American Diabetes Association, 1993) showed that this faith was well placed: type 1 diabetic patients were randomly allocated to intensive or conventional management. Mean fasting blood glucose concentration was 2.8 mmol/l lower in the intensively treated group, who had a substantial reduction in the occurrence and progression of retinopathy, nephropathy and neuropathy over a period of 4–9 years. Benefits, including reduced atheromatous as well as microvascular disease, were long-lasting and outweighed adverse effects, including a three-fold increase in severe hypoglycaemic attacks and modest excess weight gain.

The UK Prospective Diabetes Study showed that *lowering blood pressure* markedly improves outcome in type 2 diabetes. Normalisation of blood glucose was not achieved even in intensively treated patients. Better metabolic control did improve outcome, but (in contrast to lowering blood pressure) the magnitude of the benefit was disappointing and statistically significant only for microvascular complications. In long-term follow-up, patients from this study who had been allocated to intensive treatment continued to have better outcomes than patients treated with diet alone (despite diabetic control becoming similar in the two groups after the blinded treatment period had finished), suggesting that early diabetic control (within the first 12 years from diagnosis) is important (Holman et al., 2008). By contrast, studies of intensive control later in the course of the disease have been disappointing with harm from hypoglycaemia outweighing any benefit.

Realistic goals in type 2 diabetic patients are usually less ambitious than in younger type 1 patients. Dietary restriction leading to weight loss in overweight and obese patients is the cornerstone (albeit one with a tendency to crumble), combined with increased exercise. Oral agents are used to control symptoms from hyperglycaemia, as well as to limit microvascular complications, and are introduced early. Dietary measures and statins to prevent atheromatous disease (Ch. 24) are crucial. Details of dietary management and treatment for specific diabetic complications are beyond the scope of this book. Newer drugs (glitazones and drugs that mimic or potentiate incretins) have been shown to reduce glycated haemoglobin (typically by 0.5–1 percentage points) but their effects (if any) on clinical outcomes such as diabetic complications are unproven.

[5]Neuropathy ('disease of the nerves') causes dysfunction of peripheral nerve fibres, which can be motor, sensory or autonomic. Diabetic neuropathy often causes numbness in a 'stocking' distribution caused by damage to sensory fibres, and postural hypotension and erectile dysfunction due to autonomic neuropathy.

[6]Diabetic control is not easily estimated by determination of blood glucose, because this is so variable. Instead, glycated haemoglobin (haemoglobin A_{1C}) is measured. This provides an integrated measure of control over the lifespan of the red cell: approximately 120 days. In healthy individuals, 4–6% of haemoglobin is glycated; levels above 7% are indicative of diabetes.

INSULIN TREATMENT

The effects of insulin and its mechanism of action are described above. Here we describe pharmacokinetic aspects and adverse effects, both of which are central to its therapeutic use. Insulin for clinical use was once either porcine or bovine but is now almost entirely human (made by recombinant DNA technology). Animal insulins are liable to elicit an immune response; this is less of an issue with recombinant human insulins. Although recombinant insulin is more consistent in quality than insulins extracted from pancreases of freshly slaughtered animals, doses are still quantified in terms of units of activity (Ch. 7), with which doctors and patients are familiar, rather than of mass.

Pharmacokinetic aspects and insulin preparations

Insulin is destroyed in the gastrointestinal tract, and is ordinarily given by injection – usually subcutaneously, but intravenously or occasionally intramuscularly in emergencies. Intraperitoneal insulin can be used in diabetic patients with end-stage renal failure treated by ambulatory peritoneal dialysis. Pulmonary absorption of insulin occurs, but an aerosol formulation was withdrawn from therapeutic use. Other potential approaches include incorporation of insulin into biodegradable polymer microspheres as a slow-release formulation, and its encapsulation with a lectin in a glucose-permeable membrane.[7] Once absorbed, insulin has an elimination half-life of approximately 10 min. It is inactivated enzymically in the liver and kidney, and 10% is excreted in the urine. Renal impairment reduces insulin requirement.

One of the main problems in using insulin is to avoid wide fluctuations in plasma concentration and thus in blood glucose. Different formulations vary in the timing of their peak effect and duration of action. *Soluble insulin* produces a rapid and short-lived effect. Longer-acting preparations are made by precipitating insulin with protamine or zinc, thus forming finely divided amorphous solid or relatively insoluble crystals, which are injected as a suspension from which insulin is slowly absorbed. These preparations include *isophane insulin* and amorphous or crystalline *insulin zinc suspensions*. Mixtures of different forms in fixed proportions are available. **Insulin lispro** is an insulin analogue in which a lysine and a proline residue are 'switched'. It acts more rapidly but for a shorter time than natural insulin, enabling patients to inject themselves immediately before the start of a meal. **Insulin glargine** is another modified insulin analogue, designed with the opposite intention, namely to provide a constant basal insulin supply and mimic physiological postabsorptive basal insulin secretion. Insulin glargine, which is a clear solution, forms a microprecipitate at the physiological pH of subcutaneous tissue, and absorption from the subcutaneous site of injection is prolonged. Used in conjunction with short-acting insulin, it lowers postabsorptive plasma glucose.

Various dosage regimens are used. Some type 1 patients inject a combination of short- and intermediate-acting insulins twice daily, before breakfast and before the evening meal. Improved control of blood glucose can be achieved with multiple daily injections of rapid-acting insulin analogues given with meals, and a basal insulin analogue injected once daily (often at night). Insulin pumps are used in hospital to control blood glucose acutely and sometimes, by specialists, in outpatients. The most sophisticated forms of pump regulate the dose by means of a sensor that continuously measures blood glucose, but these are not used routinely – this seemingly logical approach is limited by the complexity of insulin's effects on intermediary metabolism (see Table 31.2, Fig. 31.3) which are imperfectly captured by interstitial glucose concentration, and by risks of infection.

[7]This could, in theory, provide variable release of insulin controlled by the prevailing glucose concentration, because glucose and glycated insulin compete for binding sites on the lectin.

Unwanted effects

The main undesirable effect of insulin is hypoglycaemia. This is common and, if very severe, can cause brain damage or sudden cardiac death. In the Diabetes Control and Complications Trial mentioned above, intensive insulin therapy resulted in a three-fold increase in severe hypoglycaemic episodes compared with usual care. The treatment of hypoglycaemia is to take a sweet drink or snack or, if the patient is unconscious, to give intravenous glucose or intramuscular glucagon (see clinical box, p. 385). Rebound hyperglycaemia ('Somogyi effect') can follow insulin-induced hypoglycaemia, because of the release of counter-regulatory hormones (e.g. adrenaline, glucagon and glucocorticoids). This can cause hyperglycaemia before breakfast following an unrecognised hypoglycaemic attack during sleep in the early hours of the morning. It is essential to appreciate this possibility to avoid the mistake of increasing (rather than reducing) the evening dose of insulin in this situation.

Allergy to human insulin is unusual but can occur. It may take the form of local or systemic reactions. Insulin resistance as a consequence of antibody formation is rare. Theoretical concerns regarding mitogenic effects of insulin analogues are mentioned above (p. 383).

Clinical uses of insulin and other hypoglycaemic drugs for injection

- Patients with *type 1 diabetes* require long-term **insulin**:
  - an intermediate-acting preparation (e.g. **isophane insulin**) or a long-acting analogue (e.g. **glargine**) is often combined with soluble insulin or a short-acting analogue (e.g. **lispro**) taken before meals.
- **Soluble insulin** is used (intravenously) in emergency treatment of hyperglycaemic emergencies (e.g. *diabetic ketoacidosis*).
- Approximately one-third of patients with *type 2 diabetes* ultimately benefit from **insulin**.
- Short-term treatment of patients with type 2 diabetes or impaired glucose tolerance during intercurrent events (e.g. *operations*, *infections*, *myocardial infarction*).
- During pregnancy, for *gestational diabetes* not controlled by diet alone.
- Emergency treatment of *hyperkalaemia*: **insulin** is given with glucose to lower extracellular K^+ via redistribution into cells.
- **Exenatide** for type 2 diabetes in addition to oral agents to improve control and lose weight.

OTHER HYPOGLYCAEMIC AGENTS

Biguanides

Metformin (present in French lilac, *Galega officinalis*, which was used to treat diabetes in traditional medicine for centuries) is the only biguanide used clinically to treat type 2 diabetes, for which it is now a drug of first choice.[8]

Actions and mechanism

The molecular target or targets through which biguanides act remains unclear, but their biochemical actions are well understood, and include:

- reduced hepatic glucose production (gluconeogenesis; gluconeogenesis is markedly increased in type 2 diabetes)
- increased glucose uptake and utilisation in skeletal muscle (i.e. they reduce insulin resistance)
- reduced carbohydrate absorption from the intestine
- increased fatty acid oxidation
- reduced circulating low-density and very-low-density lipoprotein (LDL and VLDL, respectively, see Ch. 23).

Reduced hepatic gluconeogenesis is especially important. The primary effect of metformin is to decrease hepatic glucose production by inhibiting the mitochondrial respiratory chain complex I (reviewed by Viollet et al., 2012). The resulting decrease in hepatic energy status activates AMPK (AMP-activated protein kinase) which is an important enzyme in metabolic control (Towler & Hardie, 2007). Activation of AMPK increases expression of a nuclear receptor that inhibits expression of genes that are important for gluconeogenesis in the liver (see Kim et al., 2008).

Metformin has a half-life of about 3 h and is excreted unchanged in the urine.

Unwanted effects

Metformin, while preventing hyperglycaemia, does *not* cause hypoglycaemia, and the commonest unwanted effects are dose-related gastrointestinal disturbances (e.g. anorexia, diarrhoea, nausea), which are usually but not always transient. Lactic acidosis is a rare but potentially fatal toxic effect, and metformin should not be given routinely to patients with renal or hepatic disease, hypoxic pulmonary disease or shock. Such patients are predisposed to lactic acidosis because of reduced drug elimination or reduced tissue oxygenation. Compensated heart failure is not a contraindication, and indeed metformin is associated with improved outcome in patients with diabetes and heart failure. It should be avoided in other situations that predispose to lactic acidosis including some forms of mitochondrial myopathy that are associated with diabetes. Long-term use may interfere with absorption of vitamin B_{12}.

Clinical use

Metformin is used to treat patients with type 2 diabetes. It does not stimulate appetite (rather the reverse; see above!) and is consequently the drug of first choice in the majority of type 2 patients who are obese, provided they have unimpaired renal and hepatic function. It can be combined with sulfonylureas, glitazones or insulin. Potential uses outside type 2 diabetes include other syndromes with accompanying insulin resistance including polycystic ovary syndrome, non-alcoholic fatty liver disease, gestational diabetes and some forms of premature puberty.

Sulfonylureas

The sulfonylureas were developed following the chance observation that a sulfonamide derivative (which was being used to treat typhoid) caused hypoglycaemia. Numerous sulfonylureas are available. The first used therapeutically were **tolbutamide** and **chlorpropamide**. Chlorpropamide has a long duration of action and a substantial fraction is excreted in the urine. Consequently, it can cause severe hypoglycaemia, especially in elderly patients in whom renal function declines inevitably but insidiously (Ch. 29). It causes flushing after alcohol because of a disulfiram-like effect (Ch. 49), and has an action like that of antidiuretic hormone on the distal nephron, giving rise to hyponatraemia and water intoxication. Williams (1994) comments that 'time honoured but idiosyncratic chlorpropamide should now be laid to rest' – a sentiment with which we concur. Tolbutamide, however, remains useful. So-called second-generation sulfonylureas (e.g. **glibenclamide**, **glipizide**; see Table 31.3) are more potent, but their maximum hypoglycaemic effect is no greater and control of blood glucose no better than with tolbutamide. These drugs all contain the sulfonylurea moiety and act in the same way, but different substitutions result in differences in pharmacokinetics and hence in duration of action (see Table 31.3).

Mechanism of action

The principal action of sulfonylureas is on B cells (Fig. 31.1), stimulating insulin secretion and thus reducing plasma glucose. High-affinity binding sites for sulfonylureas are present on the K_{ATP} channels (Ch. 4) in the surface membranes of B cells, and the binding of various sulfonylureas parallels their potency in stimulating insulin release. Block by sulfonylurea drugs of K_{ATP} channel activation causes depolarisation, Ca^{2+} entry and insulin secretion. (Compare this with the physiological control of insulin secretion, see Fig. 31.1.)

Pharmacokinetic aspects

Sulfonylureas are well absorbed after oral administration, and most reach peak plasma concentrations within 2–4 h. The duration of action varies (Table 31.3). All bind strongly to plasma albumin and are implicated in interactions with other drugs (e.g. salicylates and sulfonamides) that compete for these binding sites (see Ch. 8). Most sulfonylureas (or their active metabolites) are excreted in the urine, so their action is increased and prolonged in the elderly and in patients with renal disease.

Most sulfonylureas cross the placenta and enter breast milk and their use is contraindicated in pregnancy and in breastfeeding.

[8]Metformin had a very slow start. It was first synthesised in 1922, one of a large series of biguanides with many different pharmacological actions, which proved largely unsuitable for clinical use. Its glucose-lowering effect was noted early on, but was eclipsed by the discovery of insulin. It did not receive FDA approval until 1995. The only other biguanides in routine clinical use are the antimalarial antifolate drugs pyrimethamine and proguanil (Ch. 54).

Table 31.3 Oral hypoglycaemic sulfonylurea drugs

Drug	Relative potency[a]	Duration of action and (half-life) (hours)	Pharmacokinetic aspects[b]	General comments
Tolbutamide	1	6–12 (4)	Some converted in liver to weakly active hydroxytolbutamide; some carboxylated to inactive compound Renal excretion	A safe drug; least likely to cause hypoglycaemia May decrease iodide uptake by thyroid Contraindicated in liver failure
Glibenclamide[c]	150	18–24 (10)	Some is oxidised in the liver to moderately active products and is excreted in urine; 50% is excreted unchanged in the faeces	May cause hypoglycaemia The active metabolite accumulates in renal failure
Glipizide	100	16–24 (7)	Peak plasma levels in 1 h Most is metabolised in the liver to inactive products, which are excreted in urine; 12% is excreted in faeces	May cause hypoglycaemia Has diuretic action Only inactive products accumulate in renal failure

[a]Relative to tolbutamide.
[b]All are highly protein bound (90–95%).
[c]Termed gliburide in USA.

Unwanted effects

The sulfonylureas are usually well tolerated. Unwanted effects are specified in Table 31.3. The commonest adverse effect is hypoglycaemia, which can be severe and prolonged, the highest incidence occurring with long-acting chlorpropamide and glibenclamide and the lowest with tolbutamide. Long-acting sulfonylureas are best avoided in the elderly and in patients with even mild renal impairment because of the risk of hypoglycaemia. Sulfonylureas stimulate appetite and often cause weight gain. This is a major concern in obese diabetic patients. About 3% of patients experience gastrointestinal upsets. Allergic skin rashes can occur, and bone marrow toxicity (Ch. 57), although rare, can be severe.

During and for a few days after acute myocardial infarction in diabetic patients, insulin must be substituted for sulfonylurea treatment. This is associated with a substantial reduction in short-term mortality, although it remains unclear if this is due to a beneficial effect specific to insulin or to a detrimental effect of sulfonylurea drugs in this setting, or both. Another vexing question is whether prolonged therapy with oral hypoglycaemic drugs has adverse effects on the cardiovascular system. Blockade of K_{ATP} in heart and vascular tissue could theoretically have adverse effects, and an observational study recorded an increased risk of death and cardiovascular disease during follow up for up to 8 years in newly diagnosed type 2 diabetic patients treated with sulfonylureas compared with those treated with metformin (Evans et al., 2006).

Drug interactions

Several drugs augment the hypoglycaemic effect of sulfonylureas. Non-steroidal anti-inflammatory drugs, warfarin, some uricosuric drugs (e.g. **sulfinpyrazone**), alcohol, monoamine oxidase inhibitors, some antibacterial drugs (including sulfonamides, **trimethoprim** and **chloramphenicol**) and some imidazole antifungal drugs have all been reported to produce severe hypoglycaemia when given with a sulfonylurea. The probable basis of most of these interactions is competition for metabolising enzymes, but interference with plasma protein binding or with transport mechanisms facilitating excretion may play some part.

Agents that *decrease* the action of sulfonylureas on blood glucose include high doses of thiazide diuretics (Chs 22, 29) and glucocorticoids (pharmacodynamic interactions).

Clinical use

Sulfonylureas are used to treat type 2 diabetes in its early stages, but because they require functional B cells, they are not useful in type 1 or late-stage type 2 diabetes. They can be combined with metformin or with thiazolidinediones.

OTHER DRUGS THAT STIMULATE INSULIN SECRETION

Several drugs that act, like the sulfonylureas, by blocking the sulfonylurea receptor on K_{ATP} channels in pancreatic B cells but lack the sulfonylurea moiety have recently been developed. These include **repaglinide** and **nateglinide** which, though much less potent than most sulfonylureas, have rapid onset and offset kinetics leading to short duration of action and a low risk of hypoglycaemia.[9] These drugs are administered shortly before a meal to reduce the postprandial rise in blood glucose in type 2 diabetic patients inadequately controlled with diet and exercise. They may cause less weight gain than conventional sulfonylureas. Later in the course of the disease, they can be combined with metformin or thiazolidinediones. Unlike glibenclamide, these drugs are relatively selective for K_{ATP} channels on B cells versus K_{ATP} channels in vascular smooth muscle.

[9]It is ironic that these aggressively marketed drugs share many of the properties of tolbutamide, the oldest, least expensive and least fashionable of the sulfonylureas.

Thiazolidinediones (glitazones): pioglitazone

The thiazolidinediones (or *glitazones*) were developed following the chance observation that a **clofibrate** analogue, **ciglitazone**, which was being screened for effects on lipids, unexpectedly lowered blood glucose. Ciglitazone caused liver toxicity, and this class of drugs (despite considerable commercial success) has been dogged by adverse effects (especially cardiovascular), regulatory withdrawals and controversy. No clinical trials of these agents have demonstrated a beneficial effect on mortality, and they were licensed on the basis of statistically significant effects on haemoglobin A1c (a surrogate marker of metabolic control) of uncertain clinical significance. **Pioglitazone** is the only drug of this class that remains in clinical use, its predecessors, rosiglitazone and troglitazone, having been withdrawn because of increased risk of heart attacks and liver damage, respectively – at the time, a *cause célèbre*, and very expensive for the companies involved.

Effects

The effect of thiazolidinediones on blood glucose is slow in onset, the maximum effect being achieved only after 1–2 months of treatment. They act by enhancing the effectiveness of endogenous insulin, thereby reducing hepatic glucose output, and increasing glucose uptake into muscle.

They reduce the amount of exogenous insulin needed to maintain a given level of blood glucose by approximately 30%. Reduced blood glucose concentration is accompanied by reduced insulin and free fatty acid concentrations. Triglycerides decline, while LDL and high-density lipoprotein (HDL) are unchanged or slightly increased. The proportion of small dense LDL particles (believed to be the most atherogenic; Ch. 23) is reduced. Weight gain of 1–4 kg is common, usually stabilising in 6–12 months. Some of this is attributable to fluid retention: there is an increase in plasma volume of up to 500 ml, with a concomitant reduction in haemoglobin concentration caused by haemodilution; there is also an increase in extravascular fluid, and increased deposition of subcutaneous (as opposed to visceral) fat.

Mechanism of action

Thiazolidinediones bind to a nuclear receptor called the *peroxisome proliferator-activated receptor-γ* (PPARγ), which is complexed with retinoid X receptor (RXR; see Ch. 3).[10] PPARγ occurs mainly in adipose tissue, but also in muscle and liver. It causes differentiation of adipocytes (this contributes to the unwanted effect of weight gain), increases lipogenesis and enhances uptake of fatty acids and glucose. It also promotes amiloride-sensitive sodium ion reabsorption in renal collecting ducts, explaining the adverse effect of fluid retention (Guan et al., 2005). Endogenous agonists of PPARγ include unsaturated fatty acids and various derivatives of these, including prostaglandin J_2. Thiazolidinediones are exogenous agonists, which cause the PPARγ–RXR complex to bind to DNA, promoting transcription of several genes with products that are important in insulin signalling. These include lipoprotein lipase, fatty acid transporter protein, adipocyte fatty acid-binding protein, Glut-4, phosphoenolpyruvate carboxykinase, malic enzyme and others. It remains something of a mystery that glucose homeostasis should be so responsive to drugs that bind to receptors found mainly in fat cells; it has been suggested that the explanation may lie in resetting of the glucose–fatty acid (Randle) cycle by the reduction in circulating free fatty acids.

Pharmacokinetic aspects

Pioglitazone is rapidly and nearly completely absorbed, with time to peak plasma concentration of less than 2 h. It is highly (>99%) bound to plasma proteins, and is subject to hepatic metabolism and has a short (<7 h) elimination half-life for the parent drug, but substantially longer (up to 24 h) for the metabolite. Pioglitazone is metabolised mainly by a CYP2C isozyme and CYP3A4 to active metabolites, which are eliminated mainly in bile.

Unwanted effects

Reports of liver dysfunction caused by pioglitazone have been rare; tests of liver function are recommended before treatment and periodically thereafter, especially if symptoms such as dark urine raise the possibility of liver disease. The commonest unwanted effects of pioglitazone are weight gain and fluid retention. Fluid retention is a substantial concern, because it can precipitate or worsen heart failure, which contraindicates its use. In addition to increased cardiovascular risk, both observational studies and meta-analysis of randomised controlled trials (Loke et al., 2009) indicate an increased risk (approximately a doubling of risk) of fractures with chronic use. Its use is associated with a small increased risk of bladder cancer. Non-specific symptoms, including headache, fatigue and gastrointestinal disturbances, have been reported. Pioglitazone is contraindicated in pregnant or breastfeeding women and in children. It is theoretically possible that these drugs could cause ovulation to resume in women who are anovulatory because of insulin resistance (e.g. with polycystic ovary syndrome).

Clinical use

Pioglitazone is additive with other oral hypoglycaemic drugs in terms of effect on blood glucose, and a combination tablet with metformin is marketed. It may lessen the progression of impaired glucose tolerance to diabetes, and may reduce the need for exogenous insulin in type 2 diabetic patients. Combination with insulin may increase the risk of heart disease.

α-Glucosidase inhibitors

Acarbose, an inhibitor of intestinal α-glucosidase, is used in type 2 diabetes inadequately controlled by diet with or without other agents. It delays carbohydrate absorption, reducing the postprandial increase in blood glucose. The commonest adverse effects are related to its main action and consist of flatulence, loose stools or diarrhoea, and abdominal pain and bloating. Like metformin, it may be particularly helpful in obese type 2 patients, and it can be co-administered with metformin.

Incretin mimetics and related drugs

Exenatide is a synthetic version of *exendin-4*, a peptide found in the saliva of the Gila monster (a lizard that presumably evolved this as means to disable its prey by rendering them hypoglycaemic).

Exenatide mimics the effects of GLP-1 (see above), but is longer acting. **Liraglutide** is an alternative injectable GLP-1 agonist. These drugs lower blood glucose after a

[10] Compare with fibrates (to which thiazolidinediones are structurally related), which bind to PPARα (see Ch. 23).

meal by increasing insulin secretion, suppressing glucagon secretion and slowing gastric emptying (see above). They reduce food intake (by an effect on satiety, see Ch. 32) and are associated with modest weight loss. They reduce hepatic fat accumulation.

Exenatide is not absorbed by the gut and is administered subcutaneously. It is much more stable than GLP-1, and is administered twice daily before the first and last meal of the day. A modified release formulation is available for once weekly injection and is used in combination with metformin and a sulfonylurea in poorly controlled obese patients. It can cause hypoglycaemia and a range of gastrointestinal effects. Pancreatitis is less common but potentially severe.

Exenatide or liraglutide are used in patients with type 2 diabetes in combination with other drugs (metformin with or without a sulfonylurea, pioglitazone, insulin); they are recommended in obese patients who have failed on dual therapy and it is recommended to continue them only if they cause a drop in haemoglobin A1c of ≥1 %-age point after 6 months together with a weight loss of at least 3%. As with the glitazones evidence of cardiovascular efficacy or effect on mortality is missing so risk benefit is arguable (see Cohen, 2013 for a popular account).

Gliptins

Gliptins (e.g. **sitagliptin**, **vildagliptin**, **saxagliptin**, **linagliptin**) are synthetic drugs that competitively inhibit dipeptidylpeptidase-4 (DPP-4), thereby lowering blood glucose by potentiating endogenous incretins (GLP-1 and GIP, see p. 385) which stimulate insulin secretion. They do not cause weight loss or weight gain.

They are absorbed from the gut and administered once (or, in the case of vildagliptin, twice) daily by mouth. They are eliminated partly by renal excretion and are also metabolised by hepatic CYP enzymes. They are usually well tolerated with a range of gastrointestinal adverse effects; occasional liver disease, worsening of heart failure and pancreatitis (incidence approximately 0.1–1%) are less common but potentially serious. There is also concern that they may act as tumour promoters (see Ch. 57). Gliptins are used for type 2 diabetes in addition to other oral hypoglycaemic drugs (see clinical box on uses of oral hypoglycaemic drugs, p. 392).

POTENTIAL NEW ANTIDIABETIC DRUGS

Several agents are currently being studied, including α_2-adrenoceptor antagonists, inhibitors of fatty acid oxidation and activators of glucokinase. Lipolysis in fat cells is controlled by adrenoceptors of the β_3 subtype (see Ch. 14). The possibility of using selective β_3 agonists, currently in development, in the treatment of obese patients with type 2 diabetes is being investigated (see Ch. 32).

Drugs used in diabetes mellitus

Insulin and other injectable drugs

- Human **insulin** is made by recombinant DNA technology. For routine use, it is given subcutaneously (by intravenous infusion in emergencies).
- Different formulations of **insulin** differ in their duration of action:
  - fast- and short-acting **soluble insulin**: peak action after subcutaneous dose 2–4 h and duration 6–8 h; it is the only formulation that can be given intravenously
  - intermediate-acting insulin (e.g. **isophane insulin**)
  - long-acting forms (e.g. **insulin zinc suspension**).
- The main unwanted effect is hypoglycaemia.
- Altering the amino acid sequence (insulin analogues, e.g. **lispro** and **glargine**) can usefully alter **insulin** kinetics.
- **Insulins** are used for all type 1 diabetic patients and approximately one-third of patients with type 2 diabetes.
- **Exenatide** and **liraglutide** are injectable GLP-1 agonists used as add-on treatment in certain inadequately controlled type 2 diabetic patients. Unlike **insulin** they cause weight loss.

Oral hypoglycaemic drugs

- These are used in type 2 diabetes.
- Biguanides (e.g. **metformin**):
  - have complex peripheral actions in the presence of residual insulin, increasing glucose uptake in striated muscle and inhibiting hepatic glucose output and intestinal glucose absorption
  - cause anorexia and encourage weight loss
  - can be combined with sulfonylureas.
- Sulfonylureas and other drugs that stimulate insulin secretion (e.g. **tolbutamide**, **glibenclamide**, **nateglinide**):
  - can cause hypoglycaemia (which stimulates appetite and leads to weight gain)
  - are effective only if B cells are functional
  - block ATP-sensitive potassium channels in B cells
  - are well tolerated but promote weight gain and are associated with more cardiovascular disease than is **metformin**.
- Thiazolidinediones have been associated with serious hepatic and cardiac toxicity. **Pioglitazone** is the only one still marketed; it:
  - increases insulin sensitivity and lowers blood glucose in type 2 diabetes
  - can cause weight gain and oedema
  - increases osteoporotic fractures
  - is a peroxisome proliferator-activated receptor-γ (a nuclear receptor) agonist.
- Gliptins (e.g. **sitagliptin**):
  - potentiate endogenous incretins by blocking DPP-4
  - are added to other orally active drugs to improve control in patients with type 2 diabetes
  - are weight-neutral; they are usually well tolerated but pancreatitis is a concern.
- α-Glucosidase inhibitor, **acarbose**:
  - reduces carbohydrate absorption
  - causes flatulence and diarrhoea.

Clinical uses of oral hypoglycaemic drugs

- *Type 2 diabetes mellitus*, to reduce symptoms from hyperglycaemia (e.g. thirst, excessive urination). ('Tight' control of blood glucose has only a small effect on vascular complications in this setting.)
- **Metformin** is preferred, especially for obese patients unless contraindicated by factors that predispose to lactic acidosis (renal or liver failure, poorly compensated heart failure, hypoxaemia).
- **Acarbose** (α-glucosidase inhibitor) reduces carbohydrate absorption; it causes flatulence and diarrhoea.
- Drugs that act on the sulfonylurea receptor (e.g. **tolbutamide**, **glibenclamide**) are well tolerated but often promote weight gain. They are associated with increased cardiovascular risk compared with **metformin**.
- **Pioglitazone** improves control (reduces haemoglobin A_{1C}) but increases weight, causes fluid retention and increases risk of fractures. GLP-1 agonists (**exenatide** or **liraglutide**) are injected once daily or (**extended release exenatide**) once weekly in obese patients inadequately controlled on two hypoglycaemic drugs.
- DPP4 inhibitors (gliptins, e.g. **sitagliptin**) improve control, are well tolerated and weight-neutral, but long-term experience is lacking, as is outcome evidence. Pancreatitis is a concern.

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32 Obesity

OVERVIEW

Obesity is a growing health issue around the world and is reaching epidemic proportions in some nations. The problem is not restricted to the inhabitants of the affluent countries, to the adult population or to any one socioeconomic class. Body fat represents stored energy and obesity occurs when the homeostatic mechanisms controlling energy balance become disordered or overwhelmed. In this chapter we first outline the endogenous regulation of appetite and body mass, and then consider the main health implications of obesity and its pathophysiology. We conclude with a discussion of the drugs currently licensed for the treatment of obesity and glance at the future of pharmacological treatment of this condition.

INTRODUCTION

Survival requires a continuous provision of energy to maintain homeostasis even when the supply of food is intermittent. Evolution has furnished a mechanism for storing excess energy latent in foodstuffs in adipose tissue as energy-dense triglycerides, such that these can be easily mobilised when food is scarce. This mechanism, controlled by the so-called thrifty genes, was an obvious asset to our hunter–gatherer ancestors. However, in many societies a combination of sedentary lifestyle, genetic susceptibility, cultural influences and unrestricted access to an ample supply of calorie-dense foods has lead to a global epidemic of obesity, or 'globesity' as it is sometimes called. Obesity is one component of a cluster of disorders described in other chapters, which often coexist in the same individual, comprising what is now described as 'metabolic syndrome', a rapidly growing public health problem.

DEFINITION OF OBESITY

'Obesity' may be defined as an illness where health (and hence life expectancy) is adversely affected by excess body fat.[1] But at what point does an individual become 'obese'? The generally accepted benchmark is the body mass index (BMI). The BMI is expressed as W/h^2, where W = body weight (in kg), h = height (in metres). Although it is not a perfect index (e.g. it does not distinguish between fat and lean mass), the BMI is generally well correlated with other measurements of body fat, and it is widely utilised as a convenient index. While there are problems in defining a 'healthy' weight for a particular population, the World Health Organization (WHO) classifies adults with a BMI of ≥25 as being overweight and those with a BMI of ≥30 as obese. Childhood obesity is more difficult to assess.

