CHAPTER 1

The Molecular Basis of Hormones

Endocrine glands and their hormones

Introduction

In 1849 Claude Bernard postulated that the internal environment of the cells in the body (the *milieu intérieur*) is constantly regulated. In 1855 he also proposed that substances can be synthesised and secreted internally, within the body, by demonstrating the production and release of glucose from the liver. In these ways, perhaps he can be considered to be the 'father' of endocrinology even though we would not nowadays consider glucose to be a hormone.

While there are descriptions clearly relating to what we now know as endocrine glands going back at least 2000–3000 years in various parts of the world, it is interesting to note that endocrine glands were first classified as such only from the early 1900s. Initially, there were certain accepted methodologies which were used to ascertain whether or not tissues and organs had a true endocrine function. For example, since hormones are released into the bloodstream, certainly according to the original definition (see section 'What is a hormone?'), it is not surprising to find that endocrine glands in general have a very high blood flow per gram of tissue compared to most other organs. When assays were sufficiently developed to estimate, and later to measure, hormone levels in the blood, the concentration of a hormone would be expected to be greater in the venous effluent from an endocrine gland than in its arterial affluent. Nowadays, this general principle can be used to precisely locate the presence of an endocrine tumour.

Furthermore, removal of the endocrine gland being studied would be expected to result in observable changes to some aspect of the body's physiology. For example, one classic observation by Berthold, also in 1849, linking the testes (male sex glands) with a specific feature, was the disappearance of the comb on the head of a cockerel – a male characteristic in this species – when the testes were removed. Indeed, transplantation of the testes to another part of the body with an adequate blood circulation

Integrated Endocrinology, First Edition. John Laycock and Karim Meeran.

apparently restored the cock's comb. Likewise, the appropriate administration of an extract from the suspected endocrine gland should restore that physiological function, following the gland's removal. Clearly, such determining studies as these were essential in identifying what we can now call the 'classical' endocrine glands including the thyroid, the parathyroids, the gonads and the adrenals. However, nowadays we also recognise the much more disparate sources of hormones, from tissues not instinctively considered to have an endocrine function. For instance, the heart, lungs, kidneys and liver are better known for their other better described physiological functions than for the production of hormones, and yet this is undoubtedly one of their roles. Clearly the removal of such an organ would be problematic with regard to determining its endocrine function!

What is an endocrine gland (or tissue)?

An endocrine gland (or tissue) is usually defined as a group of cells which synthesises a chemical that is released into the surrounding medium, essentially into the blood. An endocrine gland is most easily recognised if it consists of cells with clearly identifiable, intracellular secretory machinery allowing for the expulsion of synthesised molecules out of that cell into the surrounding medium (blood). This is in contrast to an exocrine gland, such as a salivary gland or the main part of the pancreas, which secretes molecules into a duct leading to the exterior of the body. Nowadays, we know of various endocrine glands and tissues in the body which do not quite so readily fit the description given here; indeed, many of them are better known for other physiological actions. A general classification of endocrine glands would now have to include the following categories.

'Classic' endocrine glands

Various clearly defined glands have been identified as having an endocrine function and these can be classified as the 'classic' endocrine glands. They include the gonads, the thyroid, the adrenals, the parathyroids, the pancreatic islets of Langerhans and the pituitary. Each of these glands produces one or more different hormones. Only those glands producing amino acid–derived (e.g. amine, polypeptide and protein) hormones would contain secretory granules.

Gastrointestinal tract

Historically, the term hormone was actually used by two physiologists, William Bayliss and Ernest Starling, in 1909 to describe a molecule (secretin) produced by the gastrointestinal tract. This organ is extremely large with a clearly defined physiological function regarding digestion and absorption, and it is also the source of numerous hormones. While many of these hormones have clear gastrointestinal effects and fall within the

domain of gastroenterologists, some of them do have more widespread effects including on the central nervous system (CNS). These hormones are of particular interest to endocrinologists currently trying to determine the mechanisms involved in the regulation of food intake, hunger and appetite (see Chapter 16).