[1] 'Persons who are naturally very fat are apt to die earlier than those who are slender' observed Hippocrates.

Since the BMI obviously depends on the overall energy balance, another operational definition of obesity would be that it is a multifactorial disorder of energy balance in which calorie intake over the long term exceeds energy output.

OBESITY AS A HEALTH PROBLEM

Obesity is a growing and costly global health problem. The WHO in 2008 estimated that there were already more than 1.4 billion overweight adults, approximately half of whom – amounting to more than 10% of the world's population – were obese according to the criteria outlined above. National obesity levels vary enormously, being less than 5% in China, Japan and parts of Africa, and a staggering 75% in parts of Samoa. Adult obesity levels in the USA, Europe and the UK (among others) have increased threefold since 1980, with figures of 35.9% being quoted for the USA (2010 figures; Xia & Grant, 2013) and about 25% for many other industrialised nations (Padwal et al., 2003). The disease is not confined to adults: some 40 million children under 5 years old are estimated to be overweight (2011 figures). In the USA, the number of overweight children has doubled and the number of overweight adolescents has trebled since 1980. Ironically, obesity often coexists with malnutrition in many developing countries. All socioeconomic classes are affected. In the poorest countries, it is the top socioeconomic classes in whom obesity is prevalent, but in the West it is usually the reverse.

Overall, more people die in the world from being overweight and obese than being underweight. The financial burden on the healthcare system is huge. The cost of treating obesity in the USA alone was $198 billion in 2010 (Xia & Grant, 2013).

▼ While obesity itself is rarely fatal, it often coexists with metabolic and other disorders (particularly hypertension, hypercholesterolaemia and type 2 diabetes), together comprising the *metabolic syndrome*. This carries a high risk of cardiovascular conditions, strokes, cancers (particularly hormone-dependent), respiratory disorders (particularly sleep apnoea) and digestive problems, as well as osteoarthritis. One commentator (Kopelman, 2000) has remarked that obesity 'is beginning to replace under-nutrition and infectious diseases as the most significant contributor to ill health'. Increasingly, social stigma is suffered by obese individuals, leading to a sense of psychological isolation.

The risk of developing type 2 diabetes (which represents 85% of all cases of the disease) rises sharply with increasing BMI. The WHO reports that 90% of those diagnosed with the disease are obese. In a study of the disease in women, the risk of developing diabetes was closely correlated with BMI, increasing five-fold when the BMI was 25 kg/m^2, to 93-fold when the BMI was 35 kg/m^2 or above (Colditz et al., 1995). Cardiovascular disease is also increased in the obese individual, and the increased thoracic and abdominal adipose tissue reduces lung volume and makes respiration difficult. Obese subjects also have an increased risk of colon, breast, prostate, gall bladder, ovarian and uterine cancer. Numerous other disorders are associated with excess body weight, including osteoarthritis, hyperuricaemia and male hypogonadism. 'Gross' obesity (BMI ≥40 kg/m^2) is associated with a 12-fold increase in mortality in the group aged 25–35 years compared with those in this age group with a BMI of 20–25 kg/m^2.

HOMEOSTATIC MECHANISMS CONTROLLING ENERGY BALANCE

A common view, and one that is implicitly encouraged by authors of numerous books as well as the enormously lucrative dieting industry, is that obesity is simply the result of bad diet or willful overeating (hyperphagia). In truth, however, the situation is more complex. On its own, dieting seldom provides a lasting solution: the failure rate is high (probably 90%), and most dieters eventually return to their original starting weight. This suggests the operation of some intrinsic homeostatic system that operates to maintain a particular set weight. This mechanism is normally exceptionally precise, and is capable of regulating energy balance to 0.17% per decade (Weigle, 1994), a truly astonishing feat, considering the day-to-day variations in food intake.

When exposed to the same dietary choices some individuals will become obese whereas others will not. Studies of obesity in monozygotic and dizygotic twins have established a strong genetic influence on the susceptibility to the condition, and studies of rare mutations in mice (and more recently in humans) have led to the discovery and elucidation of the neuroendocrine pathways that match food intake with energy expenditure. These, in turn, have led to the concept that it is in fact disorders of these control systems that are largely responsible for the onset and maintenance of obesity.

THE ROLE OF GUT AND OTHER HORMONES IN BODY WEIGHT REGULATION

At the beginning of the 20th century it was observed that patients with damage to the hypothalamus tended to gain weight. In the 1940s it was also shown that discrete lesions in the hypothalamus of rodents caused them to become obese or exhibit unusual feeding behaviour. On the basis of experiments with rats, Kennedy proposed as early as 1953, that a hormone released from adipose tissue acted on the hypothalamus to regulate body fat and food intake. These seminal findings set the stage for future discoveries in this area.

It also was observed that mice could become obese as a result of mutations in certain genes. At least five of these have now been characterised, including the *Ob* (obesity), *Tub* (tubby), *Fat* and *Db* (diabetes) genes. Mice that are homozygous for mutant forms of these genes – *Ob/Ob* mice and *Db/Db* mice – eat excessively, have low energy expenditure, become grossly fat and have numerous metabolic and other abnormalities. Weight gain in an *Ob/Ob* mouse is suppressed if its circulation is linked to that of a normal mouse, implying that the obesity is caused by lack of a blood-borne factor.

An important conceptual breakthrough came in 1994, when Friedman and his colleagues (see Zhang et al., 1994) cloned the *Ob* gene and identified its protein product as leptin.[2] When recombinant leptin was administered systemically to *Ob/Ob* mice, it strikingly reduced food intake and body weight. It had a similar effect when injected directly into the lateral or the third ventricle, implying that it acted on the regions of the brain that control food intake and energy balance. Recombinant leptin has similar effects in humans (see Fig. 32.1).

[2]The word is derived from the Greek *leptos*, meaning thin.

Leptin mRNA is expressed in adipocytes; its synthesis is increased by glucocorticoids, insulin and oestrogens, and is reduced by β-adrenoceptor agonists. In normal human subjects, the release of leptin is pulsatile and varies according to the state of the fat stores and the BMI. Insulin (see Ch. 31) can also function in a similar manner.

Today, it is recognised that in addition to leptin and insulin, several other mediators originating mainly from the gastrointestinal (GI) tract as well as in the hypothalamus, play a crucial role in determining food intake, meal size and the feeling of satisfaction produced ('satiety').[3] Peptide hormones secreted by cells in the wall of the small intestine in response to the arrival of food (see Ch. 30) are important in this connection. Table 32.1 and Figure 32.2 summarise the chief characteristics of these mediators.

The majority of these factors are released either during, or in anticipation of, eating and most are inhibitory in nature, producing either satiety or satiation. Two exceptions are the gastric hormone, ghrelin, which promotes hunger, and leptin itself, which is controlled by the amount of adipose tissue and is thus more involved with the longer-term energy status of the individual. The main targets for these hormones are receptors on vagal afferent fibres or within the hypothalamus (or elsewhere in the central nervous system [CNS]). Here, they modulate the release of other neurotransmitters that exert a fine regulation over eating behaviour, energy expenditure and body weight. Other actions of these peptide hormones include the release of insulin by the *incretins* (see Ch. 31), which include glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP).

NEUROLOGICAL CIRCUITS THAT CONTROL BODY WEIGHT AND EATING BEHAVIOUR

CONTROL OF FOOD INTAKE

The manner in which all these hormonal signals are processed and integrated with other viscerosensory, gustatory or olfactory information within the CNS is complex. Many sites are involved in different aspects of the process and some 50 hormones and neurotransmitters are implicated. The account we present here is therefore necessarily an oversimplification: the Further Reading list should be consulted for a more complete picture.

As early lesioning studies predicted, the hypothalamus is the main brain centre that regulates appetite, feeding behaviour and energy status, although other sites in the brain such as the nucleus accumbens (NAc), the amygdala and especially the nucleus tractus solitarius (NTS) in the medulla, are also crucial. Within the hypothalamus, the arcuate nucleus (ARC), situated in the floor of the third ventricle, is a key site. It receives afferent signals originating from the GI tract and contains receptors for leptin and other significant hormones. It also has extensive reciprocal connections with other parts of the hypothalamus involved in monitoring energy status, in particular the paraventricular nuclei and the ventromedial hypothalamus. Figure 32.2 summarises in a simplified fashion some of the interactions that occur in the ARC.

Within the ARC are two groups of functionally distinct neurons that exert opposite effects on appetite. One group,

[3]The terminology can be confusing. 'Hunger' obviously refers to the desire to eat; 'satiation' is the feeling that you have eaten enough in the course of a meal. 'Satiety' refers to the feeling after a meal that you don't yet need another.

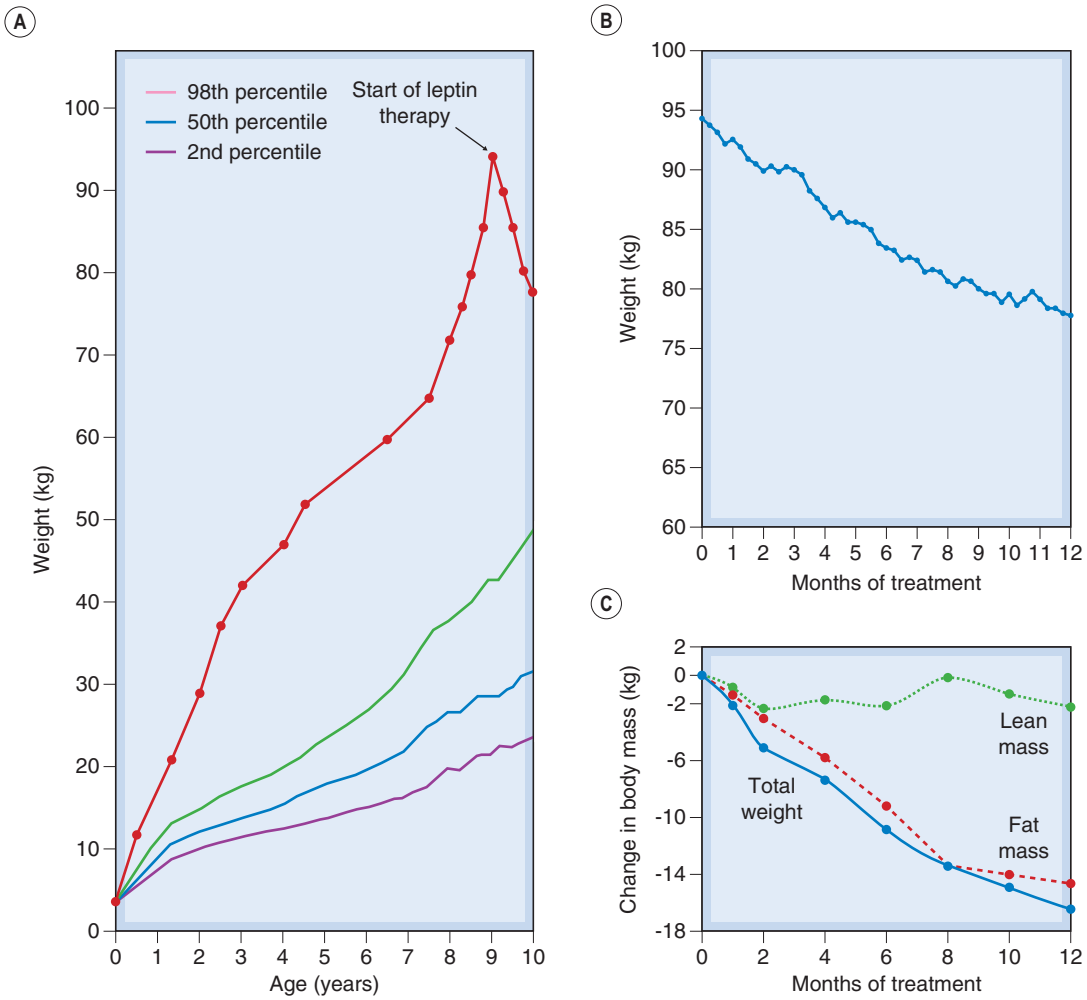

Fig. 32.1 The effect of recombinant leptin on body weight in a 9-year-old severely obese child with endogenous leptin deficiency because of a frame shift mutation in the leptin gene. Although of normal birth weight, the child began gaining weight at 4 months and was constantly demanding food. Prior to treatment, the child weighed 94.4 kg. Weight loss began after 2 weeks' treatment, and her eating pattern returned to normal. She had lost 15.6 kg of body fat after 1 year of treatment. *(Data and figure adapted from Farooqi et al. 1999.)*

Table 32.1 Some peripheral hormones that regulate eating behaviour

Hormone	Source	Stimulus to release	Target	Effect
CCK	GI tract	During feeding or just before	Vagal afferents	Limits size of meal
Amylin, insulin, glucagon	Pancreas	During feeding or just before	Vagal afferents	Limits size of meal
PYY3–36	Ileum, colon	After feeding	Brain stem, hypothalamus	Postpones need for next meal
GLP-1	Stomach	After feeding	Brain stem, hypothalamus	Postpones need for next meal
Oxcyntomodulin	Stomach	After feeding	Brain stem, hypothalamus	Postpones need for next meal
Leptin	Adipose tissue	Adiposity 'status'	Brain stem, arcuate nucleus	Longer-term regulation of food intake
Ghrelin	Stomach	Hunger, feeding	Vagus, hypothalamus	Increases food intake by increasing size and number of meals

CCK, cholecystokinin; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; PYY3–36, peptide YY.

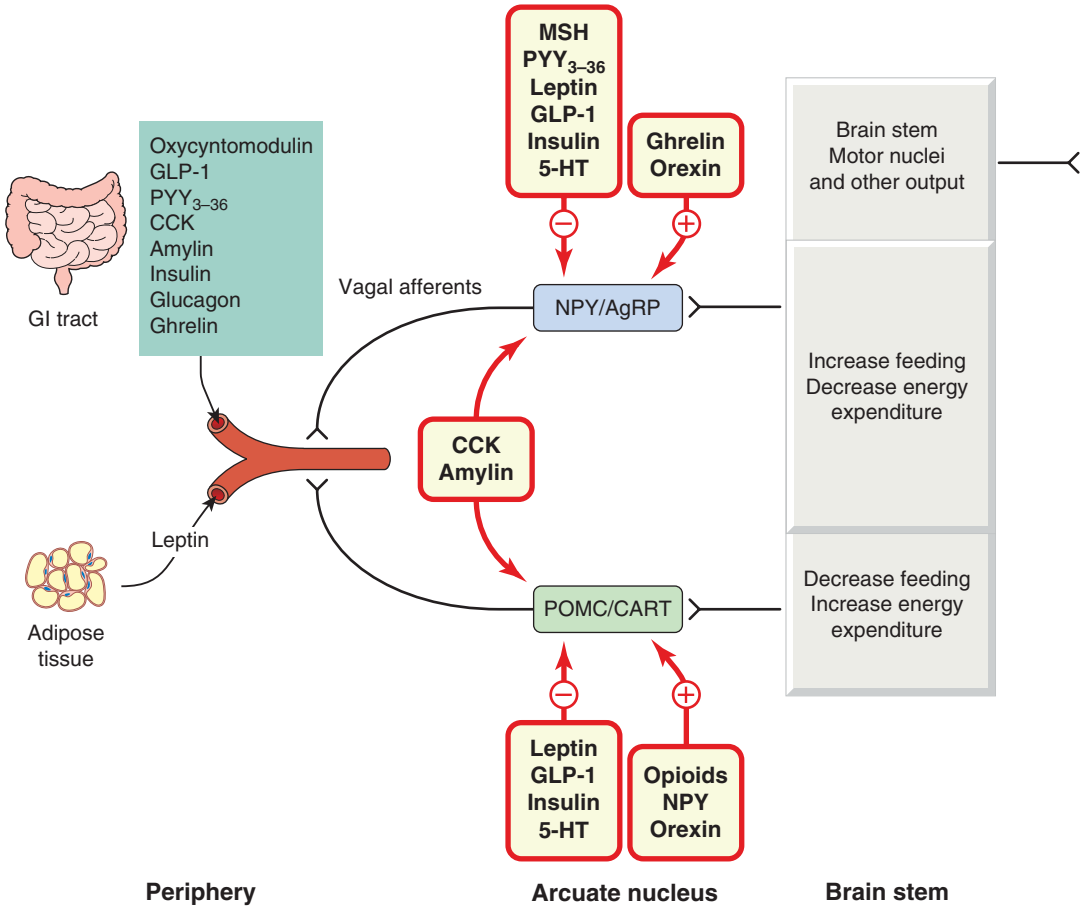

Fig. 32.2 A simplified representation of the role of peripheral hormones and other mediators in the regulation of energy balance and fat stores. The primary level of hypothalamic control is vested in two groups of neurons, with opposing actions, in the arcuate nucleus (ARC). In one group, the peptides neuropeptide Y (NPY) and agouti-related protein (AgRP) are co-localised; the other contains the polypeptides prepro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART), which release α-melanocyte-stimulating hormone (MSH). Blood-borne hormones arising from the gastrointestinal (GI) tract or adipose tissue are sensed by receptors on vagal and other afferents and are relayed through the nucleus tractus solitarius to modify the activity of these neuronal circuits. The influence of hormones on each neuronal group is indicated. Some (e.g. leptin) arise from the peripheral blood and influence the ARC neurons directly or indirectly through neuronal signals; while others (e.g. 5-hydroxytryptamine [5-HT], orexin) originate within the central nervous system itself. Activation of the NPY/AgRP group by, for example, a fall in leptin or an increase in ghrelin levels results in increased food intake and decreased energy expenditure. In the POMC/CART group of neurons, increased leptin or other hormone levels triggered by overfeeding produces a predominately inhibitory effect on feeding behaviour. A number of other hormones such as cholecystokinin (CCK) and amylin also alter the properties of the ARC neurons although the mechanism is not clear. GLP-1, glucagon-like peptide-1; PYY_{3-36}, peptide YY. *(Modified from Adan et al. 2008.)*

termed *anorexigenic* (appetite-suppressing), secrete pro-opiomelanocortin (POMC)-derived peptides (such as α-melanocyte-stimulating hormone; α-MSH) or cocaine- and amphetamine-regulated transcript (CART)[4]-derived peptides. The other group, termed *orexigenic* (appetite-promoting) neurons, secrete neuropeptide Y (NPY) or agouti-related peptide (AgRP). As these groups of neurons have opposing actions, energy homeostasis depends, in the first instance, on the balance between these actions whose final effects are transduced by the brain stem motor system and change feeding behaviour.

Monoamines such as noradrenaline, 5-hydroxytryptamine (5-HT) and dopamine also play a role in the modulation of satiety signals. Noradrenaline is co-localised with NPY in some neurons and greatly potentiates its hyperphagic action. Deficit of dopamine impairs feeding behaviour, as do agonists at the 5-HT_{2C} receptor; antagonists at this receptor have the reverse effect.

Many neural signals arising from the GI tract are integrated, and relayed on to the hypothalamus, by the NTS in the medulla. Some of these signals, including those of gustatory, olfactory, mechanical and viscerosensory signals, arise from vagal and other spinal afferents originating in the GI tract or liver. Endocrine signals have more complex signalling pathways. For example, cholecystokinin (CCK) is secreted by the duodenum in response to the process of eating and digestion of (especially fatty) foodstuffs. CCK acts locally on CCK_A receptors in the GI tract to stimulate vagal afferents and may also act on CCK_B receptors in the brain in order to function as a satiety factor. Ghrelin stimulates growth hormone release (Ch. 33) and also has a direct action on neurons in the ARC to

[4]So called because the administration of cocaine or amphetamine stimulates the transcription of this gene. Its expression in the hypothalamus is related to nutritional status implicating it in the control of appetite. Its receptor is unknown but it probably modulates the action of NPY and leptin.

modify feeding behaviour. Blood ghrelin levels normally fall after eating but not in obese individuals (English et al., 2002). Interestingly, polymorphisms in the ghrelin gene may be important in the pathogenesis of the *Prader–Willi syndrome*, a rare genetic childhood disorder that predisposes to life-threatening obesity.

Leptin also targets these neurons in the ARC. Falling leptin levels activate the orexigenic neurons, resulting in increased food intake and synthesis and storage of fat (anabolism), as well as decreased energy expenditure. Conversely, rising leptin levels activate the second group of neurons, producing the opposite anorexigenic and catabolic effect.

Inputs from other parts of the CNS also influence feeding behaviour. Of importance to us is the input from the NAc. This centre seems to regulate those aspects of eating that are driven by pleasure or reward – the so-called 'hedonic' aspects of eating (see also Ch. 49). The endocannabinoid system is important in this response. The hypothalamus contains large amounts of 2-arachidonyl glycerol and anandamide as well as the CB_1 receptor (Ch. 19). Administration of endogenous or exogenous (e.g. Δ9-THC) cannabinoids provokes a powerful feeding response.[5] This system in turn may be modulated by 'stress' and other factors in the environment.

CONTROL OF ENERGY EXPENDITURE

Balancing food intake is the energy expenditure required to maintain metabolism, physical activity and thermogenesis (heat production). The metabolic aspects include, among other things, cardiorespiratory work and the energy required by a multitude of enzymes. Physical activity increases all these, as well as increasing energy consumption by skeletal muscles. Exposure to cold also stimulates thermogenesis, and the reverse is also true. The, often dramatic (20–40% increase), thermogenic effect of feeding itself may provide a partial protection against developing obesity.

The sympathetic nervous system (sometimes in concert with thyroid hormone) plays a significant part in energy regulation in cardiovascular and skeletal muscle function during physical activity, as well as the thermogenic response of adipose tissue and the response to cold. Both 'white' and (especially) 'brown' fat cells (the colour is caused by the high density of mitochondria) play a major role in thermogenesis. Brown fat, which is densely innervated by the sympathetic nervous system, is abundant in rodents and human infants, although in human adults these cells are generally to be found more interspersed amongst white fat cells. Because of their abundant mitochondria, they are remarkable heat generators. The basis for this, as determined in mice, is the presence of mitochondrial uncoupling proteins (UCP). Three isoforms, UCP-1, -2 and -3, are known and have different distributions, although all are found in brown fat. These proteins 'uncouple' oxidative phosphorylation, so that mitochondria dissipate most energy as heat rather than producing ATP. As one might anticipate, exposure to cold or leptin administration increases both the activity and (after prolonged stimulation) the amount of UCP-1 in brown fat. Noradrenaline, acting on β adrenoceptors (mainly β_3) in brown fat, increases the activity of the peroxisome proliferator-activated receptor-γ (PPARγ) transcription factor, which, in turn, activates the gene for UCP-1. The expression of β_3 adrenoceptors is decreased in genetically obese mice.

[5]This effect is responsible for the 'munchies', a common side effect of smoking cannabis.

Energy balance

Energy balance depends on food intake, energy storage in fat and energy expenditure. In most individuals the process is tightly regulated by a homeostatic system that integrates inputs from a number of internal sensors and external factors. Important components of the system include the following:

- Hormones that signal the status of fat stores (e.g. leptin). Increasing fat storage promotes leptin release from adipocytes.
- Hormones released from the gut during feeding that convey sensations of hunger (e.g. ghrelin), satiety (e.g. CCK) or satiation (e.g. PYY3–36).
- This hormonal information together with neural, gustatory, olfactory and viscerosensory input is integrated in the hypothalamus. The arcuate nucleus is a key site.
- Two groups of opposing neurons in the arcuate nucleus sense hormonal and other signals. Those secreting POMC/CART products promote feeding while those secreting NPY/AgRP inhibit feeding. Many other CNS neurotransmitters (e.g. endocannabinoids) are involved.

The net output from this process is relayed to other sites in the brain stem motor nuclei that control feeding behaviour.

THE PATHOPHYSIOLOGY OF HUMAN OBESITY

In most adults, body fat and body weight remain more or less constant over many years, even decades, in the face of very large variations in food intake and energy expenditure amounting to about a million calories per year. The steady-state body weight and BMI of an individual, as explained, depends upon the integration of multiple interacting regulatory pathways. How, then, does obesity occur? Why is it so difficult for the obese to lose weight and maintain the lower weight?

The main determinant is manifestly a disturbance of the homeostatic mechanisms that control energy balance, and genetic endowment underlies this disturbance. Other factors, such as food availability and lack of physical activity, also contribute. Additionally, of course, there are overlaying social, cultural and psychological aspects. We discuss here the physiological and genetic mechanisms; the role of social, cultural and psychological aspects we will leave (with a profound sigh of relief) to the psychosociologists!

FOOD INTAKE AND OBESITY

As Spiegelman & Flier (1996) point out, 'one need not be a rocket scientist to notice that increased food intake tends to be associated with obesity'. A typical obese subject will usually gain 20 kg over a decade or so. This means that there has been a daily excess of energy input over energy requirement of 30–40 kcal initially, increasing gradually to maintain the increased body weight.

The type of food eaten, as well as the quantity, can disturb energy homeostasis. Fat is an energy-dense foodstuff, and it may be that the satiety mechanisms regulating appetite, which react rapidly to carbohydrate and protein, react too slowly to stop an individual consuming excess fat.

However, when obese individuals reduce their calorie intake as part of a diet regime, they shift into negative energy balance. When they lose weight, the resting metabolic rate decreases, and there is a concomitant reduction in energy expenditure. Thus an individual who was previously obese and is now of normal weight generally needs fewer calories to maintain that weight than an individual who has never been obese. The decrease in energy expenditure appears to be largely caused by an alteration in the conversion efficiency of chemical energy to mechanical work in the skeletal muscles. This adaptation to the caloric reduction contributes to the difficulty of maintaining weight loss by diet.

PHYSICAL EXERCISE AND OBESITY

It used to be said that the only exercise effective in combating obesity was pushing one's chair back from the table. It is now recognised that physical activity – i.e. increased energy expenditure – has a much more positive role in reducing fat storage and adjusting energy balance in the obese, particularly if associated with modification of the diet. An inadvertent, natural population study provides an example. Many years ago, a tribe of Pima Indians split into two groups. One group in Mexico continued to live simply at subsistence level, eating frugally and spending most of the week in hard physical labour. They are generally lean and have a low incidence of type 2 diabetes. The other group settled in the USA – an environment with easy access to calorie-rich food and less need for hard physical work. They are, on average, 57 lb (26 kg) heavier than the Mexican group and have a high incidence of early-onset type 2 diabetes.

OBESITY AS A DISORDER OF THE HOMEOSTATIC CONTROL OF ENERGY BALANCE

Because the homeostatic control of energy balance is complex, it is not easy to determine exactly what goes wrong in obesity.[6] When the leptin story unfolded, it was thought that alterations in leptin kinetics might provide a simple explanation. There is a considerable interindividual variation in sensitivity to leptin, and some individuals seem to produce insufficient amounts of this hormone. Paradoxically, however, plasma leptin is often higher in obese compared with non-obese subjects, not lower as might be expected. The reason for this is that resistance to leptin, rather than insufficient hormone, is more prevalent in obesity. Such resistance could be caused by defects in leptin carriage in the circulation, transport into the CNS, in leptin receptors in the hypothalamus (as occurs in obese *Db/Db* mice) or in post-receptor signalling.

Mediators other than leptin are also implicated. For example, tumour necrosis factor (TNF)-α, a cytokine that can relay information from fat tissue to brain, is increased in the adipose tissue of insulin-resistant obese individuals. Reduced insulin sensitivity of muscle and fat also occurs, as well as decreased β_3 adrenoceptor function in brown adipose tissue; alternatively, the uncoupling protein UCP-2 in adipocytes, may be dysfunctional.

A further suggestion is that alterations in the function of specific nuclear receptors, such as PPARα, β and γ, may play a role in obesity. These receptors regulate gene expression of enzymes associated with lipid and glucose homeostasis, and they also promote the formation of adipose tissue. PPARγ is expressed preferentially in fat cells and synergises with another transcription factor, C/EBPα, to convert precursor cells to fat cells (see Spiegelman & Flier, 1996). The gene for UCP in white fat cells also has regulatory sites that respond to PPARα and C/EBPα. **Pioglitazone**, used to treat type 2 diabetes (see Ch. 31), activates PPARγ and causes weight gain. The pathophysiology of obesity could involve disturbance(s) in any of the multitude of other factors involved in energy balance.

GENETIC FACTORS AND OBESITY

Analyses of large-scale (>100 000) studies in human monozygotic and dizygotic twin pairs indicated that 50–90% of the variance of BMI can be attributed to genetic factors, and suggested a relatively minor role for environmental influences (Barsh et al., 2000). The prevailing view is that *susceptibility* to obesity is largely determined genetically, while environmental factors regulate the *expression* of the disease.

The discovery that spontaneous mutations arising in single genes (e.g. the *Ob/Ob* genotype) produced obese phenotypes in mice led to a search for equivalent genes in humans. A review (Pérusse et al., 2005) identified over 170 human obesity cases that could be traced to single gene mutations in 10 different genes. Leptin receptor or POMC mutations are sometimes observed, but melanocortin MC_4 receptor mutations seem to be more prevalent (3–5%) in obese patients (e.g. see Barsh et al., 2000), and MC_4 agonists are being explored as potential appetite suppressants (as well as potential treatments for erectile dysfunction – another hypothalamic function in which they are implicated).

Other genes that may be involved include the neurotransmitter receptors involved in the central processing of appetite/energy expenditure (e.g. the CB_1, D_2, $5\text{-}HT_{2C}$ receptors), the β_3 adrenoceptor and the glucocorticoid receptor. Decreased function of the β_3 adrenoceptor gene could be associated with impairment of lipolysis in white fat or with thermogenesis in brown fat. A mutation of this gene has been found to be associated with abdominal obesity, insulin resistance and early-onset type 2 diabetes in some subjects and a markedly increased propensity to gain weight in a separate group of morbidly obese subjects. Alterations in the function of the glucocorticoid receptor could be associated with obesity through the permissive effect of glucocorticoids on several aspects of fat metabolism and energy balance. The significance of polymorphisms in the ghrelin gene has already been mentioned.

Overall, some 600 genes, markers and chromosomal regions are under investigation for linkage to human obesity (Pérusse et al., 2005), and it is likely (see Xia & Grant, 2013), that obesity is probably a polygenic disorder

[6]Even the type of gut flora has come under scrutiny as a potential determining factor in obesity. The notion that this could be supplemented with 'probiotics' to modify the risk is attracting attention. 'Holy shit!' was the title of one magazine article on the subject (*The Economist*, 12 November 2009).

with many genes each having a small effect. And this is without taking into account any further contributions from epigenetic changes or alterations in copy number of genes that regulate obesity. Clearly it will be a while before we have a clear appreciation of all these issues.