Central nervous system

While the hypothalamus is a part of the brain with a well-defined endocrine function, releasing molecules from nerve endings either into a specific blood portal system linking it to the anterior pituitary or into the general circulation via the posterior pituitary (see Chapter 2), it is quite possible that other parts of the brain also produce molecules which could be secreted into brain fluids such as the intracerebroventricular (icv) or general brain extravascular fluids. Certainly, the dendritic release of molecules known to be hormones into the icv fluid in the third ventricle has opened up possibilities of communication between different parts of the brain using a fluid distinct from blood. Furthermore, within the brain is a small organ which has a more clearly defined endocrine function, called the pineal gland. It is an interesting gland with an afferent nerve pathway originating from the eyes; in Hinduism and Buddhism it is known as the third (or inner) eye, and is considered to be a symbol of enlightenment. It produces melatonin, a hormone which plays a role in regulating various functions related to the internal circadian 'clock', located in the suprachiasmatic nucleus in the hypothalamus. It is released during the night and its production is inhibited by daylight.

Placenta

Another tissue which has highly specific reproductive functions, and which has a major endocrine role, is the placenta. During pregnancy many hormones are produced by this tissue, often in conjunction with the developing fetus, so that the endocrine tissue as a whole is often referred to as the feto-placental unit (see Chapter 9).

Other endocrine tissues

More recently, and as mentioned earlier, tissues better known for other physiological functions have been shown to have endocrine roles. They include the liver, the kidneys, the heart, the blood and adipose tissue. Immune (e.g. lymphoid) tissue also produces molecules which have 'endocrine' effects on their distant target cells.

The cells of an endocrine gland produce molecules for export into the general circulation as a consequence of the integration of various signals reaching those cells. At any given time, these endocrine cells may receive numerous differing signals, some stimulatory and others inhibitory.

The manner in which each endocrine cell integrates these various signals is one area of endocrinology which is just beginning to be unravelled.

What is a hormone?

A hormone is that molecule produced by a certain cell or cells (the endocrine gland or tissue) which is exported out of the cells and is transported to its target cells by a circulating fluid medium, by definition (but not necessarily) the blood. Hormones influence their target cells to respond in a specific way, normally to the benefit of the organism. It is part of the homeostatic response to an altered environment, whether internal or external. The hormone conveys a message from one part of the body to another, and can therefore be considered as a messenger molecule. The response of the target cell to that first messenger, or hormone, is often produced in response to an intracellular cascade of activated or inhibited molecules which can be considered to be 'second' messenger systems.

Molecules or metabolites that are nutrients or excretory products would have to be excluded. For this reason, Claude Bernard's original discovery that the liver releases an internal secretion which is the energy substrate glucose means that it does not actually conform to the definition of an endocrine gland on this basis; this secretory product is not a hormone. However, the liver does produce other molecules which are not simple nutrients or excretory products, but which are true messengers ('first' messengers) secreted into the bloodstream which carries them to their distant target tissues. Consequently, it can truly be considered to be an endocrine tissue (see Chapter 3). Likewise, the definition has to exclude those molecules secreted from nerve terminals and which traverse the tiny synaptic gaps between neurones to act as neurotransmitters or neuromodulators. Of course, this does not exclude the possibility that neurones, for instance in the CNS, can be endocrine cells producing true hormones. Indeed, the study of these neurones and their neurosecretions is such a rapidly expanding research area that it is now a well-established entity in its own right, called neuroendocrinology.

It is apparent that, despite the definition given here, it is not always easy to appreciate whether a molecule is a hormone or not. For example, consider the group of molecules called cytokines. These are proteins produced by cells of the immune system which clearly have a communicating function between different cells. They are transported by the blood and can have diffuse effects in the body, including the CNS, and therefore act as hormones. This group of molecules includes the interleukins (IL1-18) and various other 'factors' such as the tumour necrosis factors and interferons, all components of the immune system. Not surprisingly, endocrinologists and immunologists show much interest in them.

One way we could establish whether a molecule is a hormone or not, is to determine whether it has specific receptors on its target cells. This would certainly allow us to exclude simple nutrients and excretory products which do not have any as such. Indeed, there are occasions when a gene product leads to the discovery of a new protein, which may be a receptor protein, for instance. Such a molecule is sometimes called an orphan receptor until a ligand (a molecule that binds to a receptor, such as a hormone) for it has been identified.