Obesity

- Obesity is a multifactorial disorder of energy balance, in which long-term calorie intake exceeds energy output.
- A subject with a BMI (W/h^2) of 20–25 kg/m^2 is considered as having a healthy body weight, one with a BMI of 25–30 kg/m^2 as overweight, and one with a BMI >30 kg/m^2 as obese.
- Obesity is a growing problem in most rich nations; the incidence – at present approximately >30% in the USA and 15–20% in Europe – is increasing.
- A BMI > 30 kg/m^2 significantly increases the risk of type 2 diabetes, hypercholesterolaemia, hypertension, ischaemic heart disease, gallstones and some cancers.
- The causes of obesity include:
  - dietary, exercise, social, financial and cultural factors
  - genetic susceptibility
  - deficiencies in the synthesis or action of leptin or other gut hormone signals
  - defects in the hypothalamic neuronal systems responding to any of these signals
  - defects in the systems controlling energy expenditure (e.g. reduced sympathetic activity), decreased metabolic expenditure of energy or decreased thermogenesis caused by a reduction in β_3-adrenoceptor-mediated tone and/or dysfunction of the proteins that uncouple oxidative phosphorylation.

PHARMACOLOGICAL APPROACHES TO THE PROBLEM OF OBESITY

The first weapons in the fight against obesity are diet and exercise. Unfortunately, these often fail or show only short-term efficacy, leaving surgical techniques (such as gastric stapling or bypass) or drug therapy as a viable alternative. *Bariatric* (weight loss) surgery is much more effective than currently licensed drugs, and is believed to work not by crudely limiting gastric capacity but by its demonstrated effects on gut hormone responses to feeding, acting for example to produce earlier satiety. It is thus potentially a 'proof-of-concept' for pharmacological measures designed to interrupt these messengers.

The attempt to control body weight with drugs has had a long and, regrettably, a largely undistinguished,[7] history. Many types of 'anorectic' (e.g. appetite suppressant) agents have been tested in the past, including the uncoupling agent dinitrophenol (DNP), amphetamines, **dexfenfluramine** and **fenfluramine**. All have been withdrawn from clinical use because of serious adverse effects. DNP, an industrial chemical, is advertised online for slimmers and body-builders as a weight loss and 'fat-burning agent', and has caused deaths among those who use it for this purpose. It blocks mitochondrial ATP production, diverting energy metabolism to generate heat instead of ATP and increasing the overall metabolic rate, which can cause life-threatening hyperthermia.[8]

CENTRALLY ACTING APPETITE SUPPRESSANTS

There have been many attempts to use centrally acting drugs to control appetite. Examples include **sibutramine** and **rimonobant** (both withdrawn in most countries) and **lorcaserin**, a 5-HT$_{2C}$ receptor agonist (see Ch. 39), recently approved as an appetite suppressant. In clinical trials it enhanced weight loss through dieting, but patients regained weight after stopping the drug.

▼ Sibutramine inhibits the reuptake of 5-HT and noradrenaline at the hypothalamic sites that regulate food intake.[9] Its main effects are to reduce food intake and cause dose-dependent weight loss (see Fig. 32.3), this being associated with a decrease in obesity-related risk factors. Sibutramine enhanced satiety and was reported to produce a reduction in waist circumference (i.e. a reduction in visceral fat), a decrease in plasma triglycerides and very-low-density lipoproteins, but an increase in high-density lipoproteins. In addition, beneficial effects on hyperinsulinaemia and glucose metabolism were reported. There is some evidence that the weight loss is associated with higher energy expenditure, possibly through an increase in thermogenesis mediated by the sympathetic nervous system. Like many similar drug regimes, sibutramine was much more effective when combined with lifestyle modification (Wadden et al., 2005).

Sibutramine was withdrawn in Europe because of concerns that its cardiovascular risks outweighed its benefits.

Another novel approach to centrally acting appetite suppressants originated from research in the cannabinoid field (see Ch. 19). As noted above, the endocannabinoid system is involved in the regulation of feeding behaviour and from this observation arose the idea that this could be a useful site of pharmacological intervention. Such a drug was the CB$_1$ receptor antagonist rimonabant that was originally developed for smoking cessation. This drug was introduced as an appetite suppressant following some encouraging clinical trials but was eventually withdrawn in 2008 because of adverse effects on mood seen in some patients. A similar fate overtook another promising CB$_1$ antagonist, **taranabant**.

ORLISTAT

The only drug currently (2013) licensed in the UK for the treatment of obesity is the lipase inhibitor **orlistat**, used with concomitant dietary and other therapy (e.g. exercise).

In the intestine, orlistat reacts with serine residues at the active sites of gastric and pancreatic lipases, irreversibly inhibiting these enzymes and thereby preventing the breakdown of dietary fat to fatty acids and glycerol. It therefore decreases absorption (and correspondingly causes faecal excretion) of some 30% of dietary fat. Given in conjunction with a low-calorie diet in obese

[7] As the showman Bynum said: 'there's a sucker born every minute … and one born to take him' … thyroxine (to increase metabolic rate, Ch. 34), swallowing parasites (intestinal worms compete for ingested food), amphetamines (Ch. 58), drugs that cause malabsorption (hence leaking fat per rectum (see later in this chapter) … really!

[8] DNP is reported to have been given to Russian soldiers in the Second World War, to keep them warm.

[9] Many antidepressant drugs act by the same mechanism (see Ch. 47), and also cause weight loss by reducing appetite. However, sibutramine does not have antidepressant properties. Furthermore, depressed patients are often obese, and antidepressant drugs are used to treat both conditions (see Appolinario et al., 2004).

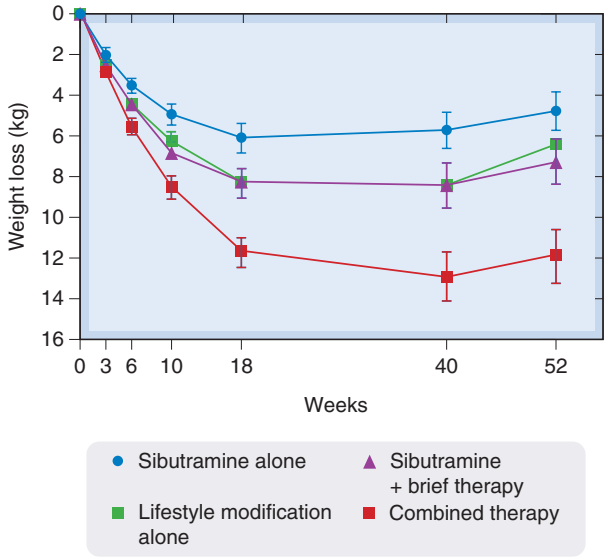

Fig. 32.3 The effect of treatment with sibutramine alone or in combination with lifestyle modification. In this study, 224 obese patient were given sibutramine alone, lifestyle modification counselling alone or sibutramine together with a 'brief' or more extensive programme of lifestyle counselling. The Y-axis shows the weight loss in kg (± SE) over time (X-axis). It is evident that sibutramine is far more effective as a weight-loss therapy when combined with lifestyle changes. This is a common experience when treating obesity. *(Modified from Wadden et al. 2005.)*

individuals, it produces a modest but consistent loss of weight compared with placebo-treated control subjects. In a meta-analysis of 11 long-term placebo-controlled trials encompassing more than 6000 patients, orlistat was found to produce a 2.9% greater reduction in body weight than in the control group, and 12% more patients lost 10% or more of their body weight compared with the controls (Padwal et al., 2003).

Orlistat is also reported to be effective in patients suffering from type 2 diabetes and other complications of obesity. It reduces leptin levels and blood pressure, protects against weight loss-induced changes in biliary secretion, delays gastric emptying and gastric secretion and improves several important metabolic parameters without interfering with the release or action of thyroid or other important hormones (Curran & Scott, 2004). It does not induce changes in energy expenditure.

PHARMACOKINETIC ASPECTS AND UNWANTED EFFECTS

Virtually all (97%) of orlistat is excreted in the faeces (83% unchanged), with only negligible amounts of the drug or its metabolites being absorbed.

Abdominal cramps, flatus with discharge and faecal incontinence can occur, as can intestinal borborygmi (rumbling) and oily spotting. Surprisingly, in view of the possibility of these antisocial effects occurring, the drug is well tolerated. Supplementary therapy with fat-soluble vitamins may be needed. The absorption of contraceptive pills and **ciclosporin** (see Ch. 26) may be decreased. The former is probably not clinically significant but the latter is potentially more serious. Given its good safety record, orlistat has recently been licensed for inclusion in some over-the-counter medicines for weight loss.

Clinical uses of anti-obesity drugs

- The main treatment of obesity is a suitable diet and increased exercise.
- **Orlistat**, which causes fat malabsorption, is considered for severely obese individuals, especially with additional cardiovascular risk factors (e.g. diabetes mellitus, hypertension).
- Many centrally acting appetite suppressants have been withdrawn because of addiction, pulmonary hypertension or other serious adverse effects.

NEW APPROACHES TO OBESITY THERAPY

As might be imagined, the quest for further effective anti-obesity agents is the subject of a prodigious effort by the pharmaceutical industry.

Rare cases of leptin deficiency in patients have been successfully treated by long-term treatment with the hormone, but this is an unusual intervention and unlikely to be of more than limited use in the future. Many other approaches are being piloted (see Kang & Park, 2012). Some of these aim to exploit the action or production of neuroendocrine satiety signals such as CCK to produce appetite suppression. Many of these GI satiety hormones produce such effects when given systemically to humans or rodents, although these are not always useful; for example, CCK reduces meal size but increases meal frequency (West et al., 1984). Glucagon-like peptides such as **liraglutide**, which are used for treating type 2 diabetes (Ch. 31), also have anorexic actions and have shown promising activity in some trials (Astrup et al., 2009). Peptide YY (PYY, Fig. 32.2) is under investigation for human use. It reduces food intake by increasing satiety; a zinc conjugate (compare zinc insulins, Ch. 31) can be administered subcutaneously and acts as a depot.

Other strategies aim to alter the CNS levels of neurotransmitters such as NPY or melanocortins, which transduce changes in these hormonal signals (Halford, 2006). The tractability of the MC_4 receptor itself as a drug target, coupled with the observation that defects in MC_4 signalling are prevalent in obesity, has attracted much interest from the pharmaceutical industry.

Given the importance of the sympathetic nervous system in the control of energy regulation, one might predict that β_3-adrenoceptor agonists might be useful therapeutics. This field has been extensively researched (see Arch, 2008) but, disappointingly, has so far failed to produce an acceptable drug.

Kang and Park (2012) highlight the likely value of combination therapies that target the complex pathways involved in appetite regulation. Most drug therapies are much more effective when used in conjunction with lifestyle and other behavioural modification. The importance of this joint approach is reviewed by Vetter et al. (2010).

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Useful Web resource

<www.who.int> (*This is the World Health Organization Web page that carries data about the prevalence of 'globesity' and its distribution around the world; click on the 'Health Topics' link and navigate to 'Obesity' in the alphabetical list of topics for further information*)

33 The pituitary and the adrenal cortex

OVERVIEW

The pituitary gland and the adrenal cortex release hormones that regulate salt and water balance, energy expenditure, growth, sexual behaviour, immune function and many other vital mechanisms. The commander-in-chief of this impressive logistical exercise is the hypothalamus and the functioning unit is known as the *hypothalamo–pituitary–adrenal (HPA) axis*. In the first part of this chapter we examine the control of pituitary function by hypothalamic hormones and review the physiological roles and clinical uses of both anterior and posterior pituitary hormones. The second part of the chapter focuses on adrenal hormones and, in particular, the anti-inflammatory effect of glucocorticoids. This should be read in conjunction with the relevant sections of Chapters 3 and 26.

THE PITUITARY GLAND

The pituitary gland comprises three different structures arising from two different embryological precursors (see Fig. 33.1). The *anterior pituitary* and the *intermediate lobe* are derived from the endoderm of the buccal cavity, while the *posterior pituitary* is derived from neural ectoderm. The anterior and posterior lobes receive independent neuronal input from the hypothalamus, with which they have an intimate functional relationship.

THE ANTERIOR PITUITARY GLAND

The anterior pituitary gland (*adenohypophysis*) secretes a number of hormones crucial for normal physiological function. Within this tissue are specialised cells such as *corticotrophs*, *lactotrophs* (*mammotrophs*), *somatotrophs*, *thyrotrophs* and *gonadotrophs*, which secrete hormones that regulate different endocrine organs of the body (Table 33.1). Interspersed among these are other cell types, including *folliculostellate cells*, which exert a nurturing and regulatory influence on the hormone-secreting endocrine cells.

Secretion from the anterior pituitary is largely regulated by the release from the hypothalamus of 'factors' – in effect local hormones – that reach the pituitary through the bloodstream.[1] The blood supply to the hypothalamus divides to form a meshwork of capillaries, the *primary plexus*, which drains into the *hypophyseal portal vessels*. These pass through the pituitary stalk to feed a *secondary plexus* of capillaries in the anterior pituitary. Peptidergic neurons in the hypothalamus secrete a variety of releasing or inhibitory hormones directly into the capillaries of the primary capillary plexus (Table 33.1 and Fig. 33.1). Most of these regulate the secretion of hormones from the anterior lobe, although the *melanocyte-stimulating hormones* (MSHs) are secreted mainly from the intermediate lobe.

The release of stimulatory hormones is regulated by negative feedback pathways between the hormones of the hypothalamus, the anterior pituitary and the peripheral endocrine glands. In *long negative feedback* pathways, hormones secreted from the peripheral glands exert regulatory actions on both the hypothalamus and the anterior pituitary. Anterior pituitary hormones acting directly on the hypothalamus comprise the *short negative feedback* pathway.

The peptidergic neurons in the hypothalamus are themselves influenced by other centres within the central nervous system (CNS) mediated through neural pathways that release dopamine, noradrenaline, 5-hydroxytryptamine and the opioid peptides (which are particularly abundant in the hypothalamus, see Ch. 15). Hypothalamic control of the anterior pituitary is also exerted through the *tuberohypophyseal dopaminergic pathway* (see Ch. 39), the neurons of which lie in close apposition to the primary capillary plexus. Dopamine secreted directly into the hypophyseal portal circulation reaches the anterior pituitary in the blood.

HYPOTHALAMIC HORMONES

The secretion of anterior pituitary hormones, then, is primarily regulated by the 'releasing factors' that originate in the hypothalamus. The most significant are described in more detail below. Somatostatin and gonadotrophin-releasing hormone are used therapeutically, the others being used mainly for diagnostic tests or as research tools. Some of these factors also function as neurotransmitters or neuromodulators elsewhere in the CNS (Ch. 39).

SOMATOSTATIN

Somatostatin is a peptide of 14 amino acid residues. It inhibits the release of growth hormone and thyroid-stimulating hormone (TSH, thyrotrophin) from the anterior pituitary (Fig. 33.2), and insulin and glucagon from the pancreas. It also decreases the release of most gastrointestinal hormones, and reduces gastric acid and pancreatic secretion.

Octreotide is a long-acting analogue of somatostatin. It is used for the treatment of *carcinoid* and other hormone-secreting tumours (Ch. 15). It also has a place in the therapy of *acromegaly* (a condition in which there is oversecretion of growth hormone in an adult). It also constricts splanchnic blood vessels, and is used to treat bleeding *oesophageal varices*. Octreotide is generally given subcutaneously. The peak action is at 2 h, and the suppressant effect lasts for up to 8 h.

Unwanted effects include pain at the injection site and gastrointestinal disturbances. Gallstones and postprandial

[1]The term 'factor' was originally coined at a time when their structure and function were not known. These are blood-borne messengers, and as such are clearly hormones. Nevertheless, the nomenclature, though irrational, lingers on.

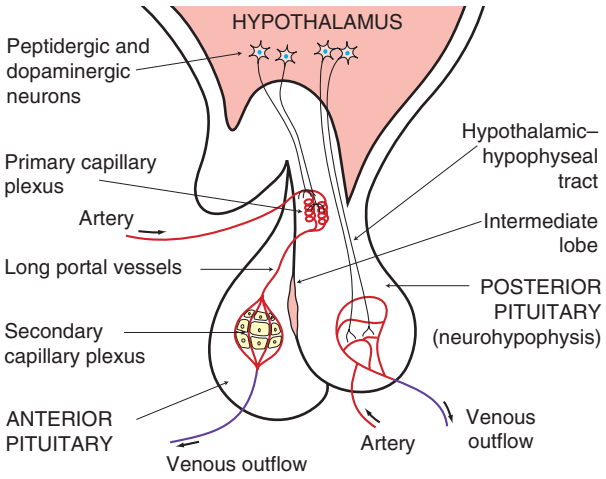

Fig. 33.1 Schematic diagram of vascular and neuronal relationships between the hypothalamus, the posterior pituitary and the anterior pituitary. The main portal vessels to the anterior pituitary lie in the pituitary stalk and arise from the primary plexus in the hypothalamus, but some (the short portal vessels) arise from the vascular bed in the posterior pituitary (not shown).

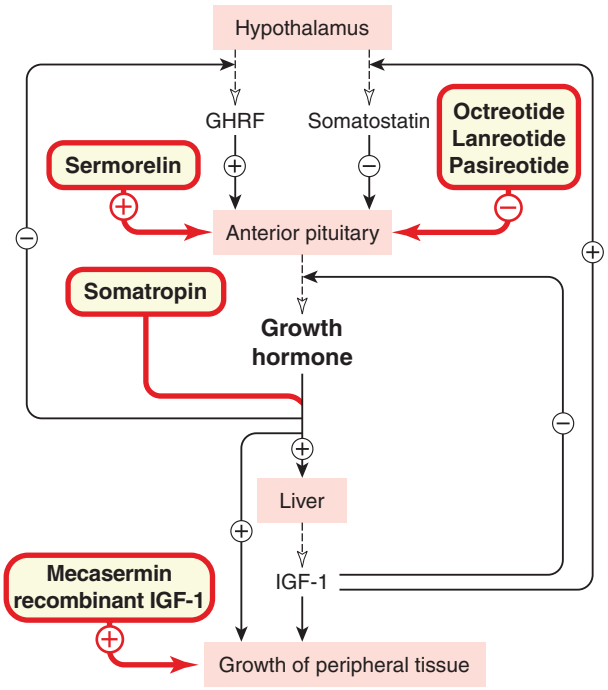

Fig. 33.2 Control of growth hormone secretion and its actions. Drugs are shown in red-bordered boxes. GHRF, growth hormone-releasing factor; IGF-1, insulin-like growth factor-1.

hyperglycaemia have also been reported, and acute hepatitis or pancreatitis has occurred in a few cases.

Lanreotide and **pasireotide** have similar effects. Lanreotide is also used in the treatment of thyroid tumours, while pasireotide, which is a particularly potent analogue, is used in the treatment of *Cushing's syndrome* when surgery is inappropriate or has been ineffective.

GONADOTROPHIN-RELEASING HORMONE

Gonadotrophin- (or luteinising hormone-) releasing hormone is a decapeptide that releases both *follicle-stimulating hormone* and *luteinising hormone* from gonadotrophs. **Gonadorelin**[2] and its analogues (**buserelin**, **goserelin**, **leuprorelin**, **nafarelin** and **triptorelin**) are used mainly in the treatment of infertility and some hormone-dependent tumours (see Ch. 35).

GROWTH HORMONE-RELEASING FACTOR (SOMATORELIN)

Growth hormone-releasing factor (GHRF) is a peptide with 44 amino acid residues. The main action of GHRF is summarised in Figure 33.2. An analogue, **sermorelin**, may be used as a diagnostic test for growth hormone secretion. Given intravenously, subcutaneously or intranasally, it causes secretion of growth hormone within minutes and peak concentrations in 1 h. The action is selective for the somatotrophs in the anterior pituitary, and no other pituitary hormones are affected. Unwanted effects are rare.

THYROTROPHIN-RELEASING HORMONE

Thyrotrophin-releasing hormone (TRH) from the hypothalamus releases TSH from the thyrotrophs.

▼ **Protirelin** is a synthetic TRH that has been used for the diagnosis of thyroid disorders (see Ch. 34). Given intravenously in normal subjects, it causes an increase in plasma TSH concentration, whereas in patients with hyperthyroidism there is a blunted response because the raised blood thyroxine concentration has a negative feedback effect on the anterior pituitary. The opposite occurs with hypothyroidism, where there is an intrinsic defect in the thyroid itself. Its use was recently discontinued in the UK.

CORTICOTROPHIN-RELEASING FACTOR

Corticotrophin-releasing factor (CRF) is a peptide that releases **adrenocorticotrophic hormone** (ACTH, corticotrophin) and β-endorphin from corticotrophs in the anterior pituitary gland. CRF acts synergistically with *antidiuretic hormone* (ADH; arginine-vasopressin), and both its action and release are inhibited by *glucocorticoids* (see Fig. 33.4). Synthetic preparations have been used to test the ability of the pituitary to secrete ACTH, and to assess whether ACTH deficiency is caused by a pituitary or a hypothalamic defect. It has also been used to evaluate hypothalamic pituitary function after therapy for Cushing's syndrome (see Fig. 33.7).

ANTERIOR PITUITARY HORMONES

The main hormones of the anterior pituitary are listed in Table 33.1. The gonadotrophins are dealt with in Chapter 35 and TSH in Chapter 34. The actions of the remainder are summarised below.

GROWTH HORMONE (SOMATOTROPHIN)

Growth hormone is secreted by the somatotroph cells and is the most abundant pituitary hormone. Secretion is high in the newborn, decreasing at 4 years to an intermediate level, which is then maintained until after puberty, after which there is a further decline. Recombinant

[2]In this context, the suffix '-relin' denotes peptides that stimulate hormone release.

Table 33.1 Hormones secreted by the hypothalamus and the anterior pituitary and related drugs.

Hypothalamic factor/hormone[a]	Effect on anterior pituitary	Main effects of anterior pituitary hormone
Corticotrophin-releasing factor (CRF)	Releases adrenocorticotrophic hormone (ACTH, corticotrophin) *Analogue*: tetracosactide	Stimulates secretion of adrenal cortical hormones (mainly glucocorticoids); maintains integrity of adrenal cortex
Thyrotrophin-releasing hormone (TRH) *Analogue*: protirelin	Releases thyroid-stimulating hormone (TSH; thyrotrophin)	Stimulates synthesis and secretion of thyroid hormones; maintains integrity of thyroid gland
Growth hormone-releasing factor (GHRF, somatorelin) *Analogue*: sermorelin	Releases growth hormone (GH; somatotrophin) *Analogue*: somatropin	Regulates growth, partly directly, partly through by releasing somatomedins from the liver and elsewhere; increases protein synthesis, increases blood glucose, stimulates lipolysis
Growth hormone release-inhibiting factor (somatostatin) *Analogues*: octreotide, lanreotide	Inhibits the release of GH	Prevents effects above as well as TSH release
Gonadotrophin (or luteinising hormone)-releasing hormone (GnRH) *Analogues*: 'gonadorelin analogues' – buserelin, goserelin, leuprorelin, naferelin, triptorelin	Releases follicle-stimulating hormone (FSH; see Ch. 35)	Stimulates the growth of the ovum and the Graafian follicle (female) and gametogenesis (male); with LH, stimulates the secretion of oestrogen throughout the menstrual cycle and progesterone in the second half
	Releases luteinising hormone (LH) or interstitial cell-stimulating hormone (see Ch. 35)	Stimulates ovulation and the development of the corpus luteum; with FSH, stimulates secretion of oestrogen and progesterone in the menstrual cycle; in male, regulates testosterone secretion
Prolactin-releasing factor (PRF)	Releases prolactin	Together with other hormones, prolactin promotes development of mammary tissue during pregnancy; stimulates milk production in the postpartum period
Prolactin release-inhibiting factor (probably dopamine)	Inhibits the release of prolactin	Prevents effects above
Melanocyte-stimulating hormone (MSH)-releasing factor	Releases α-, β- and γ-MSH	Promotes formation of melanin, which causes darkening of skin; MSH is anti-inflammatory and helps to regulate appetite/feeding
MSH release-inhibiting factor	Inhibits the release of α-, β- and γ-MSH	Prevents effects above

[a]These hormones are often spelled without the 'h' (e.g. corticotropin, thyrotropin, etc.) in contemporary texts. We have retained the original nomenclature in this edition.

human growth hormone, **somatropin**, is available for treating growth defects and other developmental problems.

Regulation of secretion

Secretion of growth hormone is regulated by the action of hypothalamic GHRF and modulated by somatostatin, as described above and outlined in Figure 33.2. A different peptide releaser of growth hormone ('ghrelin') is released from the stomach and pancreas and is implicated in the control of appetite and of body weight (Ch. 32). One of the mediators of growth hormone action, *insulin-like growth factor* (IGF)-1, which is released from the liver, has an inhibitory effect on growth hormone secretion by stimulating somatostatin release from the hypothalamus.

As with other anterior pituitary secretions, growth hormone release is pulsatile, and its plasma concentration may fluctuate 10- to 100-fold. These surges occur repeatedly during the day and night, and reflect the dynamics of hypothalamic control. Deep sleep is a potent stimulus to growth hormone secretion, particularly in children.

Actions

The main effect of growth hormone (and its analogues) is to stimulate normal growth. To do so, it acts in conjunction with other hormones secreted from the thyroid, the gonads and the adrenal cortex. It stimulates hepatic production of the IGFs – also termed *somatomedins* – which mediate most of its anabolic actions. IGF-1 (the principal mediator) mediates many of these anabolic effects and stimulates the uptake of amino acids and protein synthesis by skeletal muscle and the cartilage at the epiphyses of long bones, thus influencing bone growth. Receptors for IGF-1 exist on many other cell types, including liver cells and fat cells.

Disorders of production and clinical use

Deficiency of growth hormone (or failure of its action) results in *pituitary dwarfism*. In this condition, which may result from lack of GHRF or a lack of IGF generation or action, the normal proportions of the body are maintained. Growth hormone is used therapeutically in these patients (often children) as well as those suffering from

the short stature associated with the chromosomal disorder known as *Turner's syndrome*. It may also be used to correct short stature caused by chronic renal insufficiency in children.

Humans are insensitive to growth hormone of other species, so human growth hormone (hGH) must be used clinically. This used to be obtained from human cadavers, but this led to the spread of *Creutzfeldt–Jakob disease*, a prion-mediated neurodegenerative disorder (Ch. 40). hGH is now prepared by recombinant DNA technology (somatropin), which avoids this risk. Satisfactory linear growth can be achieved by giving somatropin subcutaneously, six to seven times per week, and therapy is most successful when started early.

hGH is also used illicitly by athletes (see Ch. 58) to increase muscle mass. The large doses used have serious side effects, causing abnormal bone growth and cardiomegaly. It has also been tested as a means of combating the bodily changes in senescence; clinical trials have shown increases in body mass, but no functional improvement.

Human recombinant IGF-1 (**mecasermin**) is also available for the treatment of growth failure in children who lack adequate amounts of this hormone.

An excessive production of growth hormone in children results in *gigantism*. An excessive production in adults, which is usually the result of a benign pituitary tumour, results in acromegaly, in which there is enlargement mainly of the jaw and of the hands and feet. The dopamine agonist **bromocriptine** and octreotide may mitigate the condition. Another useful agent is **pegvisomant**, a modified analogue of growth hormone prepared by recombinant technology that is a highly selective antagonist of growth hormone actions.

PROLACTIN

Prolactin is secreted from the anterior pituitary gland by lactotroph (mammotroph) cells. These are abundant in the gland and increase in number during pregnancy, probably under the influence of oestrogen.

Regulation of secretion

Prolactin secretion is under tonic inhibitory control by dopamine (acting on D_2 receptors on the lactotrophs) released from the hypothalamus (Fig. 33.3 and Table 33.1). The main stimulus for release is suckling; in rats, both the smell and the sounds of hungry pups are also effective triggers. Neural reflexes from the breast may stimulate the secretion from the hypothalamus of prolactin-releasing factor(s), possible candidates for which include TRH and **oxytocin**. Oestrogens increase both prolactin secretion and the proliferation of lactotrophs through release, from a subset of lactotrophs, of the neuropeptide *galanin*. Dopamine antagonists (used mainly as antipsychotic drugs; see Ch. 46) are potent stimulants of prolactin release, whereas agonists such as **bromocriptine** (Chs 39 and 46) suppress prolactin release. Bromocriptine is also used in Parkinson's disease (Ch. 40).

Actions

The prolactin receptor is a single transmembrane domain receptor related to the cytokine receptors. Several different isoforms and splice variants are known. These are found not only in the mammary gland but are widely distributed throughout the body, including the brain, ovary, heart, lungs and immune system. The main function of prolactin in women is the control of milk production. At parturition the prolactin concentration rises and lactation is initiated. Maintenance of lactation depends on suckling (see above), which causes a 10- to 100-fold increase in blood hormone levels within 30 min.

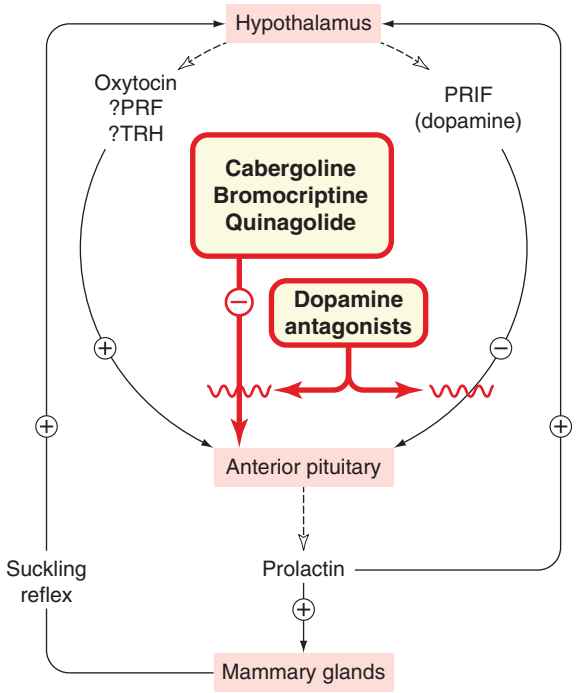

Fig. 33.3 Control of prolactin secretion. Drugs are shown in red-bordered boxes. PRF, prolactin-releasing factor(s); PRIF, prolactin release-inhibiting factor(s); TRH, thyrotrophin-releasing hormone.

Together with other hormones, prolactin is responsible for the proliferation and differentiation of mammary tissue during pregnancy. It also inhibits gonadotrophin release and/or the response of the ovaries to these trophic hormones. This is one of the reasons why ovulation does not usually occur during breastfeeding, and is believed to constitute a natural contraceptive mechanism.

▼ According to one rather appealing hypothesis, the high postpartum concentration of prolactin reflects its biological function as a 'parental' hormone. Certainly, broodiness and nest-building activity can be induced in birds, mice and rabbits by prolactin injections. Prolactin also exerts other, apparently unrelated, actions, including stimulating mitogenesis in lymphocytes. There is some evidence that it may play a part in regulating immune responses.

Modification of prolactin secretion

Prolactin itself is not used clinically. Bromocriptine, a dopamine receptor agonist, is used to decrease excessive prolactin secretion (*hyperprolactinaemia*). It is well absorbed orally, and peak concentrations occur after 2 h. Unwanted reactions include nausea and vomiting. Dizziness, constipation and postural hypotension may also occur. **Cabergoline** and **quinagolide** are similar.