As indicated earlier, hormones, by definition, are chemicals which are produced by specific cells and released into the bloodstream to exert their effects on distant target cells. However, it is quite likely that they can be released into (or enter) other circulating fluids such as the cerebrospinal fluid, seminal fluid, amniotic fluid and lymph. All these fluids are essentially made up of water containing a variety of solutes and ions, maybe cells and cell fragments. As chemicals, some of the hormones will be hydrophilic (i.e. 'water-loving') molecules such as amino acids, peptides and proteins. They can similarly be considered to be lipophobic ('lipid hating'). Other hormones, such as steroids, will be lipophilic ('lipid-loving') molecules; they can also be described as hydrophobic ('water hating'). Not surprisingly, the chemical nature of any hormone in question will have an important bearing not only on its synthesis but also on its storage, its release from the endocrine cell, its transport in the fluid medium and its mechanism of action. A relatively new consideration is that some molecules, generally considered to be gaseous, have also been shown to have an endocrine role, although because they are very short-lived in the general circulation they probably have an effect only on cells near their sites of production. Nitric oxide is the clearest example of such a molecule; it is ubiquitous, being produced in many different tissues and exerting its effects locally.

While it is clear that most hormones exert their *endocrine* effect on distant target cells, some, such as nitric oxide, have an effect on nearby adjacent cells. They are described as having a *paracrine* effect. Also, we now know that a few hormones may actually have an immediate effect on their own cells of production, influencing their own processes of synthesis, storage and release. This is called an *autocrine* effect (see Figure 1.1).

There is another term that has been used to describe the location of action for some hormones: *cryptocrine*. The term describes the actions of molecules (such as hormones) which a cell produces, and which act within a closed space associated with its cell of production. One good example of such a cryptocrine activity is that of the Sertoli cell in the testis producing factors which act on developing spermatids within that cell's enclosed environment (see Chapter 6).

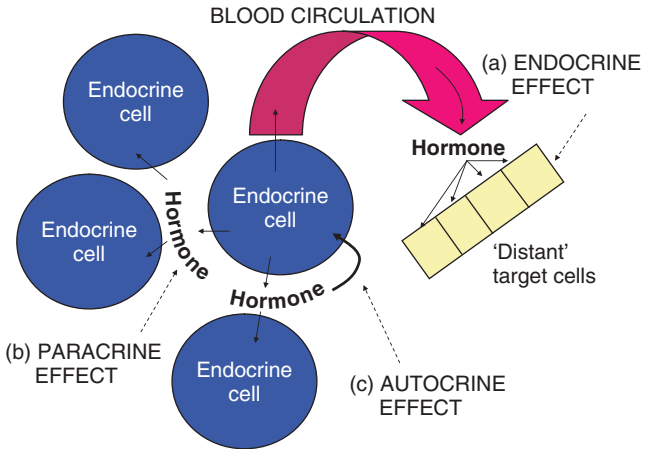

Figure 1.1 Diagram illustrating the release of a hormone from its endocrine cell and having its effect (a) on distant target cells (endocrine effect), (b) on cells nearby (paracrine effect) or (c) on its own endocrine cell of production (autocrine effect).

Interestingly, adjacent cells can have specific physical relationships between each other which permit them to 'converse'. One example is provided by gap junctions where the membranes of two cells are in contact. They are membrane proteins called connexins which contain pores (connexons) allowing small molecules such as ions and nutrients to cross from one cell to the other. Another example is the dynamic (labile) formation of tight junctions which fuse adjacent cells to each other. The tight junctions, consisting of transmembrane proteins, can actually temporarily trap extracellular fluid between the cells. Any molecule secreted by one cell into this tiny extracellular space would reach a high concentration locally, and if receptors for that molecule are present on the second cell, then an effect can be exerted which would not occur if the hormone was simply released into the general circulation to reach the second cell via a longer route involving considerable dilution (Figure 1.2).

The main hormones, their chemical nature and their main sites of synthesis are given in Table 1.1. Many of the hormones, and some of the endocrine glands, have alternative names, and they can be used interchangeably. Details of the various differing nomenclatures will be given in the relevant chapters of this text dealing with the individual glands and their hormones.