ADRENOCORTICOTROPHIC HORMONE

Adrenocorticotrophic hormone (ACTH, corticotrophin) is the anterior pituitary secretion that controls the synthesis and release of the glucocorticoids of the adrenal cortex (see Table 33.1). It is a 39-residue peptide derived from

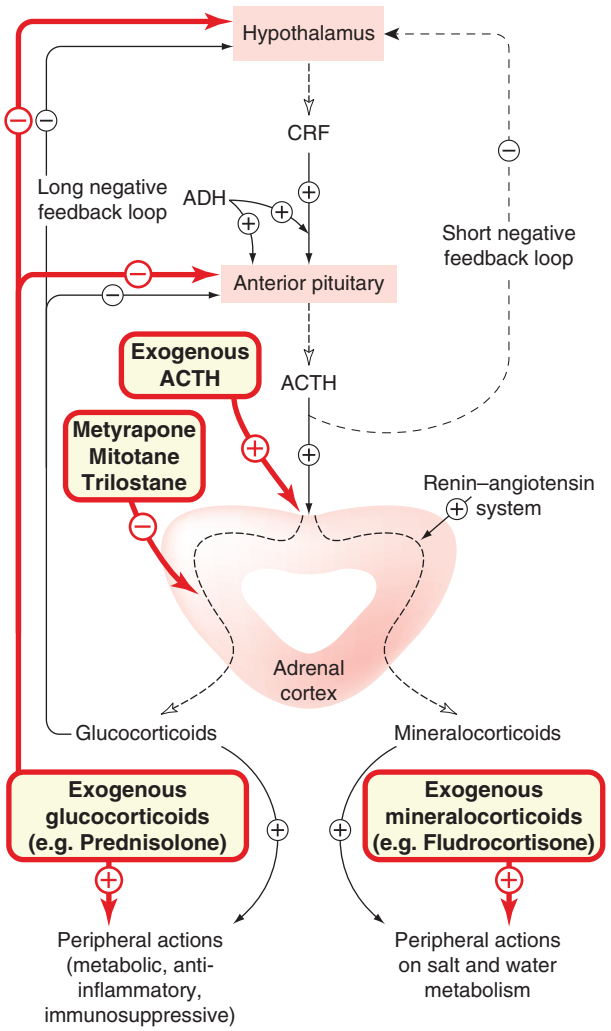

Fig. 33.4 Regulation of synthesis and secretion of adrenal corticosteroids. The long negative feedback loop is more important than the short loop (dashed lines). Adrenocorticotrophic hormone (ACTH, corticotrophin) has only a minimal effect on mineralocorticoid production. Drugs are shown in red-bordered boxes. ADH, antidiuretic hormone (vasopressin); CRF, corticotrophin-releasing factor.

Clinical uses of bromocriptine

- To prevent lactation.
- To treat galactorrhoea (i.e. non-puerperal lactation in either sex), owing to excessive prolactin secretion.
- To treat prolactin-secreting pituitary tumours (prolactinomas).
- In the treatment of Parkinson's disease (Ch. 40) and of acromegaly.

the precursor *pro-opiomelanocortin* (POMC) by sequential proteolytic processing. Failure of ACTH action because of defects in its receptor or intracellular signalling pathways can lead to severe glucocorticoid deficiency (Chan et al., 2008). Details of the regulation of ACTH secretion are shown in Figure 33.4.

▼ This hormone occupies (together with cortisone) an important place in the history of inflammation therapy because of the work of Hench and his colleagues in the 1940s, who first observed that both substances had anti-inflammatory effects in patients with rheumatoid disease. The effect of ACTH was thought to be secondary to stimulation of the adrenal cortex but, interestingly, the hormone also has anti-inflammatory actions in its own right, through activation of macrophage (melanocortin) MC_3 receptors (Getting et al., 2002).

Adrenocorticotrophic hormone itself is not often used in therapy today, because its action is less predictable than that of the corticosteroids and it may provoke antibody formation. **Tetracosactide** (**tetracosactrin**), a synthetic polypeptide that consists of the first 24 N-terminal residues of human ACTH, has the same drawbacks but is now widely used in its stead for assessing the competency of the adrenal cortex.

The concentration of ACTH in the blood is reduced by glucocorticoids, forming the basis of the ***dexamethasone suppression test***.

Actions

Acting through MC_2 receptors, tetracosactide and ACTH have two actions on the adrenal cortex:

- Stimulation of the synthesis and release of glucocorticoids. This action occurs within minutes of injection, and the ensuing biological actions are those of the steroids released.
- A trophic action on adrenal cortical cells, and regulation of the levels of key mitochondrial steroidogenic enzymes. The loss of this effect accounts for the adrenal atrophy that results from chronic glucocorticoid administration, which suppresses ACTH secretion.

The main use of tetracosactide is in the diagnosis of adrenal cortical insufficiency. The drug is given intramuscularly or intravenously, and the concentration of hydrocortisone in the plasma is measured by radioimmunoassay.

MELANOCYTE-STIMULATING HORMONE (MSH)

α-, β- and γ-MSH are peptide hormones with structural similarity to ACTH and are derived from the same precursor. Together, these peptides are referred to as *melanocortins*, because their first recognised action was to stimulate the production of melanin by specialised skin cells called *melanocytes*. As such, they play an important part in determining hair coloration, skin colour and reaction to ultraviolet light.

Melanocyte-stimulating hormone acts on melanocortin receptors, of which five (MC_{1-5}) have been cloned. These are G protein-coupled receptors (GPCRs) that activate cAMP synthesis. Melanin formation is controlled by the MC_1 receptor. Excessive α-MSH production can provoke abnormal proliferation of melanocytes and may predispose to melanoma.

▼ Melanocortins exhibit numerous other biological effects. For example, α-MSH inhibits the release of interleukin (IL)-1β and tumour necrosis factor (TNF)-α, reduces neutrophil infiltration, and exhibits anti-inflammatory and antipyretic activity. Levels of α-MSH are increased in synovial fluid of patients with rheumatoid arthritis. MC_1 and MC_3 receptors mediate the immunomodulatory effect of MSH. Agonists at these receptors with potential anti-inflammatory activity are being sought. Central injection of α-MSH also causes changes in animal behaviour, such as increased grooming and sexual activity as well as reduced feeding through actions on MC_4 receptors, and MC_4 agonists are under investigation as potential treatments for obesity and for erectile impotence.

Intracerebroventricular or intravenous injection of γ-MSH increases blood pressure, heart rate and cerebral blood flow. These effects are also likely to be mediated by the MC_4 receptor.

Two naturally occurring ligands for melanocortin receptors (*agouti-signalling protein* and *agouti-related peptide*, together called the *agouti*) have been discovered in human tissues. These are proteins that competitively antagonise the effect of MSH at melanocortin receptors.

The anterior pituitary gland and hypothalamus

- The anterior pituitary gland secretes hormones that regulate:
  - the release of *glucocorticoids* from the adrenal cortex
  - the release of *thyroid hormones*
  - the release of sex hormones: *ovulation* in the female and *spermatogenesis* in the male
  - *growth*
  - *mammary gland* structure and function.
- Each anterior pituitary hormone is regulated by a specific hypothalamic releasing factor. Feedback mechanisms govern the release of these factors. Substances available for clinical use include:
  - *growth hormone-releasing factor* (**sermorelin**) and analogues of growth hormone (**somatrophin**)
  - *thyrotrophin-releasing factor* (**protirelin**) and thyroid-stimulating hormone (thyrotrophin; used to test thyroid function)
  - **octreotide** and **lanreotide**, analogues of ***somatostatin***, which inhibit growth hormone release
  - *corticotrophin-releasing factor*, used in diagnosis
  - *gonadotrophin-releasing factor*, ***gonadorelin*** *and analogues*. Used to treat infertility and some carcinomas.

Adrenocorticotrophic hormone and the adrenal steroids

- Adrenocorticotrophic hormone (ACTH; **tetracosactrin**, **tetracosactide**) stimulates synthesis and release of glucocorticoids (e.g. **hydrocortisone**), and also some androgens, from the adrenal cortex.
- Corticotrophin-releasing factor (CRF) from the hypothalamus regulates ACTH release, and is regulated in turn by neural factors and negative feedback effects of plasma glucocorticoids.
- Mineralocorticoid (e.g. aldosterone) release from the adrenal cortex is controlled by the renin–angiotensin system.

POSTERIOR PITUITARY GLAND

The posterior pituitary gland (neurohypophysis) consists largely of the terminals of nerve cells that lie in the *supraoptic* and *paraventricular nuclei* of the hypothalamus. Their axons form the *hypothalamic–hypophyseal tract*, and the fibres terminate in dilated nerve endings in close association with capillaries in the posterior pituitary gland (Fig. 33.1). Peptides, synthesised in the hypothalamic nuclei, pass down these axons into the posterior pituitary, where they are stored and eventually secreted into the bloodstream.

The two main hormones of the posterior pituitary are **oxytocin** (which contracts the smooth muscle of the uterus; for details see Ch. 35) and **vasopressin** (antidiuretic hormone ADH; see Chs 22 and 29). They are highly homologous cyclic nonapeptides. Several analogues have been synthesised that vary in their antidiuretic, vasopressor and oxytocic (uterine stimulant) properties.

The posterior pituitary gland

- The posterior pituitary gland secretes:
  - oxytocin (see Ch. 35)
  - antidiuretic hormone (**vasopressin**), which acts on V_2 receptors in the distal kidney tubule to increase water reabsorption and, in higher concentrations, on V_{1A} receptors to cause vasoconstriction. It also stimulates adrenocorticotrophic hormone secretion.
- Substances available for clinical use are **vasopressin** and the analogues **desmopressin**, **felypressin** and **terlipressin**.

Clinical uses of antidiuretic hormone (vasopressin) and analogues

- Diabetes insipidus: **felypressin**, **desmopressin**.
- Initial treatment of bleeding oesophageal varices: **vasopressin**, **terlipressin**, **felypressin**. (**Octreotide** – a somatostatin analogue – is also used, but direct injection of sclerosant via an endoscope is the main treatment.)
- Prophylaxis against bleeding in haemophilia (e.g. before tooth extraction): **vasopressin**, **desmopressin** (by increasing the concentration of factor VIII).
- **Felypressin** is used as a vasoconstrictor with local anaesthetics (see Ch. 43).
- **Desmopressin** is used for persistent nocturnal enuresis in older children and adults.

VASOPRESSIN

Regulation of secretion and physiological role

Vasopressin released from the posterior pituitary has a crucial role in the control of the water content of the body through its action on the cells of the distal part of the nephron and the collecting tubules in the kidney (see Ch. 29). The hypothalamic nuclei that control fluid balance lie close to the nuclei that synthesise and secrete vasopressin.

One of the main stimuli for vasopressin release is an increase in plasma osmolarity (which produces a sensation of thirst). A decrease in circulating blood volume (*hypovolaemia*) is another, and here the stimuli arise from

stretch receptors in the cardiovascular system or from angiotensin release. *Diabetes insipidus* is a condition in which large volumes of dilute urine are produced because vasopressin secretion is reduced or absent, or because of a reduced sensitivity of the kidney to the hormone.

Vasopressin receptors

There are three classes of receptor: V_{1A}, V_{1B} and V_2. All are GPCRs. V_2 receptors stimulate adenylyl cyclase, which mediates the main physiological actions of vasopressin in the kidney, whereas the V_{1A} and V_{1B} receptors are coupled to the phospholipase C/inositol trisphosphate system.

The receptor for oxytocin (OT receptor) is also a GPCR, which primarily signals through phospholipase C stimulation but has a secondary action on adenylyl cyclase. Vasopressin is a partial agonist at OT but its effects are limited by the distribution of the receptor, which, as might be inferred from its classic action on the pregnant uterus, is high in the myometrium, endometrium, mammary gland and ovary. The central actions of oxytocin (and vasopressin) have also attracted attention as they are apparently involved in 'pair bonding' and the other psychosocial interactions.[3]

Actions

Renal actions

Vasopressin binds to V_2 receptors in the basolateral membrane of the cells of the distal tubule and collecting ducts of the nephron. Its main effect in the collecting duct is to increase the rate of insertion of water channels (*aquaporins*) into the lumenal membrane, thus increasing the permeability of the membrane to water (see Ch. 29). It also activates urea transporters and transiently increases Na^+ absorption, particularly in the distal tubule.

Several drugs affect the action of vasopressin. Non-steroidal anti-inflammatory drugs and **carbamazepine** increase, and **lithium**, **colchicine** and **vinca alkaloids** decrease, vasopressin effects. The effects of the last two agents are secondary to their action on the microtubules required for translocation of water channels. The antagonists **demeclocycline** and **tolvaptan** counteract the action of vasopressin on the V_2 receptor in renal tubules and can be used to treat patients with water retention combined with urinary salt loss (and thus *hyponatraemia*) caused by excessive secretion of the hormone. This *syndrome of inappropriate ADH secretion* ('SIADH') is associated with lung or other malignancies or head injury. Specific V_2 receptor antagonists are also being investigated in the treatment of heart failure (Ch. 22).

Other non-renal actions

Vasopressin causes contraction of smooth muscle, particularly in the cardiovascular system, by acting on V_{1A} receptors (see Ch. 22). The affinity of vasopressin for these receptors is lower than that for V_2 receptors, and smooth muscle effects are seen only with doses larger than those affecting the kidney. Vasopressin also stimulates blood platelet aggregation and mobilisation of coagulation factors. When released into the pituitary portal circulation it promotes the release of ACTH from the anterior pituitary by an action on V_{1B} receptors (Fig. 33.4). In the CNS, vasopressin, like oxytocin, is believed to have a role in emotional and social behaviour.

Pharmacokinetic aspects

Vasopressin, as well as various peptide analogues, is used clinically either for the treatment of diabetes insipidus or as a vasoconstrictor. Several analogues have been developed to (a) increase the duration of action and (b) shift the relative potency between the V_1 and V_2 receptors.

The main substances used are:

- vasopressin itself; short duration of action, weak selectivity for V_2 receptors, given by subcutaneous or intramuscular injection, or by intravenous infusion;
- **desmopressin**; increased duration of action, V_2-selective and therefore fewer pressor effects, can be given by several routes including nasal spray;
- **terlipressin**; increased duration of action, low but protracted vasopressor action and minimal antidiuretic properties);
- **felypressin**; a short-acting vasoconstrictor that is injected with local anaesthetics such as **prilocaine** to prolong their action (see Ch. 43).

Vasopressin itself is rapidly eliminated, with a plasma half-life less than 10 min and a short duration of action. Metabolism is by tissue peptidases, and 33% is removed by the kidney. Desmopressin is less subject to degradation by peptidases, and its plasma half-life is 75 min.

Unwanted effects

There are few unwanted effects and they are mainly cardiovascular in nature: intravenous vasopressin may cause spasm of the coronary arteries with resultant angina, but this risk can be minimised if the antidiuretic peptides are administered intranasally.

THE ADRENAL CORTEX

The adrenal glands consist of two parts: the inner *medulla*, which secretes catecholamines (see Ch. 14), and the outer *cortex*, which secretes adrenal steroids. The cortex comprises three concentric zones: the *zona glomerulosa* (the outermost layer), which elaborates mineralocorticoids, the *zona fasciculate*, which elaborates glucocorticoids, and the innermost *zona reticularis*, which produces androgen precursors. The principal adrenal steroids are those with glucocorticoid and mineralocorticoid[4] activity. Androgen secretion (see Ch. 35) by the cortex is not considered further in this chapter.

The mineralocorticoids regulate water and electrolyte balance, and the main endogenous hormone is *aldosterone*. The glucocorticoids have widespread actions on intermediate metabolism, affecting carbohydrate and protein metabolism, as well as potent regulatory effects on host defence mechanisms (Chs 6 and 26). The adrenal gland secretes a mixture of glucocorticoids; in humans the main hormone is *hydrocortisone* (also, confusingly, known as *cortisol*), and in rodents, *corticosterone*. The mineralocorticoid and glucocorticoid actions are not completely

[3]Oxytocin is released during childbirth, lactation and orgasm and has been shown to promote trust and other prosocial behaviour. This has earned it the nickname, in the popular press and in the numerous Internet discussion groups that these findings have spawned, of the 'love hormone' or, even more nauseatingly, the 'cuddle hormone'.

[4]So named because early experimenters noticed that separate fractions of adrenal gland extracts caused changes in blood glucose or salt and water retention.

Table 33.2 Comparison of the main corticosteroid agents used for systemic therapy (using hydrocortisone as a standard)

Compound	Relative affinity for receptor[a]	Approximate relative potency in clinical use		Duration of action after oral dose[b]	Comments
		Anti-inflammatory	*Sodium retaining*		
Hydrocortisone	1	1	1	Short	Drug of choice for replacement therapy (cortisol)
Cortisone	Prodrug	0.8	0.8	Short	Cheap; inactive until converted to hydrocortisone; not used as anti-inflammatory because of mineralocorticoid effects
Deflazacort	Prodrug	3	?	Short	Converted by plasma esterases into active metabolite Similar utility to prednisolone
Prednisolone	2.2	4	0.8	Intermediate	Drug of choice for systemic anti-inflammatory and immunosuppressive effects
Prednisone	Prodrug	4	0.8	Intermediate	Inactive until converted to prednisolone
Methylprednisolone	11.9	5	Minimal	Intermediate	Anti-inflammatory and immunosuppressive
Triamcinolone	1.9	5	None	Intermediate	Relatively more toxic than others
Dexamethasone	7.1	27	Minimal	Long	Anti-inflammatory and immunosuppressive, used especially where water retention is undesirable (e.g. cerebral oedema); drug of choice for suppression of ACTH production
Betamethasone	5.4	27	Negligible	Long	Anti-inflammatory and immunosuppressive, used especially when water retention is undesirable
Fludrocortisone	3.5	15	150	Short	Drug of choice for mineralocorticoid effects
Aldosterone	0.38	None	500	–	Endogenous mineralocorticoid

[a]Data obtained in human fetal lung cells.

[b]Duration of action (half-lives in hours): short, 8–12; intermediate, 12–36; long, 36–72. Some drugs are inactive until converted to active compounds *in vivo* and therefore have negligible affinity for the glucocorticoid receptor.

(Data for relative affinity obtained from Baxter & Rousseau 1979)

separated in naturally occurring steroids and some glucocorticoids have quite substantial effects on water and electrolyte balance. In fact, hydrocortisone and aldosterone are equiactive on mineralocorticoid receptors but, in mineralocorticoid-sensitive tissues such as the kidney, the action of *11β-hydroxysteroid dehydrogenase* converts hydrocortisone to the inactive metabolite cortisone,[5] thereby preventing the tissue from responding to hydrocortisone.

With the exception of *replacement therapy*, glucocorticoids are most commonly employed for their anti-inflammatory and immunosuppressive properties (see Ch. 26). Under these circumstances, their metabolic and other actions are seen as unwanted side effects. Synthetic steroids have been developed in which it has been possible to separate, to some degree, the glucocorticoid from the mineralocorticoid actions (see Table 33.2), but it has not been possible to separate the anti-inflammatory from the other actions of the glucocorticoids completely.

[5]Oddly, it was cortisone that was originally demonstrated to have potent glucocorticoid anti-inflammatory activity in the classic studies of Hench and his colleagues in 1949. The reason for this apparent anomaly is that an isoform of 11 β-hydroxysteroid dehydrogenase present in some tissues can transform cortisone back to cortisol (i.e. hydrocortisone), thus restoring its biological activity.

▼ The adrenal gland is essential to life, and animals deprived of these glands are able to survive only under rigorously controlled conditions. In humans, a deficiency in corticosteroid production, termed *Addison's disease*, is characterised by muscular weakness, low blood pressure, depression, anorexia, loss of weight and hypoglycaemia. Addison's disease may have an autoimmune aetiology, or it may result from destruction of the gland by chronic inflammatory conditions such as tuberculosis.

When corticosteroids are produced in excess, the clinical picture depends on which species predominates. Excessive *glucocorticoid* activity results in *Cushing's syndrome*, the manifestations of which are outlined in Figure 33.7. This can be caused by hypersecretion from the adrenal glands or by prolonged therapeutic use of glucocorticoids. An excessive production of *mineralocorticoids* results in retention of Na^+ and loss of K^+. This may be caused by hyperactivity or tumours of the adrenals (*primary hyperaldosteronism*, or *Conn's syndrome*, an uncommon but important cause of hypertension; see Ch. 22), or by excessive activation of the renin–angiotensin system (such as occurs in some forms of kidney disease), cirrhosis of the liver or congestive cardiac failure (*secondary hyperaldosteronism*).

GLUCOCORTICOIDS

Synthesis and release

Glucocorticoids are not stored in the adrenal gland but are synthesised under the influence of circulating ACTH secreted from the anterior pituitary gland (Fig. 33.4) and

released in a pulsatile fashion into the blood. While they are always present, there is a well-defined circadian rhythm in the secretion in healthy humans, with the net blood concentration being highest early in the morning, gradually diminishing throughout the day and reaching a low point in the evening or night. ACTH secretion itself (also pulsatile in nature) is regulated by CRF released from the hypothalamus, and by vasopressin released from the posterior pituitary gland. The release of both ACTH and CRF, in turn, is reflexly inhibited by the ensuing rising concentrations of glucocorticoids in the blood.

Opioid peptides also exercise a tonic inhibitory control on the secretion of CRF, and psychological factors, excessive heat or cold, injury or infections can also affect the release of both vasopressin and CRF. This is the principal mechanism whereby the HPA axis is activated in response to perceived threats in the external environment.

The precursor of glucocorticoids is cholesterol (Fig. 33.5). The initial conversion of cholesterol to *pregnenolone* is the rate-limiting step and is regulated by ACTH. Some biosynthetic reactions can be inhibited by drugs and these have a utility in treating Cushing's disease or adrenocortical carcinoma. **Metyrapone** prevents the β-hydroxylation at C11, and thus the formation of hydrocortisone and corticosterone. Synthesis is blocked at the 11-deoxycorticosteroid stage, leaving intermediates that have no effects on the hypothalamus and pituitary, so there is a marked increase in ACTH in the blood. Metyrapone can therefore be used to test ACTH production, and may also be used to treat patients with Cushing's syndrome. **Trilostane** (also of use in Cushing's syndrome and primary hyperaldosteronism) blocks an earlier enzyme in the pathway – the *3β-dehydrogenase*. **Aminoglutethimide** inhibits the initial step in the biosynthetic pathway and has the same overall effect as metyrapone.

Trilostane and aminoglutethamide are not currently used in the UK but **ketoconazole**, an antifungal agent (Ch. 53), also inhibits steroidogenesis and may be of value in the specialised treatment of Cushing's syndrome. **Mitotane** suppresses glucocorticoid synthesis by a direct (and unknown) mechanism on the adrenal gland. It is chiefly used to treat adrenocortical carcinomas.

Mechanism of glucocorticoid action

The glucocorticoid effects relevant to this discussion are initiated by interaction of the drugs with specific intracellular glucocorticoid receptors belonging to the nuclear receptor superfamily (although there may be other binding proteins or sites; see Norman et al., 2004). This superfamily (see Ch. 3) also includes the receptors for mineralocorticoids, the sex steroids, thyroid hormones, vitamin D_3 and retinoic acid. The actual mechanism of transcriptional control is complex, with at least four mechanisms operating within the nucleus. These are summarised diagrammatically in Figure 33.6.

When the nuclear actions of glucocorticoid receptors were first discovered it was thought that this mechanism could account for all the actions of the hormones, but a surprising discovery overturned this idea. Reichardt et al. (1998), using transgenic mice in which the glucocorticoid receptor was unable to dimerise, found that glucocorticoids were still able to exert a great many biological actions. This suggested that in addition to controlling gene expression within the nucleus, the liganded receptor itself, in either a monomeric or a dimeric form, could initiate important signal transduction events while still in the cytosolic compartment (there may even be a subpopulation of receptors that reside there permanently). One such effect seems to be interaction of the receptor with the regulatory complex, NF-κB (Ch. 3). Other important interactions may involve protein kinases/phosphatases that regulate glucocorticoid receptor behaviour and the time spent in the nuclear compartment. Some of these cytosolic actions are very rapid. For example, the glucocorticoid-induced phosphorylation by PKC and subsequent release of the protein *annexin-1*, which has potent inhibitory effects on leukocyte trafficking and other anti-inflammatory actions, occurs in minutes and could not be accounted for by changes in protein synthesis.

Glucocorticoids

Common drugs used systemically include **hydrocortisone**, **prednisolone** and **dexamethasone**.

Metabolic actions

- *Carbohydrates*: decreased uptake and utilisation of glucose accompanied by increased gluconeogenesis; this causes a tendency to hyperglycaemia.
- *Proteins*: increased catabolism, reduced anabolism.
- *Lipids*: a permissive effect on lipolytic hormones and a redistribution of fat, as observed in Cushing's syndrome.

Regulatory actions

- *Hypothalamus and anterior pituitary gland*: a negative feedback action resulting in reduced release of endogenous glucocorticoids.
- *Cardiovascular system*: reduced vasodilatation, decreased fluid exudation.
- *Musculoskeletal*: decreased osteoblast and increased osteoclast activity.
- *Inflammation and immunity:*
  - acute inflammation: decreased influx and activity of leukocytes
  - chronic inflammation: decreased activity of mononuclear cells, decreased angiogenesis, less fibrosis
  - lymphoid tissues: decreased clonal expansion of T and B cells, and decreased action of cytokine-secreting T cells. Switch from Th1 to Th2 response.
- Mediators:
  - decreased production and action of many cytokines, including interleukins, tumour necrosis factor-α and granulocyte–macrophage colony-stimulating factor
  - reduced generation of eicosanoids
  - decreased generation of IgG
  - decrease in complement components in the blood
  - increased release of anti-inflammatory factors such as interleukin (IL)-10, IL-1ra and annexin 1.
- Overall effects: reduction in the activity of the innate and acquired immune systems, but also diminution in the protective aspects of the inflammatory response and sometimes decreased healing.

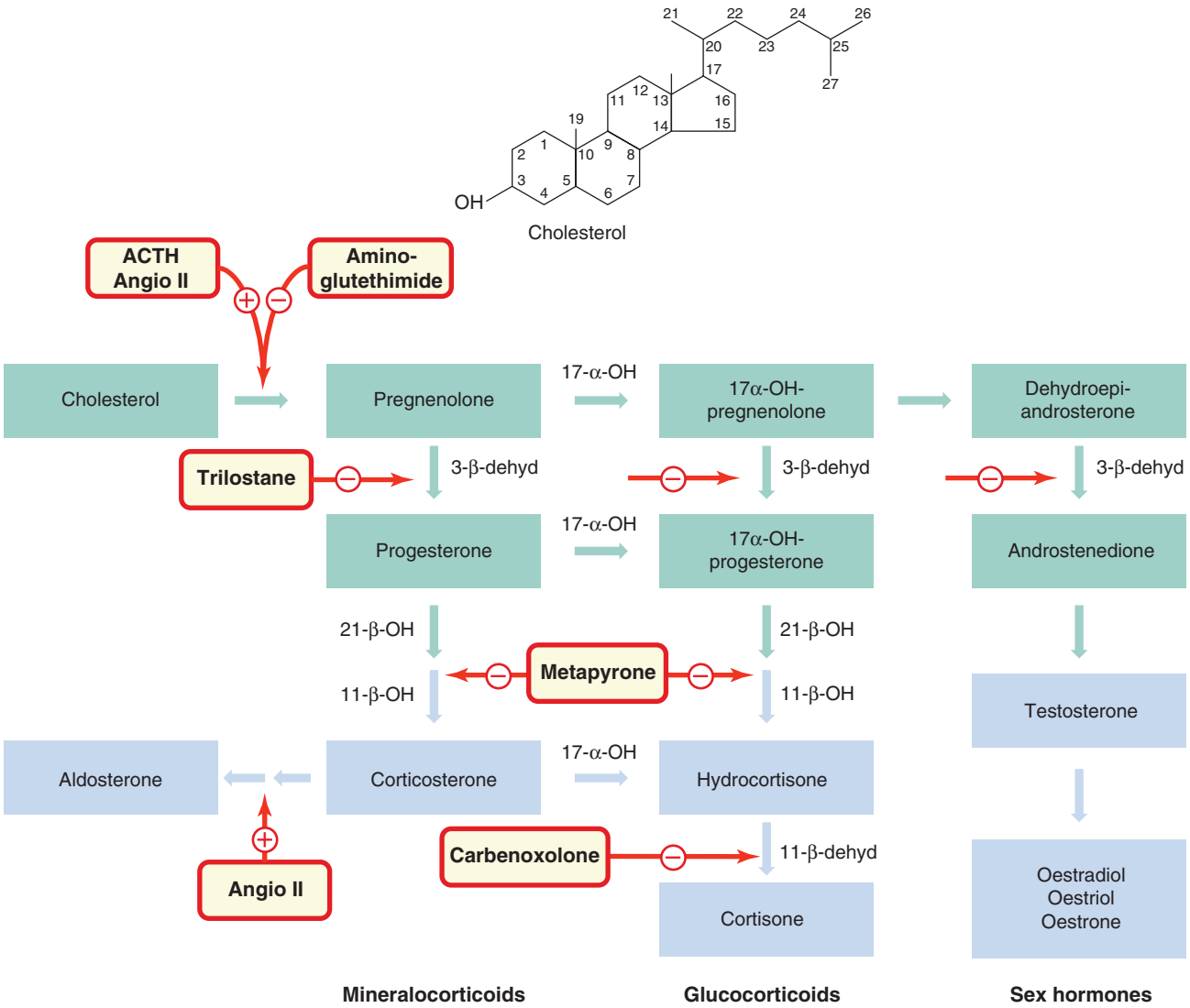

Fig. 33.5 Biosynthesis of corticosteroids, mineralocorticoids and sex hormones. All steroid hormones are synthesised from cholesterol. Successive steps of hydroxylation and dehydrogenation are important in the biosynthetic pathway and are targets for drugs. Intermediates are shown in green boxes; interconversions occur between the pathways. Blue boxes indicate circulating hormones. Drugs are shown in red-bordered boxes adjacent to their sites of action. Glucocorticoids are produced by cells of the zona fasciculata, and their synthesis is stimulated by adrenocorticotrophic hormone (ACTH); aldosterone is produced by cells of the zona glomerulosa, and its synthesis is stimulated by angiotensin II (angio II). Metyrapone inhibits glucocorticoid synthesis, aminoglutethimide and trilostane block synthesis of all three types of adrenal steroid (see text for details). Carbenoxolone inhibits the interconversion of hydrocortisone and cortisone in the kidney. Not shown is mitotane, which suppresses adrenal hormone synthesis through an unknown mechanism. Enzymes: 17-α-OH, 17-α-hydroxylase; 3-β-dehyd, 3-β-dehydrogenase; 21-β-OH, 21-β-hydroxylase; 11-β-OH, 11-β-hydroxylase; 11-β-dehyd, 11-β-hydroxysteroid dehydrogenase.