Hormones versus neurotransmitters

It will be apparent that hormones form a regulatory system which functions to maintain the body's homeostasis in response to perturbing

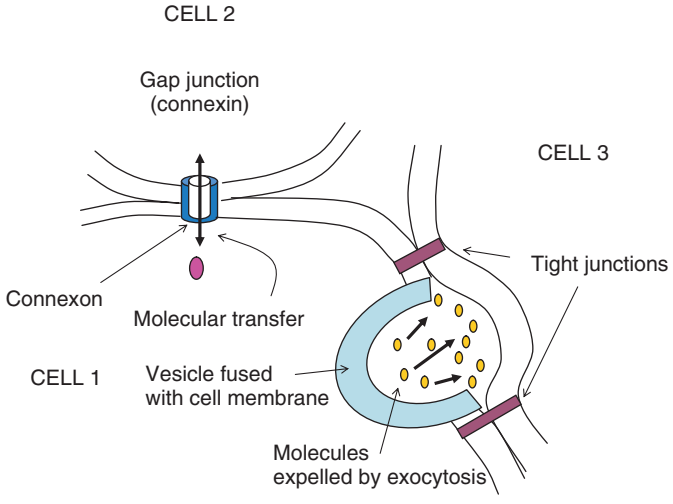

Figure 1.2 Diagram illustrating adjacent cells with gap and tight junctions, allowing paracrine communication between them. Molecules can pass from cell 1 to cell 2 via the pore (connexon) in the gap junction (connexin). They can also be released (e.g. by exocytosis) into an enclosed space defined by the presence of dynamic tight junctions, such as between cells 1 and 3 (shown) where they can reach a high concentration.

influences (stimuli) from within, as well as from outside, the body. The other major control system is the nervous system which is comprised of neurones which generally contact other neurones within the CNS via synaptic gaps, or target cells such as skeletal muscle fibres via neuromuscular junctions, for example. Both these control systems involve the use of chemical 'messengers' and have certain similarities, but there are also clear differences between them.

Neurones, when stimulated, produce neurosecretions which are released usually at the nerve terminals. Most of the time these neurosecretions are released across the synaptic gaps between neurones, and therefore they act as neurotransmitters, or at neuromuscular junctions. However, some neurones release neurosecretions into the blood, in which case they are clearly hormones. Furthermore, it is increasingly clear that some of these neurosecretory molecules could be transported to more distant target cells by the cerebrospinal fluid, or by the more general brain extracellular fluid, in which case they can also justifiably be considered to be hormones.

One important part of the brain which plays an essential role in regulating our internal environment is the hypothalamus. This part of the brain exerts many of its effects by controlling a number of peripheral endocrine glands. Some of the hypothalamic neurones release their neurosecretions into a specialised blood system. They are transported by the blood down to their target cells which comprise a 'mediating' endocrine gland called

Table 1.1 The principal hormones, together with their chemical group and main sites of synthesis.

Hormone type	Examples	Main endocrine gland
Amino acid or amino acid derived	Thyroxine (T4) and tri-iodothyronine (T3)	Thyroid
	Adrenaline and noradrenaline	Adrenal medulla
	Dopamine	Hypothalamus
Polypeptide	Insulin	Pancreas
	Glucagon	Pancreas
	Vasopressin	Neurohypophysis
	Oxytocin	Neurohypophysis
	Corticotrophin	Adenohypophysis
	Calcitonin	Thyroid parafollicular cells
	Parathormone	Parathyroid glands
	Somatostatin	Hypothalamus
	Corticotrophin-releasing hormone	Hypothalamus
	Thyrotrophin-releasing hormone	Hypothalamus
	Gonadotrophin-releasing hormone	Hypothalamus
	Inhibin	Testis, ovary
	Activin	Ovary
	Angiotensin II	Blood
	Atrial natriuretic peptide	Heart
Protein	Somatrotrophin	Adenohypophysis
	Prolactin	Adenohypophysis
	Erythropoietin	Kidney
	Cytokines (various, e.g. interleukins)	Immune system cells
	Leptin	Adipose tissue
	Ghrelin	Stomach
Glycoproteins	Thyrotrophin	Adenohypophysis
	Luteinising hormone	Adenohypophysis
	Follicle-stimulating hormone	Adenohypophysis
Steroids	Aldosterone	Adrenal
	Cortisol	Adrenal
	Testosterone	Testis
	17β-oestradiol	Ovary
	Oestrone	Ovary
	Progesterone	Ovary
	Calcitriol	Kidney
Prostaglandins	PGA1, PGA2, PGE1, PGE2, PGF1, etc.	Various
Thromboxanes and prostacyclins	TXA, TXB and PGI	Various
Gaseous molecules	Nitric oxide	Various (e.g. endothelial cells)
	Carbon monoxide	Various (e.g. hypothalamic neurones)

the pituitary (or hypophysis). Thus the hypothalamus can quite correctly be considered not only as part of the CNS but also as an endocrine gland in its own right. The hypothalamus together with the pituitary is generally called the hypothalamo-pituitary (or hypothalamo-hypophysial) system, or axis, and this will be considered in some detail in Chapters 2, 3 and 4. Indeed, the endocrine system has, rather poetically, been likened to an orchestra regulating many functions of the body, in which case the pituitary gland acts as the leader (principal first violin) while the hypothalamus is the conductor. As for all generalities, there are plenty of exceptions to this important control pathway involving the hypothalamus, and some endocrine glands have no obvious links, direct or indirect, with the hypothalamo-hypophysial system.