Actions

General metabolic and systemic effects

The main metabolic effects are on carbohydrate and protein metabolism. The glucocorticoids cause both a decrease in the uptake and utilisation of glucose and an increase in gluconeogenesis, resulting in a tendency to hyperglycaemia (see Ch. 31). There is a concomitant increase in glycogen storage, which may be a result of insulin secretion in response to the increase in blood sugar. Overall, there is decreased protein synthesis and increased protein breakdown, particularly in muscle, and this can lead to tissue wasting. Glucocorticoids also have a 'permissive' effect on the cAMP-dependent lipolytic response to catecholamines and other hormones. Such hormones cause lipase activation through a cAMP-dependent kinase, the synthesis of which requires the presence of glucocorticoids. Large doses of glucocorticoids given over a long period result in the redistribution of body fat characteristic of Cushing's syndrome (Fig. 33.7).

Glucocorticoids tend to produce a negative calcium balance by decreasing Ca^{2+} absorption in the gastrointestinal tract and increasing its excretion by the kidney. Together with increased breakdown of bone matrix

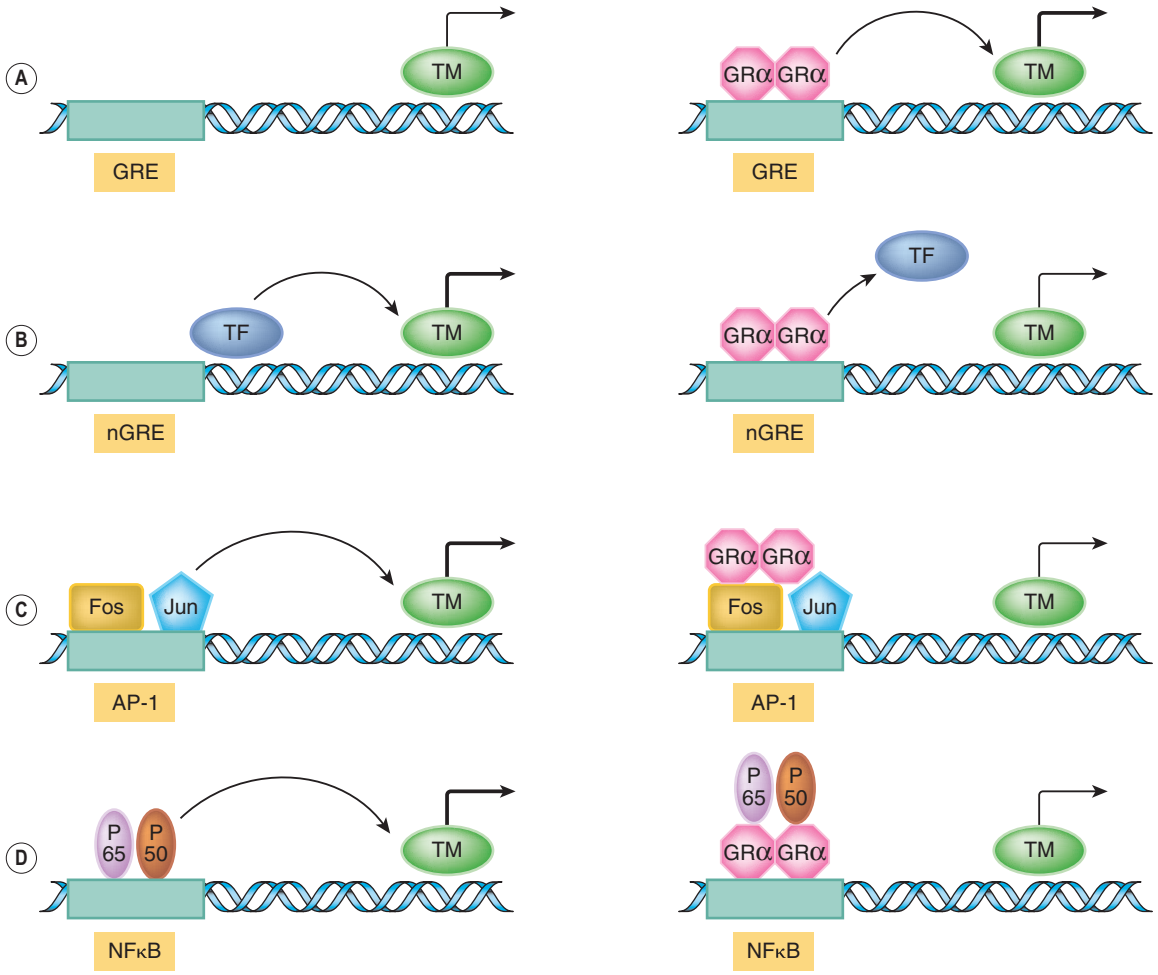

Fig. 33.6 Molecular mechanism of action of glucocorticoids. The schematic figure shows three possible ways by which the liganded glucocorticoid receptor can control gene expression following translocation to the nucleus. [**A**] Basic transactivation mechanism. Here, the transcriptional machinery (TM) is presumed to be operating at a low level. The liganded glucocorticoid receptor (GR) dimer binds to one or more 'positive' glucocorticoid response elements (GREs) within the promoter sequence (shaded zone) and upregulates transcription. [**B**] Basic transrepression mechanism. The transcriptional machinery is constitutively driven by transcription factors (TF). In binding to the 'negative' GRE (nGRE), the receptor complex displaces these factors and expression falls. [**C**] Fos/Jun mechanism. Transcription is driven at a high level by Fos/Jun transcription factors binding to their AP-1 regulatory site. This effect is reduced in the presence of the GR. [**D**] Nuclear factor (NF)κβ mechanism. The transcription factors P65 and P50 bind to the NF-κβ site, promoting gene expression. This is prevented by the presence of the GR, which binds the transcription factors, preventing their action (this may occur in the cytoplasm also). (For further details of the structure of the glucocorticoid receptor, see Ch. 3.) *(Modified from Oakley & Cidlowski 2001.)*

protein this may cause osteoporosis. In higher, non-physiological concentrations, the glucocorticoids have some mineralocorticoid actions, causing Na^+ retention and K^+ loss – possibly by swamping the protective 11β-hydroxysteroid dehydrogenase and acting at mineralocorticoid receptors.

Negative feedback effects on the anterior pituitary and hypothalamus

Both endogenous and exogenous glucocorticoids have a negative feedback effect on the secretion of CRF and ACTH (see Fig. 33.4), thus inhibiting the secretion of endogenous glucocorticoids and potentially causing atrophy of the adrenal cortex. If therapy is prolonged, it may take many months to return to normal function once the drugs are stopped.

Anti-inflammatory and immunosuppressive effects

Endogenous glucocorticoids maintain a low-level anti-inflammatory tone, and are secreted in response to inflammatory stimuli. Consequently, adrenalectomised animals show a heightened response to even mild inflammatory stimuli . On this basis, it has been suggested that a failure of appropriate secretion of glucocorticoids in response to injury or infection may underlie certain chronic inflammatory human pathologies.

Exogenous glucocorticoids are the anti-inflammatory drugs *par excellence*, and when given therapeutically inhibit the operation of both the innate and adaptive immune system. They reverse virtually all types of inflammatory reaction, whether caused by invading pathogens, by chemical or physical stimuli, or by inappropriately deployed immune responses such as are seen in hypersensitivity

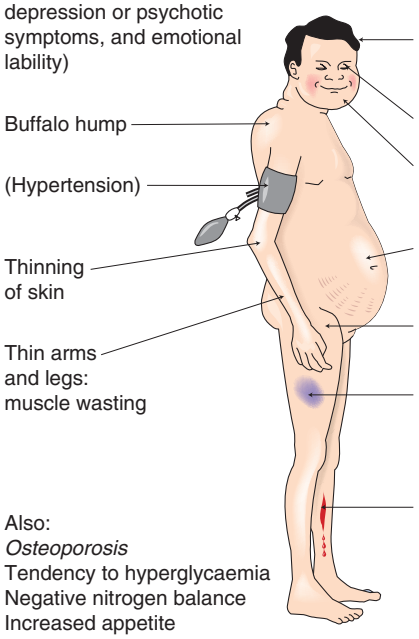

Fig. 33.7 Cushing's syndrome. This is caused by excessive exposure to glucocorticoids, and may be caused by disease (e.g. an adrenocorticotrophic hormone-secreting tumour) or by prolonged administration of glucocorticoid drugs (iatrogenic Cushing's). Italicised effects are particularly common. Less frequent effects, related to dose and duration of therapy, are shown in parentheses. *(Adapted from Baxter & Rousseau 1979)*

or autoimmune disease. When used prophylactically to suppress graft rejection, glucocorticoids are more efficient in suppressing the initiation and generation of the immune response than they are in preventing the operation of an established response where clonal proliferation has already occurred.

Given that glucocorticoids modify the expression of so many genes, and that the extent and direction of regulation varies between tissues and even at different times during disease, you will not be surprised to learn that their anti-inflammatory effects are complex.

Actions on *inflammatory* cells include:

- decreased egress of neutrophils from blood vessels and reduced activation of neutrophils, macrophages and mast cells secondary to decreased transcription of the genes for cell adhesion factors and cytokines
- decreased overall activation of T-helper (Th) cells, reduced clonal proliferation of T cells, and a 'switch' from the Th1 to the Th2 immune response (see Ch. 6)
- decreased fibroblast function, less production of collagen and glycosaminoglycans, and, under some circumstances, reduced healing and repair.

Actions on the mediators of inflammatory and immune responses (Chs 17 and 18) include:

- decreased production of prostanoids through reduced expression of cyclo-oxygenase-2
- decreased generation of many cytokines, including IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, TNF-α, cell adhesion factors and granulocyte–macrophage colony-stimulating factor. These are largely secondary to inhibition of gene transcription
- reduction in the concentration of complement components in the plasma
- decreased generation of induced nitric oxide by nitric oxide synthase 2 (NOS2)
- decreased histamine release from basophils and mast cells
- decreased immunoglobulin G (IgG) production
- increased synthesis of anti-inflammatory factors such as IL-10, IL-1-soluble receptor and annexin-1.

Potent anti-inflammatory glucocorticoids circulate constantly in the blood and are increased during inflammatory episodes. It is suggested (see Munck et al., 1984), that the anti-inflammatory and immunosuppressive actions of endogenous glucocorticoids play a crucial counter-regulatory role, in that they prevent excessive activation of inflammation and other powerful defence reactions that might, if unchecked, themselves threaten homeostasis. Certainly, this view is borne out by experimental work. While these drugs are of great value in treating conditions characterised by hypersensitivity and unwanted inflammation, they carry the hazard that they are able to suppress the same defence reactions that provide protection from infection and other insults.

Unwanted effects

Low-dose glucocorticoid replacement therapy is usually without problems but serious unwanted effects occur with large doses or prolonged administration of glucocorticoids. The major effects are as follows:

- *Suppression of the response to infection or injury*: opportunistic infection can be potentially very serious unless quickly treated with antimicrobial agents along with an increase in the dose of steroid. Oral thrush (candidiasis, a fungal infection; see Ch. 53) frequently occurs when glucocorticoids are taken by inhalation, because of suppression of local anti-infective mechanisms. Wound healing is impaired, and peptic ulceration may also occur.
- *Cushing's syndrome* (see Fig. 33.7).
- *Osteoporosis*, with the attendant hazard of fractures, is one of the main limitations to long-term glucocorticoid therapy. These drugs influence bone density both by regulation of calcium and phosphate metabolism and through effects on collagen turnover. They reduce osteoblast function (which deposits bone matrix) and increase the activity of osteoclasts (which digest bone matrix). An effect on the blood supply to bone can result in avascular necrosis of the head of the femur (see Ch. 36).
- *Hyperglycaemia* produced by exogenous glucocorticoids may develop into actual diabetes.
- *Muscle wasting* and proximal muscle weakness.
- In children, *inhibition of growth*[6] if treatment is continued for more than 6 months.

[6]However, some of the diseases for which glucocorticoids are indicated themselves retard growth. In a classical trial, glucocorticoid treatment *increased* growth in adolescents with inflammatory bowel disease as the disease resolved (Whittington et al., 1977).

- *Central nervous system effects*: euphoria, depression and psychosis.
- *Other effects*: glaucoma (in genetically predisposed persons), raised intracranial pressure and an increased incidence of cataracts.

Sudden withdrawal of the drugs after prolonged therapy may result in acute adrenal insufficiency because of suppression of the patient's capacity to synthesise corticosteroids.[7] Careful procedures for phased withdrawal should be followed. Recovery of full adrenal function usually takes about 8 weeks, although it can take 18 months or more after prolonged high-dose treatment.

Mechanism of action of the glucocorticoids

- Glucocorticoids bind intracellular receptors that then dimerise, migrate to the nucleus and interact with DNA to modify gene transcription, inducing synthesis of some proteins and inhibiting synthesis of others.
- A substantial proportion of glucocorticoid actions are mediated by interactions of regulatory factors with the receptor in the cytosol. Some are very rapid.
- *Metabolic actions*: most mediator proteins are enzymes, for example cAMP-dependent kinase, but not all actions on genes are known.
- *Anti-inflammatory and immunosuppressive actions*. Known actions include:
  - inhibition of transcription of the genes for inducible cyclo-oxygenase-2 and inducible nitric oxide synthase, cytokines and interleukins, cell adhesion molecules
  - block of vitamin D_3-mediated induction of the osteocalcin gene in osteoblasts, and modification of transcription of the collagenase genes
  - increased synthesis and release of anti-inflammatory factors including annexin-1 in cells of the innate immune system. This has potent anti-inflammatory effects on cells and mediator release, and may also mediate negative feedback at the level of the hypothalamus and anterior pituitary gland.

Pharmacokinetic aspects

There are many glucocorticoid drugs in therapeutic use. Although **cortisol** (**hydrocortisone**), the endogenous hormone, is often used, synthetic derivatives are even more common. These have different physicochemical properties as well as varying potency and have been optimised for administration by different routes. They may be administered orally, systemically or intra-articularly; given by aerosol into the respiratory tract; administered as drops into the eye or sprayed into the nose; applied as creams or ointments to the skin (see Ch. 27); or as foam enemas to the gastrointestinal tract (Ch. 30). Topical administration diminishes the likelihood of systemic toxic effects unless large quantities are used. When prolonged use of systemic glucocorticoids is necessary, therapy on alternate days may decrease suppression of the HPA axis and other unwanted effects.

As small lipophilic molecules, glucocorticoids probably enter their target cells by simple diffusion. Hydrocortisone has a plasma half-life of 90 min, although its main biological effects have a latency of 2–8 h. Biological inactivation, which occurs in liver cells and elsewhere, is initiated by reduction of the C4–C5 double bond. Cortisone and **prednisone** are inactive until converted *in vivo* to hydrocortisone and **prednisolone**, respectively.

Endogenous glucocorticoids are transported in the plasma bound to *corticosteroid-binding globulin* (CBG) and to albumin. About 77% of plasma hydrocortisone is bound to CBG, but many synthetic glucocorticoids are not bound at all. Albumin has a lower affinity for hydrocortisone but binds both natural and synthetic steroids. Both CBG-bound and albumin-bound steroids are biologically inactive.

The clinical use of systemic glucocorticoids is given in the clinical box above. Dexamethasone has a special use: it is used to test HPA axis function. In the *dexamethasone suppression test* a relatively low dose of dexamethasone, usually given at night, would be expected to suppress the hypothalamus and pituitary, and result in reduced ACTH secretion and hydrocortisone output, as measured in the plasma about 9 h later. Failure of suppression implies hypersecretion of ACTH or of glucocorticoids (Cushing's syndrome).

Clinical uses of glucocorticoids

- Replacement therapy for patients with adrenal failure (*Addison's disease*).
- Anti-inflammatory/immunosuppressive therapy (see also Ch. 26):
  - in *asthma* (Ch. 28)
  - topically in various inflammatory conditions of skin, eye, ear or nose (e.g. *eczema*, *allergic conjunctivitis* or *rhinitis*; see Ch. 27)
  - *hypersensitivity states* (e.g. severe allergic reactions)
  - in miscellaneous diseases with autoimmune and inflammatory components (e.g. *rheumatoid arthritis* and other 'connective tissue' diseases, *inflammatory bowel diseases*, some forms of *haemolytic anaemia*, *idiopathic thrombocytopenic purpura*)
  - to prevent graft-versus-host disease following organ or bone marrow transplantation.
- In neoplastic disease (Ch. 56):
  - in combination with cytotoxic drugs in treatment of specific malignancies (e.g. *Hodgkin's disease*, *acute lymphocytic leukaemia*)
  - to reduce cerebral oedema in patients with metastatic or primary *brain tumours* (**dexamethasone**).

MINERALOCORTICOIDS

The main endogenous mineralocorticoid is aldosterone. Its chief action is to increase Na^+ reabsorption by the distal tubules in the kidney, with a concomitant increase in excretion of K^+ and H^+ (see Ch. 29). An excessive secretion

[7]Patients on long-term glucocorticoid therapy are advised to carry a card stating, 'I am a patient on STEROID TREATMENT which must not be stopped abruptly'.

Pharmacokinetics and unwanted actions of the glucocorticoids

- Administration can be oral, topical or parenteral. Most naturally occurring glucocorticoids are transported in the blood by corticosteroid-binding globulin or albumen and enter cells by diffusion. They are metabolised in the liver.
- Unwanted effects are seen mainly after prolonged systemic use as anti-inflammatory or immunosuppressive agents but not usually following replacement therapy. The most important are:
  - suppression of response to infection
  - suppression of endogenous glucocorticoid synthesis
  - metabolic actions (see above)
  - osteoporosis
  - iatrogenic Cushing's syndrome (see Fig. 33.7).

of mineralocorticoids, as in *Conn's syndrome*, causes marked Na^+ and water retention, with increased extracellular fluid volume and sometimes hypokalaemia, alkalosis and hypertension. Decreased secretion, as in some patients with Addison's disease, causes Na^+ loss (desalinisation) and a marked decrease in extracellular fluid volume. There is a concomitant decrease in the excretion of K^+, resulting in hyperkalaemia.

Mineralocorticoids

Fludrocortisone is given orally to produce a mineralocorticoid effect. This drug:

- increases Na^+ reabsorption in distal tubules and increases K^+ and H^+ efflux into the tubules
- acts on intracellular receptors that modulate DNA transcription, causing synthesis of protein mediators
- is used together with a glucocorticoid in replacement therapy.

Regulation of aldosterone synthesis and release

The regulation of the synthesis and release of aldosterone depends mainly on the electrolyte composition of the plasma and on the angiotensin II system (Fig. 33.4; Chs 22 and 29). Low plasma Na^+ or high plasma K^+ concentrations affect the zona glomerulosa cells of the adrenal directly, stimulating aldosterone release. Depletion of body Na^+ also activates the renin–angiotensin system (see Ch. 22, Fig. 22.4). One of the effects of angiotensin II is to increase the synthesis and release of aldosterone (see Ch. 29, Fig. 29.5).

Mechanism of action

Like other steroid hormones, aldosterone acts through specific intracellular receptors of the nuclear receptor family. Unlike the glucocorticoid receptor, which is present in most cells, the *mineralocorticoid receptor* is restricted to a few tissues, such as the kidney and the transporting epithelia of the colon and bladder. Cells containing mineralocorticoid receptors also contain the 11β-hydroxysteroid dehydrogenase type 2 enzyme, which converts hydrocortisone (cortisol) into inactive cortisone, but does not inactivate aldosterone. This ensures that the cells are appropriately affected only by the mineralocorticoid hormone itself. Interestingly, this enzyme is inhibited by **carbenoxolone,** a compound derived from liquorice (and previously used to treat gastric ulcers; see Ch. 30). If this inhibition is marked, cortisol accumulates and acts on the mineralocorticoid receptor, producing an effect similar to Conn's syndrome (*primary hyperaldosteronism*) except that the circulating aldosterone concentration is not raised.

As with the glucocorticoids, the interaction of aldosterone with its receptor initiates transcription and translation of specific proteins, resulting in an increase in the number of sodium channels in the apical membrane of the cell, and subsequently an increase in the number of Na^+-K^+-ATPase molecules in the basolateral membrane (see Fig. 29.5), causing increased K^+ excretion (see Ch. 29). In addition to the genomic effects, there is evidence for a rapid non-genomic effect of aldosterone on Na^+ influx, through an action on the Na^+-H^+ exchanger in the apical membrane.

Clinical use of mineralocorticoids and antagonists

The main clinical use of mineralocorticoids is in replacement therapy of patients with Addison's disease. The most commonly used drug is **fludrocortisone** (Table 33.2 and Fig. 33.4), which can be taken orally to supplement the necessary glucocorticoid replacement. **Spironolactone** is a competitive antagonist of aldosterone, and it also prevents the mineralocorticoid effects of other adrenal steroids on the renal tubule (Ch. 29). Side effects include gynaecomastia and impotence, because spironolactone also has some blocking action on androgen and progesterone receptors. It is used to treat primary or secondary hyperaldosteronism and, in conjunction with other drugs, in the treatment of resistant hypertension and of heart failure (Ch. 22) and oedema (Ch. 29). **Eplerenone** has a similar indication and mechanism of action, although fewer side effects as it has lower affinity for sex hormone receptors (Ch. 22).

NEW DIRECTIONS IN GLUCOCORTICOID THERAPY

Glucocorticoids are highly effective in controlling inflammation, but severely limited by their side effects. The ideal solution would be a glucocorticoid possessing the anti-inflammatory but not the unwanted metabolic or other effects.

Following the discovery of cortisol, the pharmaceutical industry pursued this ambitious goal by testing straightforward structural analogues of cortisol. While this yielded many new active and interesting compounds (several of which are in clinical use today), none achieved a true 'separation' of the glucocorticoid actions. Many considered that the possibilities afforded by this approach had been exhausted but recently there have been fresh attempts to accomplish this. The development of structural analogues at novel sites on the steroid template (e.g. Uings et al., 2013) has met with more success and the use of X-ray crystallography has even enabled the design of non-steroidal ligands that exploit unusual binding sites on the receptor (Biggadike et al., 2009).

Another idea has been to add other functional groups on to the steroid molecule. Fiorucci et al. (2002) attached a nitric oxide donating group to prednisolone, finding augmented efficacy and reduced unwanted effects. The compound is reported to be useful in the treatment of inflammatory bowel disease (see Schacke et al., 2007).

Many investigators in this area have been influenced by the 'transrepression hypothesis': this is the notion, based upon some experimental observations, that the therapeutic effects of glucocorticoids are generally caused by the *down*-regulation (*transrepression*) of genes such as those coding for cytokines, whilst the unwanted effects are usually caused by *up*-regulation (*transactivation*) of metabolic and other genes (e.g. tyrosine amino transferase and phosphoenol pyruvate carboxykinase). This could alter intermediate metabolism and lead to, for example, diabetes. Because transactivation and transrepression utilise different molecular pathways, researchers have sought **se**lective **g**lucocorticoid **r**eceptor **a**gonists (SEGRAs) that promote one set of actions without the other. The application of this idea has been reviewed by Schacke et al. (2007) and the development of one such compound to the clinical trial stage has been reported (Schacke et al., 2009). Clark and Belvisi (2012) have reviewed the evidence for this idea and, in particular, highlighted its shortcomings.

A related idea focuses upon the histone deacetylase enzymes that are responsible for facilitating the transcriptional regulation of genes following nuclear receptor binding to response elements (Hayashi et al., 2004). One current notion is that there may be a specific isoform of this enzyme that deals with gene upregulation, and that if this could be inhibited, it would lessen the possibility of those unwanted effects. Barnes (2011) has reviewed this approach particularly as it relates to the therapy of asthma. A more general review of the whole area with particular relevance to the treatment of rheumatic diseases has been provided by Strehl et al. (2011).

The quest for the glucocorticoid magic bullet continues.

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34 The thyroid

OVERVIEW

Diseases of the thyroid gland are common, and in this chapter we deal with drug therapy used to mitigate these disorders. We set the scene by briefly outlining the structure, regulation and physiology of the thyroid, and highlight the most common abnormalities of thyroid function. We then consider the drugs that can replace the thyroid hormones when these are deficient or cease to function adequately, and the drugs that decrease thyroid function when this is excessive.

SYNTHESIS, STORAGE AND SECRETION OF THYROID HORMONES

The thyroid gland secretes three main hormones: *thyroxine* (T_4), *tri-iodothyronine* (T_3) and *calcitonin*. T_4 and T_3 are critically important for normal growth and development and for controlling energy metabolism. Calcitonin is involved in the control of plasma [Ca^{2+}] and is used to treat osteoporosis and other metabolic bone diseases. It is dealt with in Chapter 36. The term 'thyroid hormones' will be used here solely to refer to T_4 and T_3.

The functional unit of the thyroid is the follicle or acinus. Each follicle consists of a single layer of epithelial cells around a cavity, the *follicle lumen*, which is filled with a thick colloid containing thyroglobulin. *Thyroglobulin* is a large glycoprotein, each molecule of which contains about 115 tyrosine residues. It is synthesised, glycosylated and then secreted into the lumen of the follicle, where iodination of the tyrosine residues occurs. Surrounding the follicles is a dense capillary network and the blood flow through the gland is very high in comparison with other tissues. The main steps in the synthesis, storage and secretion of thyroid hormone (Fig. 34.1) are:

- uptake of plasma iodide by the follicle cells
- oxidation of iodide and iodination of tyrosine residues of thyroglobulin
- secretion of thyroid hormone.

UPTAKE OF PLASMA IODIDE BY THE FOLLICLE CELLS

Iodide uptake must occur against a concentration gradient (normally about 25:1) so it is an energy-dependent process. Iodide is captured from the blood and moved to the lumen by two transporters: the Na^+/I^- symporter (NIS) located at the basolateral surface of the thyrocytes (the energy being provided by Na^+/K^+-ATPase), and *pendrin*[1] (PDS), an I^-/Cl^- porter in the apical membranes (Nilsson, 2001). Uptake is very rapid: labelled iodide (^{125}I) is found in the lumen within 40 s of intravenous injection. Numerous mutations have been discovered in the NIS and PDS genes and these contribute to thyroid disease in some patients.

OXIDATION OF IODIDE AND IODINATION OF TYROSINE RESIDUES

The oxidation of iodide and its incorporation into thyroglobulin (termed the *organification* of iodide) is catalysed by *thyroperoxidase*, an enzyme situated at the inner surface of the cell at the interface with the colloid. The reaction requires the presence of hydrogen peroxide (H_2O_2) as an oxidising agent. Iodination occurs after the tyrosine has been incorporated into thyroglobulin. The process is shown in Figure 34.2.

Tyrosine residues are iodinated first at position 3 on the ring, forming monoiodotyrosine (MIT) and then, in some molecules, at position 5 as well, forming di-iodotyrosine (DIT). While still incorporated into thyroglobulin, these molecules are then coupled in pairs, either MIT with DIT to form T_3, or two DIT molecules to form T_4. The mechanism for coupling is believed to involve a peroxidase system similar to that involved in iodination. About one-fifth of the tyrosine residues in thyroglobulin are iodinated in this way.

The iodinated thyroglobulin of the thyroid forms a large store of thyroid hormone within the gland, with a relatively slow turnover. This is in contrast to some other endocrine secretions (e.g. the hormones of the adrenal cortex), which are not stored but synthesised and released as required.

SECRETION OF THYROID HORMONE

The thyroglobulin molecule is taken up into the follicle cell by endocytosis (Fig. 34.1). The endocytotic vesicles then fuse with lysosomes, and proteolytic enzymes act on thyroglobulin, releasing T_4 and T_3 to be secreted into the plasma. The surplus MIT and DIT, which are released at the same time, are scavenged by the cell and the iodide is removed enzymatically and reused.

REGULATION OF THYROID FUNCTION

Thyrotrophin-releasing hormone (TRH), released from the hypothalamus in response to various stimuli, releases *thyroid-stimulating hormone* (TSH; thyrotrophin) from the anterior pituitary (Fig. 34.3), as does the synthetic tripeptide **protirelin** (pyroglutamyl-histidyl-proline amide), which is used in this way for diagnostic purposes. TSH acts on receptors on the membrane of thyroid follicle cells through a mechanism that involves cAMP and phosphatidylinositol 3-kinase. It has a trophic action on thyroid cells and controls all aspects of thyroid hormone synthesis, including:

- the uptake of iodide by follicle cells, by stimulating transcription of the iodide transporter genes; this is

[1]So called because it is implicated in the pathophysiology of *Pendred's syndrome*, named after the eponymous English physician who first described this autosomal recessive form of familial goitre in association with sensorineural deafness.

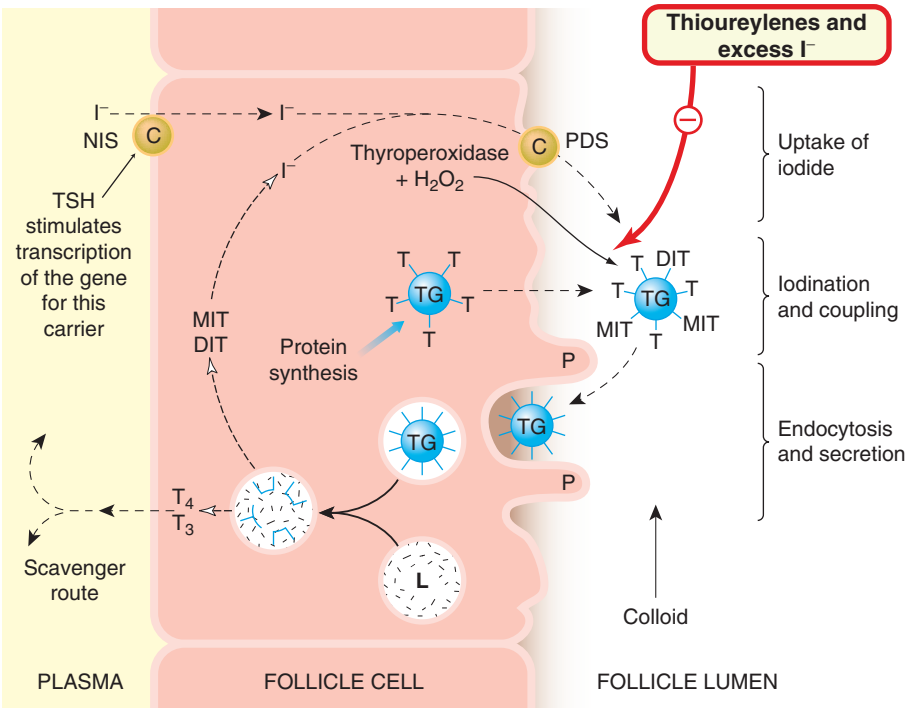

Fig. 34.1 Diagram of thyroid hormone synthesis and secretion, with the sites of action of some drugs used in the treatment of thyroid disorders. Iodide in the blood is transported by the carriers NIS and pendrin (PDS) through the follicular cell and into the colloid-rich lumen, where it is incorporated into thyroglobulin under the influence of the thyroperoxidase enzyme (see text for details). The hormones are produced by processing of the endocytosed thyroglobulin and exported into the blood. DIT, di-iodotyrosine; L, lysosome; MIT, monoiodotyrosine; P, pseudopod; T, tyrosine; T_3, tri-iodothyronine; T_4, thyroxine; TG, thyroglobulin; TSH, thyroid-stimulating hormone (thyrotrophin).