Both neural and endocrine systems are essential in regulating the various differing activities of the body, and both are dependent on the release of specific chemicals as either neurotransmitters or hormones, so what are their distinct characteristics?

There are many similarities between them. For instance:

Neurotransmitters have been generally considered to be small molecules such as acetylcholine, amino acids (e.g. glutamine) or amino acid–derived molecules (e.g. gamma-amino butyric acid, or GABA), while hormones can also be amino acid derived, but also include polypeptides, steroids and larger proteins and glycoproteins. This difference now seems to be much less clear-cut: neurones can also produce polypeptides, and even steroids (neurosteroids) which have direct or modifying effects on adjacent or other postsynaptic neurones. Some hormones, initially thought to be synthesised only in peripheral endocrine cells, are now known to be produced in the CNS, by either neurones or other cells such as glia. The opposite is also true, with some molecules originally being clearly identified as neurotransmitters (e.g. in the CNS) also shown to be produced by typical secretory cells in peripheral tissues.

Neurotransmitters and many hormones are essentially released from vesicles into the surrounding fluid by very similar mechanisms, involving calcium ions and an expulsion system called exocytosis which involves intracellular microtubules and filaments.

Their mechanisms of action are generally similar, essentially involving either ion channels or G protein–related receptors.

While there are similarities between neurotransmitters and hormones, there are also some crucial differences. The more obvious differences are given in Table 1.2.

Table 1.2 Some differences between neurotransmitters and hormones.

	Neurotransmitters	Hormones
Transmission	Across synaptic cleft	By blood (or other fluid)
Target cells	Direct to specific neurones or other cells	Can be some distance from source cells
Speed of action	Milliseconds	From seconds up to days

Synthesis of hormones

Many cells synthesise molecules which are necessary for the proper functioning of those cells some of which are intracellular (e.g. enzymes), while others may be molecules (e.g. glucose) destined for export to other cells in the body. Intracellular molecules can be carbohydrates (e.g. energy substrates), lipids (e.g. for membranes) or amino acid–derived polypeptides and larger proteins (e.g. enzymes), for example. One group of molecules synthesised for export to other cells consists of hormones, and these can be amines, polypeptides, proteins or steroids. Some of the proteins may well have carbohydrate components attached to them, and they are then called glycoproteins.

Most hormones fall into one of two groups because of the chemical nature of their structures: they are either 'water-loving, lipid-hating' (hydrophilic, lipophobic) molecules, or are the opposite and are 'water-hating, lipid-loving' (hydrophobic, lipophilic). The former group of molecules generally consists of the polypeptide and protein hormones, while the steroid hormones mainly comprise the latter. This difference between the two types of molecules has much relevance regarding their synthesis, storage and release and also influences their transport in the blood or other fluids, their binding to receptors and their mechanisms of action. However, there are other molecules which are very small, can have very short lives and do not readily fall into either of these two main categories. This third group includes gaseous molecules such as nitric oxide and carbon monoxide, and amines which are amino acid–derived hormones such as the iodothyronines and catecholamines which share some of the properties of both of the other two main groups.

Polypeptide and protein hormones

These hormones consist of chains of amino acids. Polypeptides are often, arbitrarily, described as being chains of 2–100 amino acids, while proteins are generally taken to be longer chains of 100 or more amino acids. Some proteins are glycosylated, having carbohydrate residues attached

to them (glycoproteins), and can also comprise more than one chain linked together. All these hormones are often synthesised initially as larger precursor molecules called prohormones which are cleaved to form more than one product, including the known hormone(s).