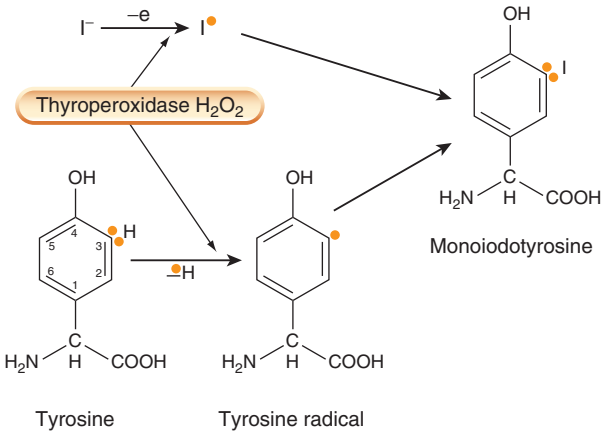

Fig. 34.2 Iodination of tyrosyl residues by the thyroperoxidase–H_2O_2 complex. This probably involves two sites on the enzyme, one of which removes an electron from iodide to give the free radical I•; another removes an electron from tyrosine to give the tyrosyl radical (shown by orange dot). Monoiodotyrosine results from the addition of the two radicals.

the main mechanism by which it regulates thyroid function and controls all aspects of thyroid hormone synthesis including:

- the synthesis and secretion of thyroglobulin
- the generation of H_2O_2 and the iodination of tyrosine
- the endocytosis and proteolysis of thyroglobulin
- the actual secretion of T_3 and T_4
- the blood flow through the gland.

The production of TSH is also regulated by a negative feedback effect of thyroid hormones on the anterior pituitary gland; T_3 is more active than T_4 in this respect. The peptide **somatostatin** also reduces basal TSH release. The control of the secretion of TSH thus depends on a balance between the actions of T_3/T_4 and TRH (and probably also somatostatin) on the pituitary.[2]

The other main factor influencing thyroid function is the plasma iodide concentration. About 100 nmol of T_4 is synthesised daily, necessitating uptake by the gland of approximately 500 nmol of iodide each day (equivalent to about 70 µg of iodine). A reduced iodine intake, with reduced plasma iodide concentration, will result in a decrease of hormone production and an increase in TSH secretion. An increased plasma iodide has the opposite effect, although this may be modified by other factors. The overall feedback mechanism responds to changes of iodide slowly over fairly long periods of days or weeks, because there is a large reserve capacity for the binding and uptake of iodide in the thyroid. The size and vascularity of the thyroid are reduced by an increase in plasma iodide and this is exploited therapeutically in preparing

[2]Other control systems may also operate under some circumstances. A 'long feedback' loop through which T_3/T_4 can act on the hypothalamus to reduce TSH has been demonstrated in some animals.

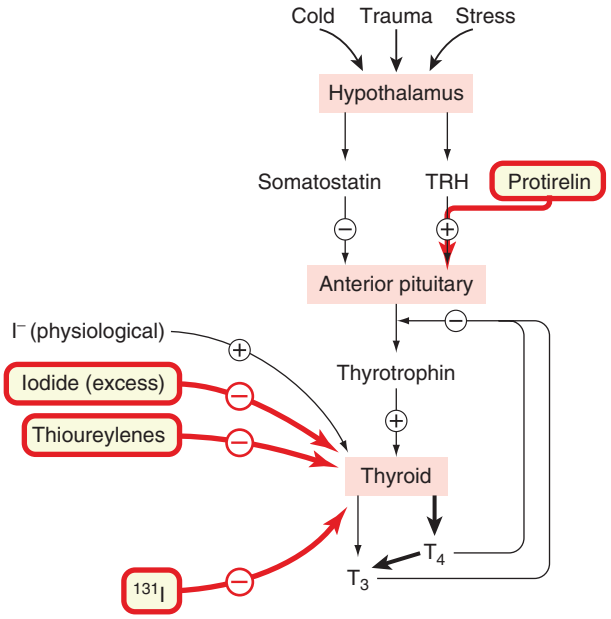

Fig. 34.3 Regulation of thyroid hormone secretion. Iodide (I^-) is essential for thyroid hormone synthesis, but excess of endogenous or exogenous iodide (30 times the daily requirement of iodine) may be used to inhibit the increased thyroid hormone production of thyrotoxicosis. Protirelin as well as recombinant thyrotrophin-releasing hormone (TRH) is sometimes used to stimulate the system for diagnostic purposes. Larger amounts of this isotope are used for ablation of thyroid tissue (see text for details). T_3, tri-iodothyronine; T_4, thyroxine.

hyperthyroid patients for surgery to the gland. Diets deficient in iodine eventually result in a continuous excessive compensatory secretion of TSH, and eventually in an increase in vascularity and (sometimes gross) hypertrophy of the gland.[3]

ACTIONS OF THE THYROID HORMONES

The physiological actions of the thyroid hormones fall into two main categories: those affecting metabolism and those affecting growth and development.

EFFECTS ON METABOLISM

The thyroid hormones produce a general increase in the metabolism of carbohydrates, fats and proteins, and regulate these processes in most tissues, T_3 being three to five times more active than T_4 in this respect (Fig. 34.4). Although the thyroid hormones directly control the activity of some of the enzymes of carbohydrate metabolism, most effects are brought about in conjunction with other hormones, such as insulin, glucagon, the glucocorticoids and the catecholamines. There is an increase in oxygen consumption and heat production, which is manifested as an increase in the measured basal metabolic rate. This reflects the action of these hormones on tissues such as heart, kidney, liver and muscle, although not on others,

[3]'Derbyshire neck' was the name given to this condition in a part of the UK where sources of dietary iodine were once scarce.

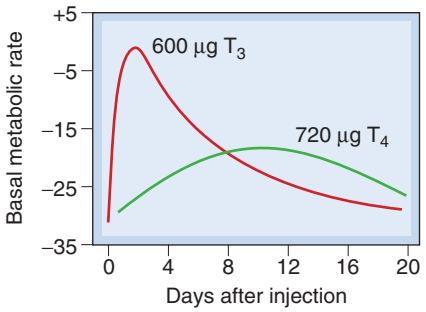

Fig. 34.4 The effect of equimolar doses of tri-iodothyronine (T_3) and thyroxine (T_4) on basal metabolic rate (BMR) in a hypothyroid subject. Note that this figure is meant only to illustrate overall differences in effect; thyroxine is not given clinically in a single bolus dose as here, but in regular daily doses so that the effect builds up to a plateau. The apparent differences in potency really represent differences in kinetics, reflecting the prehormone role of T_4. *(Modified from Blackburn et al., 1954.)*

such as the gonads, brain or spleen. The calorigenic action is important as part of the response to a cold environment. Administration of thyroid hormone results in augmented cardiac rate and output, and increased tendency to dysrhythmias such as atrial fibrillation.

EFFECTS ON GROWTH AND DEVELOPMENT

The thyroid hormones have a critical effect on growth, partly by a direct action on cells, and also indirectly by influencing growth hormone production and potentiating its effects on its target tissues. The hormones are important for a normal response to parathormone (Ch. 36) and calcitonin as well as for skeletal development; they are also essential for normal growth and maturation of the central nervous system.

MECHANISM OF ACTION

While there is some evidence for non-genomic actions (see Bassett et al., 2003), thyroid hormones act mainly through a specific nuclear receptor, TR (Ch. 3). Two distinct genes, TRα and TRβ, code for several receptor isoforms that have distinct functions. T_4 may be regarded as a prohormone, because when it enters the cell, it is converted to T_3, which then binds with high affinity to TR. This interaction is likely to take place in the nucleus, where TR isoforms generally act as a constitutive repressor of target genes. When T_3 is bound, these receptors change conformation, the co-repressor complex is released and a co-activator complex is recruited, which then activates transcription, resulting in generation of mRNA and protein synthesis.

TRANSPORT AND METABOLISM OF THYROID HORMONES

Both thyroid hormones are transported in the blood mainly bound to thyroxine-binding globulin (TBG). Plasma concentrations of these hormones can be measured by radioimmunoassay, and are approximately 1×10^{-7} mol/l (T_4) and 2×10^{-9} mol/l (T_3). Both are eventually metabolised in their target tissues by deiodination, deamination, decarboxylation and conjugation with

glucuronic and sulfuric acids. The liver is a major site of metabolism and the free and conjugated forms are excreted partly in the bile and partly in the urine. The half-life of T_3 is a few hours, whereas that of T_4 varies between 3–4 days in hyperthyroidism, and 9–10 days in hypothyroidism.[4] Abnormalities in the metabolism of these hormones may occur naturally or be induced by drugs or heavy metals, and this may give rise to a variety of (uncommon) clinical conditions such as the 'low T_3 syndrome'.

ABNORMALITIES OF THYROID FUNCTION

Thyroid disorders are among the most common endocrine diseases, and subclinical thyroid disease is particularly prevalent in the middle-aged and elderly. They are accompanied by many extrathyroidal symptoms, particularly in the heart and skin. One (rare) cause of organ dysfunction is thyroid cancer. Many other thyroid disorders have an autoimmune basis. The reason for this is not clear, although it may be linked to polymorphisms in the PDS, tumour necrosis factor (TNF)-α or other genes. Regardless of causation, thyroid dysfunction is often associated with enlargement of the gland, known as *goitre*. Like other autoimmune diseases, such thyroid disorders are more common in women than men and occur with increased frequency during pregnancy (Cignini et al., 2012).

HYPERTHYROIDISM (THYROTOXICOSIS)

In thyrotoxicosis there is excessive secretion and activity of the thyroid hormones, resulting in a high metabolic rate, an increase in skin temperature and sweating, and heat intolerance. Nervousness, tremor, tachycardia and increased appetite associated with loss of weight occur. There are several types of hyperthyroidism, but only two are common: diffuse toxic goitre (also called *Graves' disease*[5] or exophthalmic goitre) and toxic nodular goitre.

Diffuse toxic goitre is an organ-specific autoimmune disease caused by autoantibodies to the TSH receptor which activate it, increasing thyroxine secretion. Constitutively active mutations of the TRH receptor may also be involved. As is indicated by the name, patients with exophthalmic goitre have protrusion of the eyeballs. The pathogenesis of this condition is not fully understood, but it is thought to be caused by the presence of TSH receptor-like proteins in orbital tissues. There is also an enhanced sensitivity to catecholamines. Toxic nodular goitre is caused by a benign neoplasm or adenoma, and may develop in patients with long-standing simple goitre. This condition does not usually have concomitant exophthalmos. The antidysrhythmic drug **amiodarone** (Ch. 21) is rich in iodine and can cause either hyperthyroidism or hypothyroidism. Some iodine-containing radiocontrast agents, such as **iopanoic acid** and its congeners, used as imaging agents to visualise the gall bladder, may also interfere with thyroid function. The chronic use of psychotropic agents may precipitate a variety of thyroid abnormalities (Bou Khalil & Richa, 2011).

SIMPLE, NON-TOXIC GOITRE

A dietary deficiency of iodine, if prolonged, causes a rise in plasma TRH and eventually an increase in the size of the gland. This condition is known as simple or non-toxic goitre. Another cause is ingestion of *goitrogens* (e.g. from cassava root). The enlarged thyroid usually manages to produce normal amounts of thyroid hormone, although if the iodine deficiency is very severe, hypothyroidism may supervene.

HYPOTHYROIDISM

A decreased activity of the thyroid results in hypothyroidism, and in severe cases *myxoedema*. Once again, this disease is immunological in origin, and the manifestations include low metabolic rate, slow speech, deep hoarse voice, lethargy, bradycardia, sensitivity to cold and mental impairment. Patients also develop a characteristic thickening of the skin (caused by the subcutaneous deposition of glycosaminoglycans), which gives myxoedema its name. *Hashimoto's thyroiditis*, a chronic autoimmune disease in which there is an immune reaction against thyroglobulin or some other component of thyroid tissue, can lead to both hypothyroidism and myxoedema. Genetic factors play an important role. Therapy of thyroid tumours with radioiodine is another cause of hypothyroidism.

Thyroid deficiency during development, which is the most prevalent endocrine disorder in the newborn (1 in 3000–4000 births) causes congenital hypothyroidism,[6] characterised by gross retardation of growth and mental deficiency.

DRUGS USED IN DISEASES OF THE THYROID

HYPERTHYROIDISM

Hyperthyroidism may be treated pharmacologically or surgically. In general, surgery is now used only when there are mechanical problems resulting from compression of the trachea by the thyroid. Under such circumstances it is usual to remove only part of the organ. Although the condition of hyperthyroidism can be controlled with antithyroid drugs, these drugs do not alter the underlying autoimmune mechanisms or improve the exophthalmos associated with Graves' disease.

RADIOIODINE

Radioiodine is a first-line treatment for hyperthyroidism (particularly in the USA). The isotope used is ^{131}I (usually as the sodium salt), and the dose generally 5–15 mCi. Given orally, it is taken up and processed by the thyroid in the same way as the stable form of iodide, eventually becoming incorporated into thyroglobulin. The isotope emits both β and γ radiation. The γ rays pass through the tissue without causing damage, but the β particles have a very short range; they are absorbed by the tissue and exert a powerful cytotoxic action that is restricted to the cells of the thyroid follicles, resulting in significant destruction of the tissue. ^{131}I has a half-life of 8 days, so by 2 months its radioactivity has effectively disappeared. It is given as one single dose, but its cytotoxic effect on the gland is delayed for 1–2 months and does not reach its maximum for a further 2 months.

[4]Correcting hypothyroidism by administration of T_4 therefore takes 2–3 weeks to reach equilibrium.

[5]After a Dublin physician who connected 'violent and long continued palpitations in females' with enlargement of the thyroid gland. Their complaints of fluttering hearts and lumps in their throats had previously been attributed to hysteria.

[6]An older term for this condition, *cretinism*, has been dropped.

The thyroid

- Thyroid hormones, tri-iodothyronine (T_3) and thyroxine (T_4), are synthesised by iodination of tyrosine residues on thyroglobulin within the lumen of the thyroid follicle.
- Hormone synthesis and secretion are regulated by thyroid-stimulating hormone (thyrotrophin) and influenced by plasma iodide.
- There is a large pool of T_4 in the body; it has a low turnover rate and is found mainly in the circulation.
- There is a small pool of T_3 in the body; it has a fast turnover rate and is found mainly intracellularly.
- Within target cells, the T_4 is converted to T_3, which interacts with a nuclear receptor to regulate gene transcription.
- T_3 and T_4 actions:
  - stimulation of metabolism, causing increased oxygen consumption and increased metabolic rate
  - regulation of growth and development.
- Abnormalities of thyroid function include:
  - hyperthyroidism (thyrotoxicosis); either diffuse toxic goitre or toxic nodular goitre
  - hypothyroidism; in adults this causes myxoedema, in infants, gross retardation of growth and mental deficiency
  - simple non-toxic goitre caused by dietary iodine deficiency, usually with normal thyroid function.

Hypothyroidism will eventually occur after treatment with radioiodine, particularly in patients with Graves' disease, but is easily managed by replacement therapy with T_4. Radioiodine is best avoided in children and also in pregnant patients because of potential damage to the fetus. There is theoretically an increased risk of thyroid cancer but this has not been seen following therapeutic treatment.

The uptake of ^{131}I and other isotopes of iodine is also used diagnostically as a test of thyroid function. A tracer dose of the isotope is given orally or intravenously and the amount accumulated by the thyroid is measured by a γ-scintillation counter placed over the gland. ^{131}I is also used for the treatment of thyroid cancer.

THIOUREYLENES

This group of drugs comprises **carbimazole**, **methimazole** and **propylthiouracil**. Chemically, they are related to thiourea, and the thiocarbamide (S–C–N) group is essential for antithyroid activity.

Mechanism of action

Thioureylenes decrease the output of thyroid hormones from the gland, and cause a gradual reduction in the signs and symptoms of thyrotoxicosis, the basal metabolic rate and pulse rate returning to normal over a period of 3–4 weeks. Their mode of action is not completely understood, but there is evidence that they inhibit the iodination of tyrosyl residues in thyroglobulin (see Figs 34.1 and 34.2). It is thought that they inhibit the thyroperoxidase-catalysed oxidation reactions by acting as substrates for the postulated peroxidase–iodinium complex, thus competitively inhibiting the interaction with tyrosine. Propylthiouracil has the additional effect of reducing the deiodination of T_4 to T_3 in peripheral tissues.

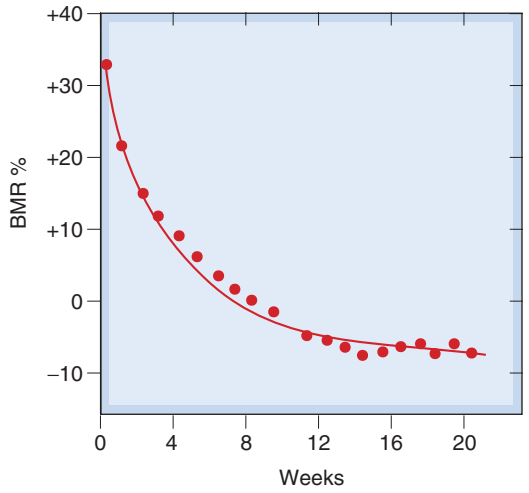

Fig. 34.5 Time course of fall of basal metabolic rate (BMR) during treatment with an antithyroid drug, carbimazole. The curve is exponential, corresponding to a daily decrease in BMR of 3.4%. *(Modified from Furth et al., 1963.)*

Pharmacokinetic aspects

Thioureylenes are given orally. Carbimazole is rapidly converted to its active metabolite methimazole, which is distributed throughout the body water and has a plasma half-life of 6–15 h. An average dose of carbimazole produces more than 90% inhibition of thyroid incorporation of iodine within 12 h. The full clinical response to this and other antithyroid drugs, however, may take several weeks (Fig. 34.5), partly because T_4 has a long half-life, and also because the thyroid may have large stores of hormone, which need to be depleted before the drug's action can be fully manifest. Propylthiouracil is thought to act somewhat more rapidly because of its additional effect as an inhibitor of the peripheral conversion of T_4 to T_3.

Both methimazole and propylthiouracil cross the placenta and also appear in the milk, but this effect is less pronounced with propylthiouracil, because it is more strongly bound to plasma protein. After degradation, the metabolites are excreted in the urine, propylthiouracil being excreted more rapidly than methimazole. The thioureylenes may be concentrated in the thyroid.

Unwanted effects

The most dangerous unwanted effect of thioureylene drugs is neutropenia and agranulocytosis (see Ch. 24). This is relatively rare, having an incidence of 0.1–1.2%, and is reversible on cessation of treatment. Patients must be warned to report symptoms (especially sore throat) immediately and have a blood count. Rashes (2–25%) and other symptoms including headaches, nausea, jaundice and pain in the joints, can also occur.

IODINE/IODIDE

Iodine is converted *in vivo* to iodide (I^-), which temporarily inhibits the release of thyroid hormones. When high doses of iodine are given to thyrotoxic patients, the symptoms subside within 1–2 days. There is inhibition of the secretion of thyroid hormones and, over a period of 10–14

days, a marked reduction in vascularity of the gland, which becomes smaller and firmer. Iodine is often given orally in a solution with potassium iodide (*'Lugol's iodine'*). With continuous administration, its effect reaches maximum within 10–15 days and then decreases. The mechanism of action is not entirely clear; it may inhibit iodination of thyroglobulin, possibly by reducing the H_2O_2 generation that is necessary for this process.

The main uses of iodine/iodide are for the preparation of hyperthyroid subjects for surgical resection of the gland, and as part of the treatment of severe thyrotoxic crisis (*thyroid storm*). It is also used following exposure to accidental leakage of radioactive iodine from nuclear reactors, to reduce uptake of the radioactive isotope in the thyroid. Allergic reactions can occur; these include angioedema, rashes and drug fever. Lacrimation, conjunctivitis, pain in the salivary glands and a cold-like syndrome are dose-related adverse effects connected to the concentration of iodide by transport mechanisms in tears and saliva.

OTHER DRUGS USED

The β-adrenoceptor antagonists, for example **propranolol** and **nadolol** (Ch. 14), are not antithyroid agents as such, but they are useful for decreasing many of the signs and symptoms of hyperthyroidism – the tachycardia, dysrhythmias, tremor and agitation. They are used during the preparation of thyrotoxic patients for surgery, as well as in most hyperthyroid patients during the initial treatment period while the thioureylenes or radioiodine take effect, or as part of the treatment of acute hyperthyroid crisis. Eye drops containing **guanethidine**, a noradrenergic-blocking agent (Ch. 14), are used to mitigate the exophthalmos of hyperthyroidism (which is not relieved by antithyroid drugs); it acts by relaxing the sympathetically innervated smooth muscle that causes eyelid retraction. Glucocorticoids (e.g. **prednisolone** or **hydrocortisone**) or surgical decompression may be needed to mitigate severe exophthalmia in Graves' disease. Some other drugs (e.g. cholecystographic agents or antiepileptic drugs) as well as environmental 'endocrine disruptors'[7] may interfere with the normal production of thyroid hormones.

HYPOTHYROIDISM

There are no drugs that specifically augment the synthesis or release of thyroid hormones. The only effective treatment for hypothyroidism, unless it is caused by iodine deficiency (which is treated with iodide), is to administer the thyroid hormones themselves as replacement therapy. Synthetic T_4 (official name: **levothyroxine**) and T_3 (official name: **liothyronine**), identical to the natural hormones, are given orally. Levothyroxine, as the sodium salt in doses of 50–100 µg/day, is the usual first-line drug of choice. Liothyronine has a faster onset but a shorter duration of action, and is generally reserved for acute emergencies such as the rare condition of myxoedema coma, where these properties are an advantage.

Unwanted effects may occur with overdose, and in addition to the signs and symptoms of hyperthyroidism there is a risk of precipitating angina pectoris, cardiac dysrhythmias or even cardiac failure. The effects of less severe overdose are more insidious; the patient feels well but bone resorption is increased, leading to osteoporosis (Ch. 36).

The use of drugs to treat thyroid cancer (see Kojic et al., 2012) is a specialist subject and will not be covered here.

The use of drugs acting on the thyroid is summarised in the clinical box.

Drugs in thyroid disease

Drugs for hyperthyroidism

- *Radioiodine* (^{131}I), given orally, is selectively taken up by thyroid and damages cells; it emits short-range β radiation, which affects only thyroid follicle cells. Hypothyroidism will eventually occur.
- Thioureylenes (e.g. **carbimazole**, **propylthiouracil**) decrease the synthesis of thyroid hormones; the mechanism is through inhibition of thyroperoxidase, thus reducing iodination of thyroglobulin. They are given orally.
- *Iodine*, given orally in high doses, transiently reduces thyroid hormone secretion and decreases vascularity of the gland.

Drugs for hypothyroidism

- **Levothyroxine** has all the actions of endogenous thyroxine; it is given orally.
- **Liothyronine** has all the actions of endogenous tri-iodothyronine; it is given intravenously.

Clinical use of drugs acting on the thyroid

Radioiodine

- Hyperthyroidism (Graves' disease, multinodular toxic goitre).
- Relapse of hyperthyroidism after failed medical or surgical treatment.

Carbimazole or propylthiouracil

- Hyperthyroidism (diffuse toxic goitre); at least 1 year of treatment is needed.
- Preliminary to surgery for toxic goitre.
- Part of the treatment of thyroid storm (very severe hyperthyroidism); **propylthiouracil** is preferred. The β-adrenoceptor antagonists (e.g. **propranolol**) are also used.

Thyroid hormones and iodine

- **Levothyroxine** (T_4) is the standard replacement therapy for hypothyroidism.
- **Liothyronine** (T_3) is the treatment of choice for myxoedema coma.
- Iodine dissolved in aqueous potassium iodide (**'Lugol's iodine'**) is used short term to control thyrotoxicosis preoperatively. It reduces the vascularity of the gland.

[7]These are man-made chemicals such as pesticides or herbicides (e.g. polychlorinated biphenyls) that linger in the environment and are ingested in foodstuffs. The endocrine system is particularly sensitive to these, especially during development.

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35 The reproductive system

OVERVIEW

In this chapter, we describe the endocrine control of the female and male reproductive systems as the basis for understanding drug actions in sex hormone replacement, contraception, treatment of infertility, management of labour and treatment of erectile dysfunction.

INTRODUCTION

Drugs that affect reproduction (both by preventing conception and more recently for treating infertility) transformed society in the latter half of the last century. In this chapter, we briefly summarise salient points in reproductive endocrinology as a basis for understanding the numerous important drugs that work on the male and female reproductive systems. Such drugs are used for contraception, to treat infertility, as sex hormone replacement and in obstetric practice to influence labour. The principle of negative feedback is stressed and is central to understanding how hormones interact to control reproduction[1] – many drugs, including agents used to prevent or assist conception, work by influencing negative feedback mechanisms. The chapter concludes with a short section on erectile dysfunction.

ENDOCRINE CONTROL OF REPRODUCTION

Hormonal control of the reproductive systems in men and women involves sex steroids from the gonads, hypothalamic peptides and glycoprotein gonadotrophins from the anterior pituitary gland.

NEUROHORMONAL CONTROL OF THE FEMALE REPRODUCTIVE SYSTEM

Increased secretion of hypothalamic and anterior pituitary hormones occurs in girls at puberty and stimulates secretion of oestrogen from the ovaries. This causes maturation of the reproductive organs and development of secondary sexual characteristics, and also accelerated growth followed by closure of the epiphyses of the long bones. Sex steroids, *oestrogens* and *progesterone*, are thereafter involved in the menstrual cycle, and in pregnancy. A simplified outline is given in Figures 35.1 and 35.2.

The menstrual cycle begins with menstruation, which lasts for 3–6 days, during which the superficial layer of uterine endometrium is shed. The endometrium regenerates during the follicular phase of the cycle after menstrual flow has stopped. A releasing factor, *gonadotrophin-releasing hormone* (GnRH), is secreted from peptidergic neurons in the hypothalamus which discharge in a pulsatile fashion, approximately one burst per hour. GnRH stimulates the anterior pituitary to release gonadotrophic hormones (Fig. 35.1) – *follicle-stimulating hormone* (FSH) and *luteinising hormone* (LH). These act on the ovaries to promote development of small groups of follicles, each of which contains an ovum. One follicle develops faster than the others and forms the Graafian follicle (Figs 35.1 and 35.2E), which secretes oestrogens, and the rest degenerate. The ripening Graafian follicle consists of thecal and granulosa cells surrounding a fluid-filled centre, within which lies an ovum. Oestrogens are responsible for the proliferative phase of endometrial regeneration, which occurs from day 5 or 6 until mid-cycle (Fig. 35.2B,F). During this phase, the endometrium increases in thickness and vascularity, and at the peak of oestrogen secretion there is a prolific cervical secretion of mucus of pH 8–9, rich in protein and carbohydrate, which facilitates entry of spermatozoa. Oestrogen has a negative feedback effect on the anterior pituitary, decreasing gonadotrophin release during chronic administration of oestrogen as oral contraception (see p. 433-434). In contrast, the spike of endogenous oestrogen secretion just before mid-cycle sensitises LH-releasing cells of the pituitary to the action of the GnRH and causes the mid-cycle surge of LH secretion (Fig. 35.2C). This, in turn, causes rapid swelling and rupture of the Graafian follicle, resulting in ovulation. If fertilisation occurs, the fertilised ovum passes down the fallopian tubes to the uterus, starting to divide as it goes.

Stimulated by LH, cells of the ruptured follicle proliferate and develop into the *corpus luteum*, which secretes progesterone. Progesterone acts, in turn, on oestrogen-primed endometrium, stimulating the secretory phase of the cycle, which renders the endometrium suitable for the implantation of a fertilised ovum. During this phase, cervical mucus becomes more viscous, less alkaline, less copious and in general less welcoming for sperm. Progesterone exerts negative feedback on the hypothalamus and pituitary, decreasing the release of LH. It also has a thermogenic effect, causing a rise in body temperature of about 0.5 °C at ovulation, which is maintained until the end of the cycle.

If implantation of a fertilised ovum does not occur, progesterone secretion stops, triggering menstruation. If implantation does occur the corpus luteum continues to secrete progesterone which, by its effect on the hypothalamus and anterior pituitary, prevents further ovulation. The chorion (an antecedent of the placenta) secretes human chorionic gonadotrophin (HCG), which maintains the lining of the uterus during pregnancy. For reasons that are not physiologically obvious HCG has an additional pharmacological action, exploited therapeutically in treating infertility (see p. 433), of stimulating ovulation. As

[1]Recognition that negative feedback is central to endocrine control was a profound insight, made in 1930 by Dorothy Price, a laboratory assistant in the University of Chicago experimenting on effects of testosterone in rats. She referred to it as 'reciprocal influence' and it helps in understanding how many reproductive hormones seem, confusingly, to cause both an effect and its opposite if given in different doses or over different time courses.

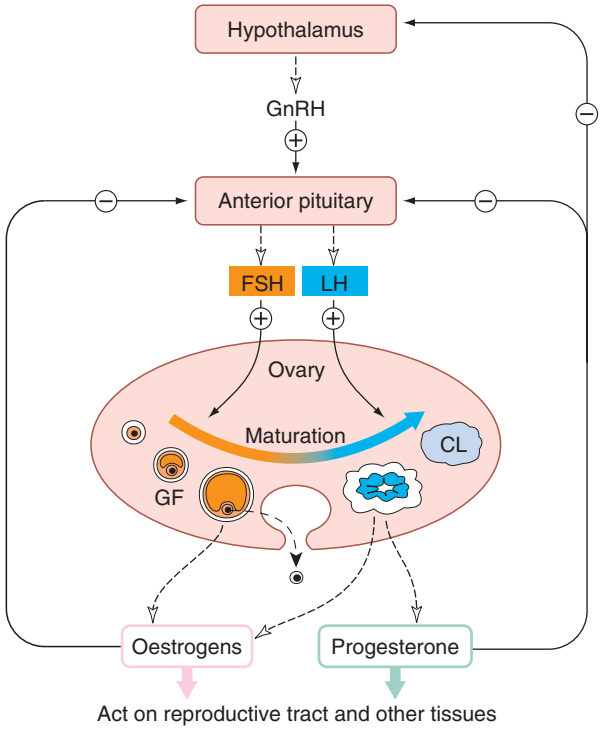

Fig. 35.1 Hormonal control of the female reproductive system. The Graafian follicle (GF) is shown developing on the left, then involuting to form the corpus luteum (CL) on the right, after the ovum (•) has been released. FSH, follicle-stimulating hormone; GnRH, gonadotrophin-releasing hormone; LH, luteinising hormone.

pregnancy proceeds, the placenta develops further hormonal functions and secretes a variety of hormones, including gonadotrophins, progesterone and oestrogens. Progesterone secreted during pregnancy controls the development of the secretory alveoli in the mammary gland, while oestrogen stimulates the lactiferous ducts. After parturition oestrogen, along with prolactin (see Ch. 33), is responsible for stimulating and maintaining lactation, whereas supraphysiological doses of oestrogen suppress lactation.

Oestrogens, progestogens (progesterone-like drugs), androgens and the gonadotrophins are described below – see Figure 35.3 for biosynthetic pathways.

NEUROHORMONAL CONTROL OF THE MALE REPRODUCTIVE SYSTEM

As in women, hypothalamic, anterior pituitary and gonadal hormones control the male reproductive system. A simplified outline is given in Figure 35.4. GnRH controls the secretion of gonadotrophins by the anterior pituitary. This secretion is not cyclical as in menstruating women, although it is pulsatile in both sexes, as with other anterior pituitary hormones (see Ch. 33). FSH is responsible for the integrity of the seminiferous tubules, and after puberty is important in gametogenesis through an action on Sertoli cells, which nourish and support developing spermatozoa. LH, which in the male is also called *interstitial cell-stimulating hormone* (ICSH), stimulates the interstitial cells (Leydig cells) to secrete androgens – in particular *testosterone*. LH/ICSH secretion begins at puberty, and the consequent secretion of testosterone causes maturation of

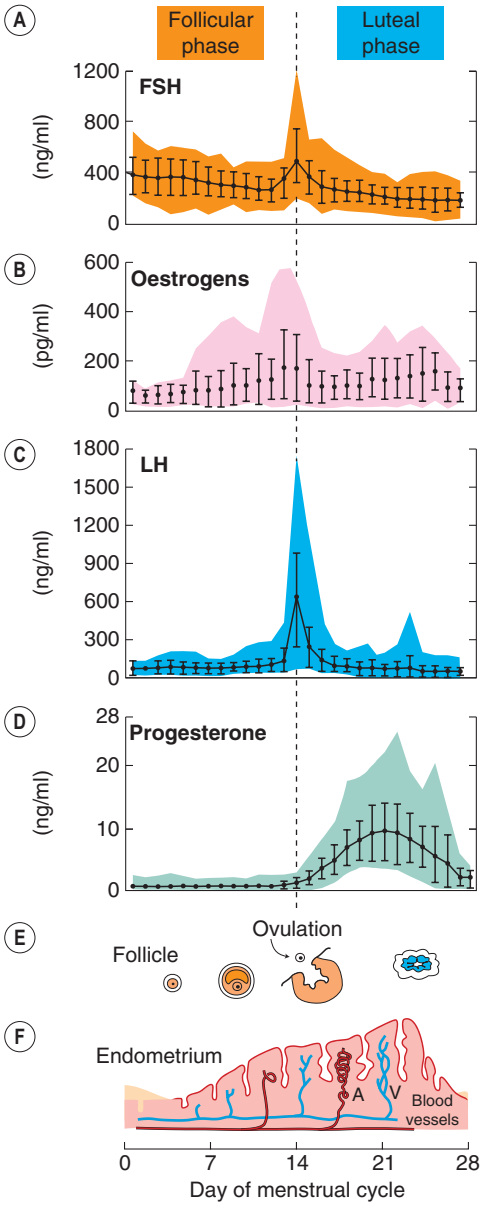

Fig. 35.2 Plasma concentrations of ovarian hormones and gonadotrophins in women during normal menstrual cycles. Values are the mean ± standard deviation of 40 women. The shaded areas indicate the entire range of observations. Day 1 is the onset of menstruation. E and F show diagrammatically the changes in the ovarian follicle and the endometrium during the cycle. Ovulation on day 14 of the menstrual cycle occurs with the mid-cycle peak of luteinising hormone (LH), represented by the vertical dashed line. A, arterioles; FSH, follicle-stimulating hormone; V, venules. *(After van de Wiele R L, Dyrenfurth I 1974 Pharmacol Rev 25, 189–217.)*

the reproductive organs and development of secondary sexual characteristics. Thereafter, the primary function of testosterone is the maintenance of spermatogenesis and hence fertility – an action mediated by Sertoli cells. Testosterone is also important in the maturation of spermatozoa as they pass through the epididymis and vas deferens. A further action is a feedback effect on the anterior pituitary, modulating its sensitivity to GnRH and thus influencing secretion of LH/ICSH. Testosterone has marked anabolic effects, causing development of the musculature

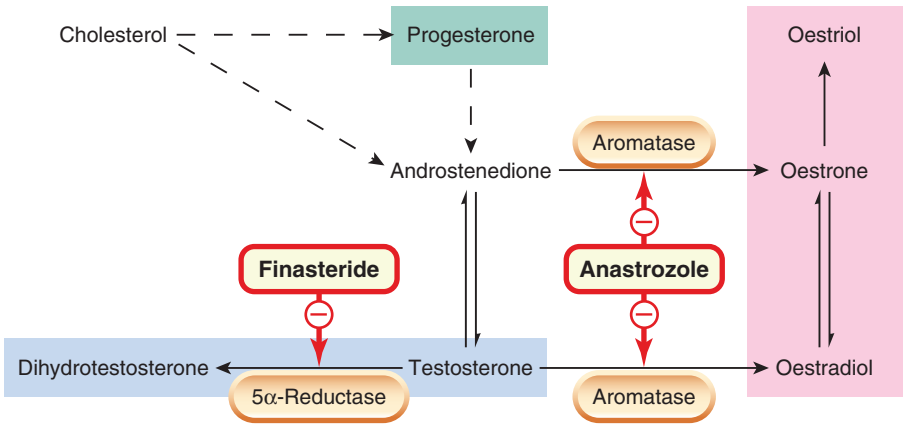

Fig. 35.3 The biosynthetic pathway for the androgens and oestrogens, with sites of drug action. (See also Fig. 33.5.) Finasteride is used in benign prostatic hyperplasia, and anastrozole to treat breast cancer in postmenopausal women.

Hormonal control of the female reproductive system

- The menstrual cycle starts with menstruation.
- Gonadotrophin-releasing hormone, released from the hypothalamus, acts on the anterior pituitary to release follicle-stimulating hormone (FSH) and luteinising hormone (LH).
- FSH and LH stimulate follicle development in the ovary. FSH is the main hormone stimulating oestrogen release. LH stimulates ovulation at mid-cycle and is the main hormone controlling subsequent progesterone secretion from the corpus luteum.
- Oestrogen controls the proliferative phase of the endometrium and has negative feedback effects on the anterior pituitary. Progesterone controls the later secretory phase, and has negative feedback effects on both the hypothalamus and anterior pituitary.
- If a fertilised ovum is implanted, the corpus luteum continues to secrete progesterone.
- After implantation, human chorionic gonadotrophin (HCG) from the chorion becomes important, and later in pregnancy progesterone, HCG and other hormones are secreted by the placenta.

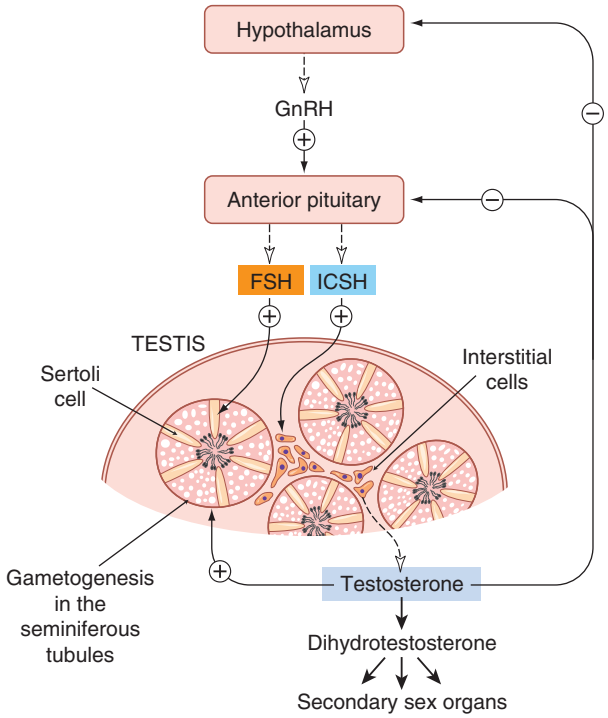

Fig. 35.4 Hormonal control of the male reproductive system. FSH, follicle-stimulating hormone; GnRH, gonadotrophin-releasing hormone; ICSH, interstitial cell-stimulating hormone.

and increased bone growth which results in the pubertal growth spurt, followed by closure of the epiphyses of the long bones.

Secretion of testosterone is mainly controlled by LH/ICSH, but FSH also plays a part, possibly by releasing a factor similar to GnRH from the Sertoli cells which are its primary target. The interstitial cells that synthesise testosterone also have receptors for prolactin, which may influence testosterone production by increasing the number of receptors for LH/ICSH.

BEHAVIOURAL EFFECTS OF SEX HORMONES

As well as controlling the menstrual cycle, sex steroids affect sexual behaviour. Two types of control are recognised: *organisational* and *activational*.

Organisational control refers to the fact that sexual differentiation of the brain can be permanently altered by the presence or absence of sex steroids at key stages in development. In rats, administration of androgens to females within a few days of birth results in long-term virilisation of behaviour. Conversely, neonatal castration of male rats causes them to develop behaviourally as females. Brain development in the absence of sex steroids follows female lines, but is switched to the male pattern by exposure of the hypothalamus to androgen at a key stage of development. Similar but less complete behavioural virilisation of female offspring has been demonstrated following androgen administration in non-human primates, and probably also occurs in humans if pregnant women are exposed to excessive androgen.

The *activational* effect of sex steroids refers to their ability to modify sexual behaviour after brain development is complete. In general, oestrogens and androgens

increase sexual activity in the appropriate sex. **Oxytocin**, which is important during parturition (see p. 435), also has roles in mating and parenting behaviours, its action in the central nervous system being regulated by oestrogen (see Ch. 33).

DRUGS AFFECTING REPRODUCTIVE FUNCTION

OESTROGENS

Oestrogens are synthesised by the ovary and placenta, and in small amounts by the testis and adrenal cortex. The starting substance for synthesis of oestrogen (and other steroids) is cholesterol. The immediate precursors to the oestrogens are androgenic substances – androstenedione or testosterone (Fig. 35.3). There are three main endogenous oestrogens in humans: *oestradiol*, *oestrone* and *oestriol* (Fig. 35.3). Oestradiol is the most potent and is the principal oestrogen secreted by the ovary. At the beginning of the menstrual cycle, the plasma concentration is 0.2 nmol/l, rising to ~2.2 nmol/l in mid-cycle.

Actions

Oestrogen acts in concert with progesterone, and induces synthesis of progesterone receptors in uterus, vagina, anterior pituitary and hypothalamus. Conversely, progesterone decreases oestrogen receptor expression in the reproductive tract. *Prolactin* (see Ch. 33) also influences oestrogen action by increasing the numbers of oestrogen receptors in the mammary gland, but has no effect on oestrogen receptor expression in the uterus.

Effects of exogenous oestrogen depend on the state of sexual maturity when the oestrogen is administered:

- In primary hypogonadism: oestrogen stimulates development of secondary sexual characteristics and accelerates growth.
- In adults with primary amenorrhoea: oestrogen, given cyclically with a progestogen, induces an artificial cycle.
- In sexually mature women: oestrogen (with a progestogen) is contraceptive.
- At or after the menopause: oestrogen replacement prevents menopausal symptoms and bone loss.

Oestrogens have several metabolic actions, including mineralocorticoid (retention of salt and water) and mild anabolic actions. They increase plasma concentrations of high-density lipoproteins, a potentially beneficial effect (Ch. 23) that may contribute to the relatively low risk of atheromatous disease in premenopausal women compared with men of the same age. However, oestrogens also increase the coagulability of blood, and increase the risk of thromboembolism.

Mechanism of action

Oestrogen binds to nuclear receptors, as do other steroid hormones (Ch. 3). There are at least two types of oestrogen receptor, termed ERα and ERβ. Binding is followed by interaction of the resultant complexes with nuclear sites and subsequent genomic effects. In addition to these 'classic' intracellular receptors, some oestrogen effects, in particular its rapid vascular actions, are initiated by interaction with membrane receptors, including a G protein-coupled [o]estrogen receptor ('GPER', see review by Nilsson et al., 2011). Acute vasodilatation caused by 17-β-oestradiol is mediated by nitric oxide, and a plant-derived (phyto-) oestrogen called **genistein** (which is selective for ERβ, as well as having quite distinct effects due to inhibition of protein kinase C) is as potent as 17-β-oestradiol in this regard (Walker et al., 2001). Oestrogen receptor modulators (receptor-selective oestrogen agonists or antagonists) are mentioned below.

Preparations

Many preparations (oral, transdermal, intramuscular, implantable and topical) of oestrogens are available for a wide range of indications. These include natural (e.g. **oestradiol**, **oestriol**) and synthetic (e.g. **mestranol**, **ethinylestradiol**, **diethylstilbestrol**) oestrogens. Oestrogens are presented either as single agents or combined with progestogen.

Pharmacokinetic aspects

Natural and synthetic oestrogens are well absorbed in the gastrointestinal tract, but after absorption the natural oestrogens are rapidly metabolised in the liver, whereas synthetic oestrogens are degraded less rapidly. There is variable enterohepatic cycling. Most oestrogens are readily absorbed from skin and mucous membranes. They may be given as intravaginal creams or pessaries for local effect. In the plasma, natural oestrogens are bound to albumin and to a sex steroid-binding globulin. Natural oestrogens are excreted in the urine as glucuronides and sulfates.

Unwanted effects

Unwanted effects of oestrogens range from the common and tiresome to the life-threatening but rare: breast tenderness, nausea, vomiting, anorexia, retention of salt and water with resultant oedema, and increased risk of thromboembolism. More details of the unwanted effects of oral contraceptives are given below.

Used intermittently for postmenopausal replacement therapy, oestrogens cause menstruation-like bleeding. Oestrogen causes endometrial hyperplasia unless given cyclically with a progestogen. When administered to males, oestrogens result in feminisation.

Oestrogen administration to pregnant women can cause genital abnormalities in their offspring: carcinoma of the vagina was more common in young women whose mothers were given diethylstilbestrol in early pregnancy in a misguided attempt to prevent miscarriage (see Ch. 57).

The clinical uses of oestrogens and antioestrogens are summarised in the box (p. 429). In addition, see the section below on postmenopausal hormone replacement therapy (HRT).

OESTROGEN RECEPTOR MODULATORS

Raloxifene, a 'selective [o]estrogen receptor modulator' (SERM), has antioestrogenic effects on breast and uterus but oestrogenic effects on bone, lipid metabolism and blood coagulation. It is used for prevention and treatment of postmenopausal osteoporosis (Ch. 36) and reduces the incidence of oestrogen receptor-positive breast cancer similarly to **tamoxifen** but with fewer adverse events (Barret-Connor et al., 2006; Vogel et al., 2006). The US Food and Drug Administration has supported its use to reduce the risk of invasive breast cancer in postmenopausal women with osteoporosis and in postmenopausal women at high risk for invasive breast

cancer. Unlike oestrogen, it does not prevent menopausal flushes.

Tamoxifen has an antioestrogenic action on mammary tissue but oestrogenic actions on plasma lipids, endometrium and bone. It produces mild oestrogen-like adverse effects consistent with partial agonist activity. The tamoxifen–oestrogen receptor complex does not readily dissociate, so there is interference with the recycling of receptors.

Tamoxifen upregulates transforming growth factor-β, a cytokine that retards the progression of malignancy, and that also has a role in controlling the balance between bone-producing osteoblasts and bone-resorbing osteoclasts (Ch. 36).

The use of tamoxifen to treat and prevent breast cancer is discussed further in Chapter 56.

ANTIOESTROGENS

Antioestrogens compete with natural oestrogens for receptors in target organs; in addition to SERMs (raloxifene, tamoxifen), which are partial agonists in some tissues and antagonists in others, there are drugs that are pure oestrogen-receptor antagonists.

Clomiphene inhibits oestrogen binding in the anterior pituitary, so preventing negative feedback and acutely increasing secretion of GnRH and gonadotrophins. This stimulates and enlarges the ovaries, increases oestrogen secretion and induces ovulation. It is used in treating infertility caused by lack of ovulation. Twins are common, but multiple pregnancy is unusual.

See the clinical box on oestrogens and antioestrogens for a summary of clinical uses.

PROGESTOGENS

The natural progestational hormone (progestogen) is *progesterone* (see Figs 35.2 and 35.3). This is secreted by the corpus luteum in the second part of the menstrual cycle, and by the placenta during pregnancy. Small amounts are also secreted by the testis and adrenal cortex.

Progestogens act, as do other steroid hormones, on nuclear receptors. The density of progesterone receptors is controlled by oestrogens (see p. 428).

Preparations

There are two main groups of progestogens:

1. The naturally occurring hormone and its derivatives (e.g. **hydroxyprogesterone**, **medroxyprogesterone**, **dydrogesterone**). Progesterone itself is virtually inactive orally, because of presystemic hepatic metabolism. Other derivatives are available for oral administration, intramuscular injection or administration via the vagina or rectum.
2. Testosterone derivatives (e.g. **norethisterone**, **norgestrel** and **ethynodiol**) can be given orally. The first two have some androgenic activity and are metabolised to give oestrogenic products. Newer progestogens used in contraception include **desogestrel** and **gestodene**; they may have fewer adverse effects on lipids than ethynodiol and may be considered for women who experience side effects such as acne, depression or breakthrough bleeding with the older drugs. However, these newer drugs have been associated with higher risks of venous thromboembolic disease (see below).

Oestrogens and antioestrogens

- The endogenous oestrogens are oestradiol (the most potent), oestrone and oestriol; there are numerous exogenous synthetic forms (e.g. **ethinylestradiol**).
- Mechanism of action involves interaction with nuclear receptors (ERα or ERβ) in target tissues, resulting in modification of gene transcription. Some of the rapid vascular effects of oestrogens are mediated by a G protein-coupled [o]estrogen receptor (GPER).
- Their pharmacological effects depend on the sexual maturity of the recipient:
  - before puberty, they stimulate development of secondary sexual characteristics
  - given cyclically in the female adult, they induce an artificial menstrual cycle and are used for contraception
  - given at or after the menopause, they prevent menopausal symptoms and protect against osteoporosis, but increase thromboembolism.
- Antioestrogens are competitive antagonists or partial agonists. **Tamoxifen** is used in oestrogen-dependent breast cancer. **Clomiphene** induces ovulation by inhibiting the negative feedback effects on the hypothalamus and anterior pituitary.
- Selective drugs that are oestrogen agonists in some tissues but antagonists in others are being developed. **Raloxifene** (one such drug) is used to treat and prevent osteoporosis.

Clinical uses of oestrogens and antioestrogens

Oestrogens

- Replacement therapy:
  - primary ovarian failure (e.g. Turner's syndrome)
  - secondary ovarian failure (menopause) for flushing, vaginal dryness and to preserve bone mass.
- Contraception.
- Prostate and breast cancer (these uses have largely been superseded by other hormonal manipulations; see Ch. 56).

Antioestrogens

- To treat oestrogen-sensitive breast cancer (**tamoxifen**).
- To induce ovulation (**clomiphene**) in treating infertility.

Actions

The pharmacological actions of the progestogens are in essence the same as the physiological actions of progesterone described above. Specific effects relevant to contraception are detailed below.

Pharmacokinetic aspects

Injected progesterone is bound to albumin, not to the sex steroid-binding globulin. Some is stored in adipose tissue. It is metabolised in the liver, and the products, pregnanolone and pregnanediol, are conjugated with glucuronic acid and excreted in the urine.

Unwanted effects

Unwanted effects of progestogens include weak androgenic actions. Other unwanted effects include acne, fluid retention, weight change, depression, change in libido, breast discomfort, premenstrual symptoms, irregular menstrual cycles and breakthrough bleeding. There is an increased incidence of thromboembolism.

Clinical uses of progestogens are summarised in the box below.

ANTIPROGESTOGENS

Mifepristone is a partial agonist at progesterone receptors. It sensitises the uterus to the action of prostaglandins. It is given orally and has a plasma half-life of 21 h. Mifepristone is used, in combination with a prostaglandin (e.g. **gemeprost**; see p. 436), as a medical alternative to surgical termination of pregnancy (see clinical box, opposite).

POSTMENOPAUSAL HORMONE REPLACEMENT THERAPY

At the menopause, whether natural or surgically induced, ovarian function decreases and oestrogen levels fall. There is a long history of disagreement regarding the pros and cons of hormone replacement therapy (HRT) in this context, with the prevailing wisdom undergoing several revisions over the years (see Davis et al., 2005). HRT normally involves the cyclic or continuous administration of low doses of one or more oestrogens, with or without a progestogen. Short-term HRT has some clear-cut benefits:

- improvement of symptoms caused by reduced oestrogen, for example hot flushes and vaginal dryness
- prevention and treatment of osteoporosis, but other drugs are usually preferable for this (Ch. 36).

Oestrogen replacement does not reduce the risk of coronary heart disease, despite earlier hopes, nor is there evidence that it reduces age-related decline in cognitive function. Drawbacks include:

- cyclical withdrawal bleeding
- adverse effects related to progestogen (see below)
- increased risk of endometrial cancer if oestrogen is given unopposed by progestogen
- increased risk of breast cancer, related to the duration of HRT use and disappearing within 5 years of stopping
- increased risk of venous thromboembolism (risk approximately doubled in women using combined HRT for 5 years).

See Web links in the reference list for best estimates of risks of cancer (breast, endometrium, ovary), venous thromboembolism, stroke and coronary artery disease in relation to age and duration of HRT use.

Oestrogens used in HRT can be given orally (conjugated oestrogens, oestradiol, oestriol), vaginally (oestriol), by transdermal patch (oestradiol) or by subcutaneous implant (oestradiol). **Tibolone** is marketed for the short-term treatment of symptoms of oestrogen deficiency. It has oestrogenic, progestogenic and weak androgenic activity, and can be used continuously without cyclical progesterone (avoiding the inconvenience of withdrawal bleeding).

Progestogens and antiprogestogens

- The endogenous hormone is progesterone. Examples of synthetic drugs are the progesterone derivative **medroxyprogesterone** and the testosterone derivative **norethisterone**.
- Mechanism of action involves intracellular receptor/altered gene expression, as for other steroid hormones. Oestrogen stimulates synthesis of progesterone receptors, whereas progesterone inhibits synthesis of oestrogen receptors.
- Main therapeutic uses are in oral contraception and oestrogen replacement regimens, and to treat endometriosis.
- The antiprogestogen **mifepristone**, in combination with prostaglandin analogues, is an effective medical alternative to surgical termination of early pregnancy.

Clinical uses of progestogens and antiprogestogens

Progestogens

- Contraception:
  - with **oestrogen** in *combined oral contraceptive pill*
  - as *progesterone-only contraceptive pill*
  - as *injectable* or *implantable* progesterone-only contraception
  - as part of an *intrauterine* contraceptive system.
- Combined with **oestrogen** for *oestrogen replacement therapy* in women with an intact uterus, to prevent endometrial hyperplasia and carcinoma.
- For *endometriosis*.
- In *endometrial carcinoma*; use in breast and renal cancer has declined.
- Poorly validated uses have included various menstrual disorders.

Antiprogestogens

- Medical termination of pregnancy: **mifepristone** (partial agonist) combined with a prostaglandin (e.g. **gemeprost**).

ANDROGENS

Testosterone is the main natural androgen. It is synthesised mainly by the interstitial cells of the testis, and in smaller amounts by the ovaries and adrenal cortex. Adrenal androgen production is influenced by adrenocorticotrophic hormone (ACTH, corticotrophin). As for other steroid hormones, cholesterol is the starting substance. Dehydroepiandrosterone and androstenedione are important intermediates. They are released from the gonads and the adrenal cortex, and converted to testosterone in the liver (see Fig. 35.3).

Actions

In general, the effects of exogenous androgens are the same as those of testosterone, and depend on the age and

sex of the recipient. If given to prepubertal boys, the individuals concerned do not reach their full predicted height because of premature closure of the epiphyses of the long bones. In boys at the age of puberty, there is rapid development of secondary sexual characteristics (i.e. growth of facial, axillary and pubic hair, deepening of the voice), maturation of the reproductive organs and a marked increase in muscular strength. There is a growth spurt with an acceleration in the usual increase in height that occurs year on year in younger children, followed by cessation of linear growth. In adults, the anabolic effects can be accompanied by retention of salt and water. The skin thickens and may darken, and sebaceous glands become more active which can result in acne. Body weight and muscle mass increase, partly due to water retention. Androgens cause a feeling of well-being and an increase in physical vigour, and may increase libido. Whether they are responsible for sexual behaviour as such is controversial, as is their contribution to aggressive behaviour. Paradoxically, testosterone administration inhibits spermatogenesis, so reducing male fertility.

Administration of 'male' doses to women results in masculinisation, but lower doses (e.g. patches that release 300 mg of testosterone/day) restore plasma testosterone to normal female concentrations and improve sexual dysfunction in women following ovariectomy, without adverse effects (Braunstein et al., 2005).

Mechanism of action

In most target cells, testosterone works through an active metabolite, dihydrotestosterone, to which it is converted locally by a 5α-reductase enzyme. In contrast, testosterone itself causes virilisation of the genital tract in the male embryo and regulates LH/ICSH production in anterior pituitary cells. Testosterone and dihydrotestosterone modify gene transcription by interacting with nuclear receptors.

Preparations

Testosterone itself can be given by subcutaneous implantation or by transdermal patches (male replacement dose approximately 2.5 mg/day). Various esters (e.g. enanthate and proprionate) are given by intramuscular depot injection. Testosterone undecanoate and mesterolone can be given orally.

Pharmacokinetic aspects

If given orally, testosterone is rapidly metabolised in the liver. Virtually all testosterone in the circulation is bound to plasma protein – mainly to the sex steroid-binding globulin. Approximately 90% of endogenous testosterone is eliminated as metabolites. The elimination half-life of the free hormone is short (10–20 min). It is converted in the liver to androstenedione (see Fig. 35.3), which has weak androgenic activity. Synthetic androgens are less rapidly metabolised, and some are excreted in the urine unchanged.

Unwanted effects

Unwanted effects of androgens include decreased gonadotrophin release during continued use, with resultant infertility, and salt and water retention leading to oedema. Adenocarcinoma of the liver has been reported. Androgens impair growth in children (via premature fusion of epiphyses), cause acne and lead to masculinisation in girls. Adverse effects of testosterone replacement and monitoring for these are reviewed by Rhoden & Morgentaler (2004).

Androgens and the hormonal control of the male reproductive system

- Gonadotrophin-releasing hormone from the hypothalamus acts on the anterior pituitary to release both follicle-stimulating hormone, which stimulates gametogenesis, and luteinising hormone (also called interstitial cell-stimulating hormone), which stimulates androgen secretion.
- The endogenous hormone is testosterone; intramuscular depot injections of testosterone esters are used for replacement therapy.
- Mechanism of action is via intracellular receptors.
- Effects depend on age/sex, and include development of male secondary sexual characteristics in prepubertal boys and masculinisation in women.

Clinical uses of androgens and antiandrogens

- Androgens (**testosterone** preparations) as hormone replacement in:
  - male hypogonadism due to pituitary or testicular disease (e.g. 50–100 mg per day as gel applied to the skin)
  - female hyposexuality following ovariectomy (e.g. 300 μg/day patches).
- Antiandrogens (e.g. **flutamide**, **cyproterone**) are used as part of the treatment of prostatic cancer.
- 5α-Reductase inhibitors (e.g. **finasteride**) are used in benign prostatic hyperplasia.

The clinical uses of androgens are given in the clinical box above.

ANABOLIC STEROIDS

Androgens can be modified chemically to alter the balance of anabolic and other effects. 'Anabolic steroids' (e.g. **nandrolone**) increase protein synthesis and muscle development disproportionately, but clinical use (e.g. in debilitating disease) has been disappointing. They are used in the therapy of aplastic anaemia and (notoriously) abused by some athletes (Ch. 58), as is testosterone itself. Unwanted effects are described above, under Androgens. In addition, cholestatic jaundice, liver tumours and increased risk of coronary heart disease are recognised adverse effects of high-dose anabolic steroids.

ANTIANDROGENS

Both oestrogens and progestogens have antiandrogen activity, oestrogens mainly by inhibiting gonadotrophin secretion and progestogens by competing at androgen receptors in target organs. **Cyproterone** is a derivative of progesterone and has weak progestational activity. It is a partial agonist at androgen receptors, competing with dihydrotestosterone for receptors in androgen-sensitive target tissues. Through its effect in the hypothalamus, it

depresses the synthesis of gonadotrophins. It is used as an adjunct in the treatment of prostatic cancer during initiation of GnRH agonist treatment (see below). It is also used in the therapy of precocious puberty in males, and of masculinisation and acne in women. It also has a central nervous system effect, decreasing libido, and has been used to treat hypersexuality in male sexual offenders.[2]

Flutamide is a non-steroidal antiandrogen used with GnRH agonists in the treatment of prostate cancer.

Drugs can have antiandrogen action by inhibiting synthetic enzymes. **Finasteride** inhibits the enzyme (5α-reductase) that converts testosterone to dihydrotestosterone (Fig. 35.3). This active metabolite has greater affinity than testosterone for androgen receptors in the prostate gland. Finasteride is well absorbed after oral administration, has a half-life of about 7 h, and is excreted in the urine and faeces. It is used to treat benign prostatic hyperplasia, although α_1-adrenoceptor antagonists, for example **terazosin** or **tamsulosin** (Chs 14 and 29), are more effective (working by the entirely different mechanism of relaxing smooth muscle in the capsule of the prostate gland and opposing α_1-adrenoceptor-mediated prostatic growth). Surgery is another option.

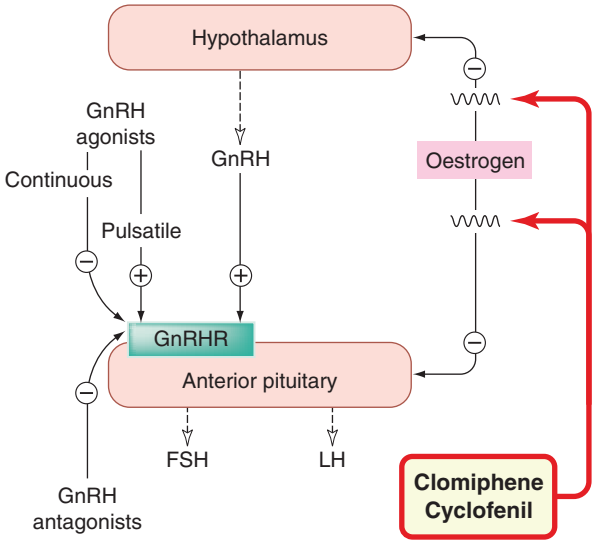

Fig. 35.5 Regulation of gonadotrophin (follicle-stimulating hormone, FSH; luteinising hormone, LH) release from the anterior pituitary. GnRHR, GnRH receptor.

GONADOTROPHIN-RELEASING HORMONE: AGONISTS AND ANTAGONISTS

Gonadotrophin-releasing hormone is a decapeptide that controls the secretion of FSH and LH by the anterior pituitary. Secretion of GnRH is controlled by neural input from other parts of the brain, and through negative feedback by the sex steroids (Figs 35.1 and 35.5). Exogenous androgens, oestrogens and progestogens all inhibit GnRH secretion, but only progestogens exert this effect at doses that do not have marked hormonal actions on peripheral tissues, presumably because progesterone receptors in the reproductive tract are sparse unless they have been induced by previous exposure to oestrogen. **Danazol** (see below) is a synthetic steroid that inhibits release of GnRH and, consequently, of gonadotrophins (FSH and LH). **Clomiphene** is an oestrogen antagonist that stimulates gonadotrophin release by inhibiting the negative feedback effects of endogenous oestrogen; it is used to treat infertility (see clinical box, p. 429, and Fig. 35.5).