The endocrine cells which synthesise and export polypeptide and protein molecules contain a number of well-developed intracellular organelles which are involved in the processing and packaging of these molecules. These are the endoplasmic reticulum, the Golgi complex and the secretory granules. The synthesis process begins with the activation (or de-repression) of a specific gene on a chromosome within the nucleus of the endocrine cell by a transcription-stimulating (or -inhibiting) factor. The human genome contains approximately 30,000 genes, each one containing the code for a specific protein. When a specific gene is activated, resulting in a new protein molecule being synthesised, we talk about the gene having been 'expressed' (i.e. it is a process of gene expression). The gene, which consists of a segment of deoxyribonucleic acid (DNA), contains the nuclear code for a chain of amino acids. Nuclear chromosomes are made up of long strands of DNA. Each strand of DNA is made up of two chains of nucleotides linked together into the shape of a double helix. Each nucleotide consists of a base which projects towards the centre of the double helix, and an associated pentose sugar and phosphate which together form the backbone of the structure. Other molecules are also associated with the helix. The genetic code for the protein to be synthesised is provided by a series of base triplets, each one coding for an amino acid. Transcription of the gene code sequence into a corresponding molecule of messenger ribonucleic acid (mRNA) is initiated by an enzyme called RNA polymerase. Only one of the two DNA strands is used as a template for any given protein, so unzipping of the strands is an initial step. The start of the relevant segment of DNA is identified by the presence of a special nucleotide sequence called the promoter which is where the RNA polymerase attaches. Another sequence called a terminator identifies the end of the gene sequence. There are four DNA bases – adenine, cytosine, guanine and thymine – each pairing with a different base in a complementary manner. During transcription, each cytosine, guanine and thymine in the DNA template pairs with a guanine, cytosine and adenine respectively in the RNA strand, while adenine pairs with uracil (not thymine, as in the other DNA strand in the double helix). Each triplet of transcription nucleotides, called a codon, is associated with a specific amino acid.

Not all the DNA along a gene is transcribed into protein. Each gene is composed of exons which do transcribe into proteins, and introns which do not. As the complementary mRNA strand is synthesised within the nucleus, the introns are removed by enzymes called small nuclear ribonucleoproteins (snRNPs, called 'snurps'). The product of this process

is a functional strand of mRNA which passes through a pore in the double membrane comprising the nuclear envelope. The transport process is active, and selective, for that mRNA molecule. The mRNA molecule that is synthesised initially from a gene's DNA can then be split (or 'spliced') into more than one mRNA component, each of which can be translated into a different protein. This process is called alternative splicing.

To get from the mRNA molecule to a new protein, the coded information provided in the nucleotide sequence needs to be translated into a complementary sequence of amino acids, linked by peptide bonds. The translation process occurs in ribosomes which are either present free in the cytoplasm or bound to the membrane of the rough endoplasmic reticulum (RER) which encircles, and is attached to, the nucleus. For proteins due for export such as hormones, the translation process is usually associated with the ribosomes on the endoplasmic reticulum. Each ribosome consists of two subunits. The small subunit has a binding site for the mRNA, while the larger subunit has two binding sites for other small RNA molecules which bind specific amino acids and *transfer* them to the ribosome (transfer RNA, or tRNA).

The translation process begins with an mRNA molecule binding to the small ribosomal subunit at the mRNA-binding site. An initiator tRNA then binds to the promoter codon on the mRNA strand and this is where translation begins. The initiator tRNA is complementary to the mRNA codon, and is called the anticodon. The tRNA molecule is also bound to the larger ribosomal subunit where the anticodon is translated into methionine amino acid, which is always the starter sequence for the subsequent growing chain of amino acids. The next mRNA codon pairs with the subsequent tRNA molecule anticodon, and its attached amino acid then binds with the previous amino acid by a peptide bond, and so on (Figure 1.3).

The peptide bonding is catalysed by an enzyme component of the ribosome larger subunit. The subsequent arrival of other tRNA molecules ensures that peptides are linked to each other according to the code provided by the mRNA. This polymerising process of amino acids results in the formation of the new protein molecule. The synthesis of the protein is completed when a stop codon is reached; the protein molecule then separates from the final tRNA and the ribosome itself separates into its large and small subunits. More complete details of this process can be found in specific textbooks on molecular biology.