Synthetic GnRH is termed **gonadorelin**. Numerous analogues of GnRH, both agonists and antagonists, have been synthesised. **Buserelin**, **leuprorelin**, **goserelin** and **nafarelin** are agonists, the last being 200 times more potent than endogenous GnRH.

Pharmacokinetics and clinical use

Gonadotrophin-releasing hormone agonists, given by subcutaneous infusion in pulses to mimic physiological secretion of GnRH, stimulate gonadotrophin release (Fig. 35.5) and induce ovulation. They are absorbed intact following nasal administration (Ch. 8). Continuous use, by nasal spray or as depot preparations, stimulates gonadotrophin release transiently, but then paradoxically inhibits gonadotrophin release (Fig. 35.5) because of downregulation (desensitisation) of GnRH receptors in the pituitary. GnRH analogues are given in this fashion to cause gonadal suppression in various sex hormone-dependent conditions, including prostate and breast cancers, endometriosis (endometrial tissue outside the uterine cavity) and large uterine fibroids. Continuous, non-pulsatile administration inhibits spermatogenesis and ovulation. GnRH agonists are used by specialists in infertility treatment, not to stimulate ovulation (which is achieved using gonadotrophin preparations) but to suppress the pituitary before administration of FSH or HCG.

Unwanted effects of GnRH analogues

Unwanted effects of GnRH agonists in women, for example flushing, vaginal dryness and bone loss, result from hypo-oestrogenism. The initial stimulation of gonadotrophin secretion on starting treatment can cause transient worsening of pain from bone metastases in men with prostate cancer, so treatment is started only after the patient has received an androgen receptor antagonist such as **flutamide** (see above and Ch. 56).

DANAZOL

Actions and pharmacokinetics

Danazol inhibits gonadotrophin secretion (especially the mid-cycle surge), and consequently reduces oestrogen synthesis in the ovary (Fig. 35.5). In men, it reduces androgen synthesis and spermatogenesis. It has androgenic activity. It is orally active and metabolised in the liver.

Danazol is used in sex hormone-dependent conditions including endometriosis, breast dysplasia and gynaecomastia. An additional special use is to reduce attacks of swelling in hereditary angio-oedema (Ch. 28).

Unwanted effects are common, and include gastrointestinal disturbances, weight gain, fluid retention, dizziness, menopausal symptoms, muscle cramps and headache. Danazol is virilising in women.

GONADOTROPHINS AND ANALOGUES

Gonadotrophins (FSH, LH and HCG) are glycoproteins produced and secreted by the anterior pituitary (FSH and LH see Ch. 33) or chorion and placenta (HCG). Large amounts of gonadotrophins are present in the urine of

[2]Very different doses are used for these different conditions, for example 2 mg/day for acne, 100 mg/day for hypersexuality and 300 mg/day for prostatic cancer.

women following the menopause, in whom oestrogen no longer exerts feedback inhibition on the pituitary, which consequently secretes large amounts of FSH and LH.[3]

Preparations

Gonadotrophins are extracted from urine of pregnant (HCG) or postmenopausal women (human menopausal gonadotrophin, which contains a mixture of FSH and LH). Recombinant FSH (**follitropin**) and LH (**lutropin**) are also available.

Pharmacokinetics and clinical use

Gonadotrophin preparations are given by injection. They are used to treat infertility caused by lack of ovulation as a result of hypopituitarism, or following failure of treatment with **clomiphene**; they are also used by specialists to induce ovulation to enable eggs to be collected for in vitro fertilisation. For this use, gonadotrophin is usually administered after secretion of endogenous FSH and LH has been suppressed (see p. 432). Gonadotrophins are also sometimes used in men with infertility caused by a low sperm count as a result of hypogonadotrophic hypogonadism (a disorder that is sometimes accompanied by lifelong anosmia, i.e. lack of sense of smell). (Gonadotrophins do not, of course, work for patients whose low sperm count is the result of primary testicular failure.) HCG has been used to stimulate testosterone synthesis in boys with delayed puberty, but testosterone is usually preferred.

Gonadotrophin-releasing hormone and gonadotrophins

- Gonadotrophin-releasing hormone is a decapeptide; **gonadorelin** is the synthetic form. **Nafarelin** is a potent analogue.
- Given in pulsatile fashion, they stimulate gonadotrophin release; given continuously, they inhibit it.
- The gonadotrophins, follicle-stimulating hormone and luteinising hormone, are glycoproteins.
- Preparations of gonadotrophins (e.g. chorionic gonadotrophin) are used to treat infertility caused by lack of ovulation.
- **Danazol** is a modified progestogen that inhibits gonadotrophin production by an action on the hypothalamus and anterior pituitary.

DRUGS USED FOR CONTRACEPTION

ORAL CONTRACEPTIVES

There are two main types of oral contraceptives:

1. Combinations of an oestrogen with a progestogen (the combined pill).
2. Progestogen alone (the progestogen-only pill).

THE COMBINED PILL

The combined oral contraceptive pill is extremely effective, at least in the absence of intercurrent illness and of treatment with potentially interacting drugs (see p. 434). The oestrogen in most combined preparations (second-generation pills)[4] is **ethinylestradiol**, although a few preparations contain **mestranol** instead. The progestogen may be **norethisterone**, **levonorgestrel**, **ethynodiol**, or – in 'third-generation' pills – **desogestrel** or **gestodene**, which are more potent, have less androgenic action and cause less change in lipoprotein metabolism, but which probably cause a greater risk of thromboembolism than do second-generation preparations. The oestrogen content is generally 20–50 µg of ethinylestradiol or its equivalent, and a preparation is chosen with the lowest oestrogen and progestogen content that is well tolerated and gives good cycle control in the individual woman. This combined pill is taken for 21 consecutive days followed by 7 pill-free days, which causes a withdrawal bleed. Normal cycles of menstruation usually commence fairly soon after discontinuing treatment, and permanent loss of fertility (which may be a result of early menopause rather than a long-term consequence of the contraceptive pill) is rare.

The mode of action is as follows:

- Oestrogen inhibits secretion of FSH via negative feedback on the anterior pituitary, and thus suppresses development of the ovarian follicle.
- Progestogen inhibits secretion of LH and thus prevents ovulation; it also makes the cervical mucus less suitable for the passage of sperm.
- Oestrogen and progestogen act in concert to alter the endometrium in such a way as to discourage implantation.

They may also interfere with the coordinated contractions of the cervix, uterus and fallopian tubes that facilitate fertilisation and implantation.

Hundreds of millions of women worldwide have used this method since the 1960s, and in general the combined pill constitutes a safe and effective method of contraception. There are distinct health benefits from taking the pill (see p. 434), and serious adverse effects are rare. However, minor unwanted effects constitute drawbacks to its use, and several important questions need to be considered.

Common adverse effects

The common adverse effects are:

- weight gain, owing to fluid retention or an anabolic effect, or both
- mild nausea, flushing, dizziness, depression or irritability
- skin changes (e.g. acne and/or an increase in pigmentation)
- amenorrhoea of variable duration on cessation of taking the pill.

Questions that need to be considered

Is there an increased risk of cardiovascular disease (venous thromboembolism, myocardial infarction, stroke)? With second-generation pills (oestrogen content less than 50 µg), the risk of thromboembolism is small (incidence approximately 15 per 100 000 users per year, compared with 5 per 100 000 non-pregnant non-users per year or 60 episodes of thromboembolism per

[3]This forms the basis for the standard blood test, estimation of plasma LH/FSH concentrations, to confirm whether a woman is postmenopausal.

[4]The first-generation pills, containing more than 50 µg of oestrogen, were shown in the 1970s to be associated with an increased risk of deep vein thrombosis and pulmonary embolism.

100 000 pregnancies). The risk is greatest in subgroups with additional factors, such as smoking (which increases risk substantially) and long-continued use of the pill, especially in women over 35 years of age. The incidence of thromboembolic disease is approximately 25 per 100 000 users per year in users of preparations containing **desogestrel** or **gestodene**, which is still a small absolute risk compared with the risk of thromboembolism in an unwanted pregnancy. In general, provided risk factors, e.g. smoking, hypertension and obesity, have been identified, combined oral contraceptives are safe for most women for most of their reproductive lives.

Is cancer risk affected? Ovarian and endometrial cancer risk is *reduced*.

Is blood pressure increased? A marked increase in arterial blood pressure occurs in a small percentage of women shortly after starting the combined oral contraceptive pill. This is associated with increased circulating angiotensinogen, and disappears when treatment is stopped. Blood pressure is therefore monitored carefully when oral contraceptive treatment is started, and an alternative contraceptive substituted if necessary.

Beneficial effects

Besides avoiding unwanted pregnancy, other desirable effects of the combined contraceptive pill include decreased menstrual symptoms such as irregular periods and intermenstrual bleeding. Iron deficiency anaemia and premenstrual tension are reduced, as are benign breast disease, uterine fibroids and functional cysts of the ovaries.

THE PROGESTOGEN-ONLY PILL

The drugs used in progestogen-only pills include **norethisterone**, **levonorgestrel** or **ethynodiol**. The pill is taken daily without interruption. The mode of action is primarily on the cervical mucus, which is made inhospitable to sperm. The progestogen probably also hinders implantation through its effect on the endometrium (Fig. 35.2) and on the motility and secretions of the fallopian tubes (see p. 433).

Potential beneficial and unwanted effects

Progestogen-only contraceptives offer a suitable alternative to the combined pill for some women in whom oestrogen is contraindicated, and are suitable for women whose blood pressure increases unacceptably during treatment with oestrogen. However, their contraceptive effect is less reliable than that of the combination pill, and missing a dose may result in conception. Disturbances of menstruation (especially irregular bleeding) are common. Only a small proportion of women use this form of contraception, so long-term safety data are less reliable than for the combined pill.

Pharmacokinetics of oral contraceptives: drug interactions

Combined and progestogen-only oral contraceptives are metabolised by hepatic cytochrome P450 enzymes. Because the minimum effective dose of oestrogen is used (in order to avoid excess risk of thromboembolism), any increase in its clearance may result in contraceptive failure, and indeed enzyme-inducing drugs can have this effect not only for combined but also for progesterone-only pills. Such drugs include **rifampicin** and **rifabutin**, as well as **carbamazepine**, **phenytoin** and others, including the herbal preparation St John's Wort (Ch. 47).

Oral contraceptives

The combined pill

- The combined pill contains an oestrogen and a progestogen. It is taken for 21 consecutive days out of 28.
- Mode of action: the oestrogen inhibits follicle-stimulating hormone release and therefore follicle development; the progestogen inhibits luteinising hormone release and therefore ovulation, and makes cervical mucus inhospitable for sperm; together, they render the endometrium unsuitable for implantation.
- Drawbacks: weight gain, nausea, mood changes and skin pigmentation can occur.
- Serious unwanted effects are rare. A small proportion of women develop reversible hypertension; there is a small increase in diagnosis of breast cancer, possibly attributable to earlier diagnosis, and of cervical cancer. There is an increased risk of thromboembolism with third-generation pills especially in women with additional risk factors (e.g. smoking) and with prolonged use.
- There are several beneficial effects, not least the avoidance of unwanted pregnancy, which itself carries risks to health.

The progestogen-only pill

- The progestogen-only pill is taken continuously. It differs from the combined pill in that the contraceptive effect is less reliable and is mainly a result of the alteration of cervical mucus. Irregular bleeding is common.

OTHER DRUG REGIMENS USED FOR CONTRACEPTION

POSTCOITAL (EMERGENCY) CONTRACEPTION

Oral administration of **levonorgestrel**, alone or combined with oestrogen, is effective if taken within 72 h of unprotected intercourse and repeated 12 h later. Nausea and vomiting are common (and the pills may then be lost: replacement tablets can be taken with an antiemetic such as **domperidone**). Insertion of an intrauterine device is more effective than hormonal methods, and works up to 5 days after intercourse.

LONG-ACTING PROGESTOGEN-ONLY CONTRACEPTION

Medroxyprogesterone can be given intramuscularly as a contraceptive. This is effective and safe. However, menstrual irregularities are common, and infertility may persist for many months after cessation of treatment.

Levonorgestrel implanted subcutaneously in non-biodegradable capsules is used by approximately 3 million women worldwide. This route of administration avoids first-pass metabolism. The capsules release their progestogen content slowly over 5 years. Irregular bleeding and headache are common.

A levonorgestrel-impregnated intrauterine system provides prolonged, reliable contraception and, in contrast to standard copper containing devices, *reduces* menstrual bleeding.

THE UTERUS

The physiological and pharmacological responses of the uterus vary at different stages of the menstrual cycle and during pregnancy.

THE MOTILITY OF THE UTERUS

Uterine muscle contracts rhythmically both *in vitro* and *in vivo*, contractions originating in the muscle itself. Myometrial cells in the fundus act as pacemakers and give rise to conducted action potentials. The electrophysiological activity of these pacemaker cells is regulated by the sex hormones.

The non-pregnant human uterus contracts spontaneously but weakly during the first part of the cycle, and more strongly during the luteal phase and during menstruation. Uterine movements are depressed in early pregnancy because oestrogen, potentiated by progesterone, hyperpolarises myometrial cells. This suppresses spontaneous contractions. Towards the end of gestation, however, contractions recommence; these increase in force and frequency, and become fully coordinated during parturition. The nerve supply to the uterus includes both excitatory and inhibitory sympathetic components: adrenaline, acting on β_2 adrenoceptors, inhibits uterine contraction, whereas noradrenaline, acting on α adrenoceptors, stimulates contraction.

DRUGS THAT STIMULATE THE UTERUS

Drugs that stimulate the pregnant uterus and are important in obstetrics include **oxytocin**, **ergometrine** and prostaglandins.

OXYTOCIN

The neurohypophyseal hormone oxytocin (an octapeptide) regulates myometrial activity, causing uterine contraction. Oxytocin release is stimulated by cervical dilatation, and by suckling; its role in parturition is incompletely understood but the fact that an antagonist (**atosiban**, see below) is effective in delaying the onset of labour implicates it in the physiology of parturition.

Oestrogen induces oxytocin receptor synthesis and, consequently, the uterus at term is highly sensitive to this hormone. Given by slow intravenous infusion to induce labour, oxytocin causes regular coordinated contractions that travel from fundus to cervix. Both amplitude and frequency of these contractions are related to dose, the uterus relaxing completely between contractions during low-dose infusion. Larger doses further increase the frequency of the contractions, and there is incomplete relaxation between them. Still higher doses cause sustained contractions that interfere with blood flow through the placenta and cause fetal distress or death.

Oxytocin contracts myoepithelial cells in the mammary gland, which causes 'milk let-down' – the expression of milk from the alveoli and ducts. It also has a vasodilator action. A weak antidiuretic action can result in water retention, which can be problematic in patients with cardiac or renal disease, or with pre-eclampsia.[5] Oxytocin and oxytocin receptors are also found in the brain, particularly in the limbic system, and are believed to play a role in mating and parenting behaviour.

The clinical use of synthetic oxytocin is given in the box on p. 436.

Oxytocin can be given by intravenous injection or intramuscularly, but is most often given by intravenous infusion. It is inactivated in the liver and kidneys, and by circulating placental oxytocinase.

Unwanted effects of oxytocin include dose-related hypotension, due to vasodilatation, with associated reflex tachycardia. Its antidiuretic hormone-like effect on water excretion by the kidney causes water retention and, unless water intake is curtailed, consequent hyponatraemia.

ERGOMETRINE

Ergot (*Claviceps purpurea*) is a fungus that grows on rye and contains a surprising variety of pharmacologically active substances (see Ch. 15). Ergot poisoning, which was once common, was often associated with abortion. In 1935, **ergometrine** was isolated and was recognised as the oxytocic principle in ergot.

Ergometrine contracts the human uterus. This action depends partly on the contractile state of the organ. On a contracted uterus (the normal state following delivery), ergometrine has relatively little effect. However, if the uterus is inappropriately relaxed, ergometrine initiates strong contraction and reduces bleeding from the placental bed (the raw surface from which the placenta has detached). Ergometrine also has a moderate vasoconstrictor action.

The mechanism of action of ergometrine on smooth muscle is not understood. It is possible that it acts partly on α adrenoceptors, like the related alkaloid ergotamine (see Ch. 14), and partly on 5-hydroxytryptamine receptors.

The clinical use of ergometrine is given in the box on p. 436.

Ergometrine can be given orally, intramuscularly or intravenously. It has a very rapid onset of action and its effect lasts for 3–6 h.

Ergometrine can produce vomiting, probably by an effect on dopamine D_2 receptors in the chemoreceptor trigger zone (see Ch. 30, Fig. 30.5). Vasoconstriction with an increase in blood pressure associated with nausea, blurred vision and headache can occur, as can vasospasm of the coronary arteries, resulting in angina.

PROSTAGLANDINS

Prostaglandins are discussed in detail in Chapter 17. The endometrium and myometrium have substantial prostaglandin-synthesising capacity, particularly in the second, proliferative phase of the menstrual cycle. Prostaglandin (PG)$F_{2\alpha}$ is generated in large amounts, and has been implicated in the ischaemic necrosis of the endometrium that precedes menstruation (although it has relatively little vasoconstrictor action on many human blood vessels, in contrast to some other mammalian species). Vasodilator prostaglandins, PGE_2 and PGI_2 (prostacyclin), are also generated by the uterus.

In addition to their vasoactive properties, the E and F prostaglandins contract uterine smooth muscle whose sensitivity to these prostaglandins increases during gestation. Their role in parturition is not fully understood, but

[5]Eclampsia is a pathological condition (involving, among other things, high blood pressure, swelling and seizures) that occurs in pregnant women.

as cyclo-oxygenase inhibitors can delay labour (see below), they probably play some part in this.

Prostaglandins also play a part in two of the main disorders of menstruation: dysmenorrhoea (painful menstruation) and menorrhagia (excessive blood loss). Dysmenorrhoea is associated with increased production of PGE_2 and $PGF_{2\alpha}$; non-steroidal anti-inflammatory drugs, which inhibit prostaglandin biosynthesis (see Ch. 26), are used to treat dysmenorrhoea. Menorrhagia, in the absence of uterine pathology, may be caused by a combination of increased vasodilatation and reduced haemostasis. Increased generation by the uterus of PGI_2 (which inhibits platelet aggregation) could impair haemostasis as well as causing vasodilatation. Non-steroidal anti-inflammatory drugs (e.g. **mefenamic acid**) are used to treat menorrhagia as well as dysmenorrhoea.

Prostaglandin preparations

Prostaglandins of the E and F series promote coordinated contractions of the body of the pregnant uterus, while relaxing the cervix. E and F prostaglandins reliably cause abortion in early and middle pregnancy, unlike oxytocin which generally does not cause expulsion of the uterine contents at this stage. The prostaglandins used in obstetrics are **dinoprostone** (PGE_2), **carboprost** (15-methyl $PGF_{2\alpha}$) and **gemeprost** or **misoprostol** (PGE_1 analogues). Dinoprostone can be given intravaginally as a gel or as tablets. Carboprost is given by deep intramuscular injection. Gemeprost or misoprostol are given intravaginally.

Unwanted effects

Unwanted effects include uterine pain, nausea and vomiting, and diarrhoea. Dinoprost can cause hypotension. When combined with mifepristone, a progestogen antagonist that sensitises the uterus to prostaglandins, lower doses of the prostaglandins (e.g. misoprostol) can be used to terminate pregnancy and side effects are reduced.

See the clinical box for the clinical uses of prostaglandins (see Ch. 17).

DRUGS THAT INHIBIT UTERINE CONTRACTION

Selective β_2-adrenoceptor agonists, such as **ritodrine** or **salbutamol**, inhibit spontaneous or oxytocin-induced contractions of the pregnant uterus. These uterine relaxants are used in selected patients to prevent premature labour occurring between 22 and 33 weeks of gestation in otherwise uncomplicated pregnancies. They can delay delivery by 48 h, time that can be used to administer glucocorticoid therapy to the mother so as to mature the lungs of the baby and reduce neonatal respiratory distress. It has been difficult to demonstrate that any of the drugs used to delay labour improve the outcome for the baby. Risks to the mother, especially pulmonary oedema, increase after 48 h, and myometrial response is reduced, so prolonged treatment is avoided. Cyclo-oxygenase inhibitors (e.g. **indometacin**) inhibit labour, but their use could cause problems in the baby, including renal dysfunction and delayed closure of the ductus arteriosus, both of which are influenced by endogenous prostaglandins.

An oxytocin receptor antagonist, **atosiban**, provides an alternative to a β_2-adrenoceptor agonist. It is given as an intravenous bolus followed by an intravenous infusion for not more than 48 h. Adverse effects include vasodilatation, nausea, vomiting and hyperglycaemia.

Clinical uses of drugs acting on the uterus

Myometrial stimulants (oxytocics)

- **Oxytocin** is used to *induce or augment labour* when the uterine muscle is not functioning adequately. It can also be used to treat *postpartum haemorrhage*.
- **Ergometrine** can be used to treat *postpartum haemorrhage*. **Carboprost** can be used if patients do not respond to **ergometrine**.
- A preparation containing both **oxytocin** and **ergometrine** is used for the management of the third stage of labour; the two agents together can also be used, prior to surgery, to control bleeding due to incomplete abortion.
- **Gemeprost** (intravaginally) or **misoprostol** (intravaginally or by mouth) are used in *therapeutic abortion* and **misoprostol** (unlicensed use) in *induction of labour*.
- **Gemeprost**, given as vaginal pessary following **mifepristone**, is used as a medical alternative to surgical *termination of pregnancy* (up to 63 days of gestation).

Myometrial relaxants

- The β-adrenoceptor agonists (e.g. **ritodrine**) are used to delay *preterm labour*.
- **Atosiban** (oxytocin antagonist) also delays preterm labour.

Drugs acting on the uterus

- At parturition, **oxytocin** causes regular coordinated uterine contractions, each followed by relaxation; **ergometrine**, an ergot alkaloid, causes uterine contractions with an increase in basal tone. **Atosiban**, an antagonist of oxytocin, delays labour.
- Prostaglandin (PG) analogues, for example **dinoprostone** (PGE_2) and **dinoprost** ($PGF_{2\alpha}$), contract the pregnant uterus but relax the cervix. Cyclo-oxygenase inhibitors inhibit PG synthesis and delay labour. They also alleviate symptoms of dysmenorrhoea and menorrhagia.
- The β_2-adrenoceptor agonists (e.g. **ritodrine**) inhibit spontaneous and oxytocin-induced contractions of the pregnant uterus.

ERECTILE DYSFUNCTION

Erectile function depends on complex interactions between physiological and psychological factors. Erection is caused by vasorelaxation in the arteries and arterioles supplying the erectile tissue. This increases penile blood flow; the consequent increase in sinusoidal filling compresses the venules, occluding venous outflow and causing erection. During sexual intercourse, reflex contraction of the ischiocavernosus muscles compresses the base of the corpora cavernosa, and the intracavernosal

pressure can reach several hundred millimetres of mercury during this phase of rigid erection. Innervation of the penis includes autonomic and somatic nerves. Nitric oxide is probably the main mediator of erection and is released both from nitrergic nerves and from endothelium (Ch. 20; Fig. 20.6).

Erectile function is adversely affected by several therapeutic drugs (including many antipsychotic, antidepressant and antihypertensive agents), and psychiatric and vascular disease (especially in association with endothelial dysfunction) can themselves cause erectile dysfunction, which is common in middle-aged and older men, even if they have no psychiatric or cardiovascular problems.[6] There are several organic causes, including hypogonadism (see clinical box, p. 431), hyperprolactinaemia (see Ch. 33), arterial disease and various causes of neuropathy (most commonly diabetes), but often no organic cause is identified.

Over the centuries, there has been a huge trade in parts of various creatures that have the misfortune to bear some fancied resemblance to human genitalia, in the pathetic belief that consuming these will restore virility or act as an aphrodisiac (i.e. a drug that stimulates libido). Alcohol (Ch. 49) 'provokes the desire but takes away the performance', and cannabis (Ch. 19) can also release inhibitions and probably does the same. **Yohimbine** (an α_2-adrenoceptor antagonist; Ch. 14) may have some positive effect in this regard, but trials have proved inconclusive. **Apomorphine** (a dopamine agonist; Ch. 40) causes erections in humans as well as in rodents when injected subcutaneously, but it is a powerful emetic, a disadvantage in this context. The picture picked up somewhat when it was found that injecting vasodilator drugs directly into the corpora cavernosa causes penile erection. **Papaverine** (Ch. 22), if necessary with the addition of **phentolamine**, was used in this way. The route of administration is not acceptable to most men, but diabetics in particular are often not needle-shy, and this approach was a real boon to many such patients. **PGE_1** (**alprostadil**) is often combined with other vasodilators when given intracavernosally. It can also be given transurethrally as an alternative (albeit still a somewhat unromantic one) to injection. Adverse effects of all these drugs include priapism (prolonged and painful erection with risk of permanent tissue damage), which is no joke. Treatment consists of aspiration of blood (using sterile technique) and, if necessary, cautious intracavernosal administration of a vasoconstrictor such as **phenylephrine**. Intracavernosal and transurethral preparations are still available, but orally active phosphodiesterase inhibitors are now generally the drugs of choice.

PHOSPHODIESTERASE TYPE V INHIBITORS

Sildenafil, the first selective phosphodiesterase type V inhibitor (see also Chs 20 and 22), was found accidentally to influence erectile function.[7] **Tadalafil** and **vardenafil** are also phosphodiesterase type V inhibitors licensed to treat erectile dysfunction. Tadalafil is longer acting than sildenafil. In contrast to intracavernosal vasodilators, phosphodiesterase type V inhibitors do not cause erection independent of sexual desire, but enhance the erectile response to sexual stimulation. They have transformed the treatment of erectile dysfunction.

Mechanism of action

Phosphodiesterase V is the isoform that inactivates cGMP. Nitrergic nerves release nitric oxide (or a related nitrosothiol) which diffuses into smooth muscle cells, where it activates guanylyl cyclase. The resulting increase in cytoplasmic cGMP mediates vasodilatation via activation of protein kinase G (Ch. 4, Fig. 4.10). Consequently, inhibition of phosphodiesterase V potentiates the effect on penile vascular smooth muscle of endothelium-derived nitric oxide and of nitrergic nerves that are activated by sexual stimulation (Fig. 35.6). Other vascular beds are also affected, suggesting other possible uses, notably in pulmonary hypertension (Ch. 22).

Pharmacokinetic aspects and drug interactions

Peak plasma concentrations of sildenafil occur approximately 30–120 min after an oral dose and are delayed by eating, so it is taken an hour or more before sexual activity. It is given as a single dose as needed. It is metabolised by CYP3A4, which is induced by **carbamazepine**, **rifampicin** and **barbiturates**, and inhibited by **cimetidine**, macrolide antibiotics, antifungal imidazolines and some antiviral drugs (such as **ritonavir**). These drugs can

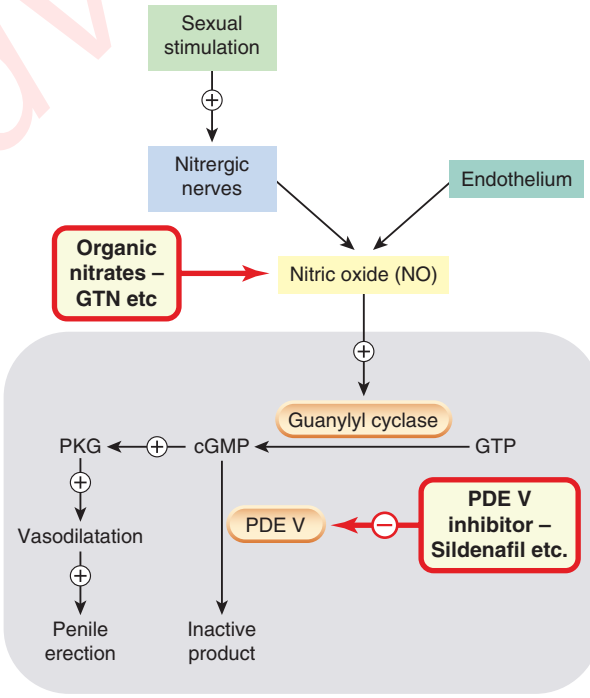

Fig. 35.6 Mechanism of phosphodiesterase V (PDE V) inhibitors on penile erection, and of the interaction of PDE V inhibitors with organic nitrates. The large grey rectangle denotes a vascular smooth muscle cell in the corpora cavernosa. Sexual stimulation releases nitric oxide (NO) from nitrergic nerves and this activates guanylyl cyclase, increasing cGMP production and hence activating protein kinase G (PKG), causing vasodilatation and penile erection. cGMP is inactivated by PDE V, so PDE V inhibitors (e.g. sildenafil) potentiate NO and promote penile erection. NO from organic nitrates such as glyceryl trinitrate (GTN) is also potentiated leading to generalised vasodilatation and hypotension.

[6] In randomised controlled trials, an appreciable proportion of men who discontinued treatment because of erectile dysfunction had been receiving placebo.

[7] Sildenafil was originally intended to treat angina, but volunteers in early phase trials reported an effect on affairs of the heart in a quite different anatomical region from the precordium.

interact with sildenafil. Tadalafil has a longer half-life than sildenafil, so can be taken longer before sexual activity. A clinically important pharmacodynamic interaction of all phosphodiesterase V inhibitors occurs with all organic nitrates, which work through increasing cGMP (Ch. 20) and are therefore markedly potentiated by sildenafil (Ch. 35, Fig. 35.6). Consequently, concurrent nitrate use, including use of **nicorandil**, contraindicates the use of any phosphodiesterase type V inhibitor.[8]

[8]This is important not only for sufferers from angina who take nitrates such as glyceryl trinitrate or isosorbide mononitrate therapeutically or prophylactically and are at risk of hypotension because of coronary artery disease, but also asymptomatic individuals who take amyl nitrate recreationally ('poppers') because of its effect on pelvic musculature.

Unwanted effects

Many of the unwanted effects of phosphodiesterase type V inhibitors are caused by vasodilatation in other vascular beds; these effects include hypotension, flushing and headache. Visual disturbances have occasionally been reported and are of concern because sildenafil has some action on phosphodiesterase VI, which is present in the retina and important in vision. The manufacturers advise that sildenafil should not be used in patients with hereditary retinal degenerative diseases (such as retinitis pigmentosa) because of the theoretical risk posed by this. Vardenafil is more selective for the type V isozyme than is sildenafil (reviewed by Doggrell, 2005), but is also contraindicated in patients with hereditary retinal disorders.

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Useful Web resource

www.mhra.gov.uk/home/groups/pl-p/documents/websiteresources/con2032228.pdf (*Risks of cancer [breast, endometrium, ovary], venous thromboembolism, stroke and coronary artery disease in relation to age and duration of HRT use*)