The synthesis of a polypeptide hormone generally begins with the formation of an initial larger protein called a pre-prohormone. This molecule moves from its ribosome through the membrane of the RER. The transfer

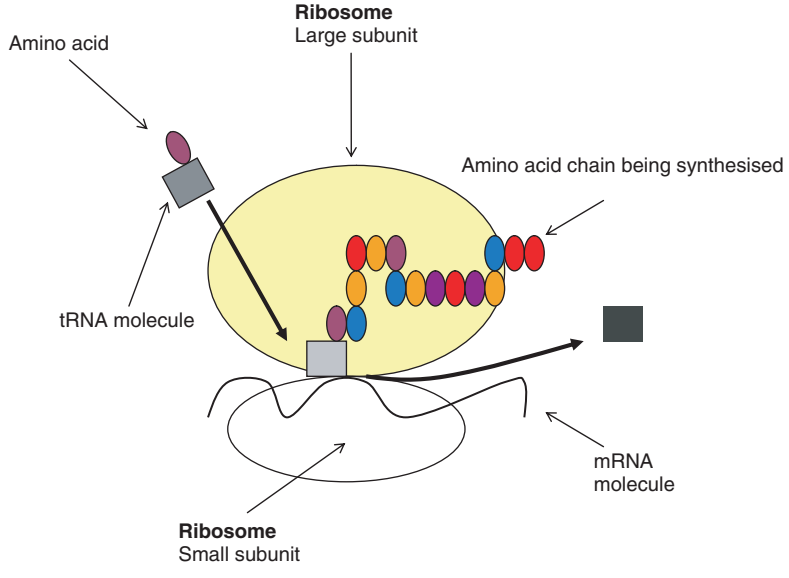

Figure 1.3 Diagram illustrating the arrival of a tRNA molecule, with its specific amino acid, at its binding site on the large ribosomal subunit where the amino acid links to the previous amino acid in the sequence by a peptide bond.

into the RER follows recognition of the initial segment of the molecule which is called the signal peptide. This is subsequently cleaved off the main molecule on entry into the sacs, or tubules, comprising the RER, producing the prohormone precursor. Subsequently, this precursor passes from the RER into the adjacent Golgi complex where it can be further modified. For example, here a prohormone can be enzymatically cleaved to form more than one protein or polypeptide, or it can be glycosylated, or otherwise modified. The final stage in the process of peptide hormone synthesis is the incorporation of the prohormone breakdown products into vesicles, which bud off from the Golgi membrane. There may be various peptide breakdown products from the same prohormone precursor, including the known biologically active hormone itself. In many cases, the other peptides have no known function although as research progresses they are likely to be shown to have some role, or biological activity, somewhere in the body.

The vesicles, invariably present in protein hormone–secreting endocrine cells, are an important storage source of the hormone (Figure 1.4).

Interestingly, because all eukaryotic cells within an individual contain the same genetic material, with relevant genes normally expressed only in specific tissues, it is apparent that abnormal polypeptide hormone

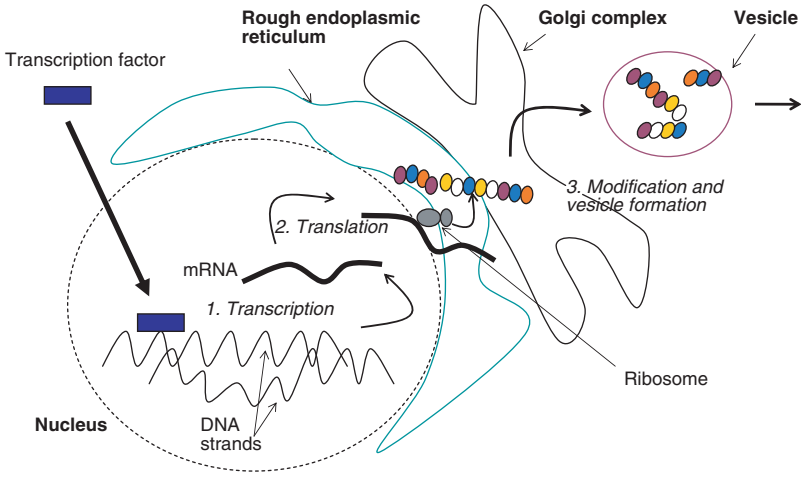

Figure 1.4 Diagram illustrating the three major steps involved in the synthesis of a hormone following the binding of the transcription factor to its site on a DNA strand: (1) Transcription of the protein 'code' from DNA to mRNA, (2) translation from mRNA to protein and (3) processing (chemical modification) and packaging of protein hormone into vesicles ready for export from the endocrine cell.

production is possible by non-endocrine tissue should that gene expression become activated. This is what can happen when a cell becomes abnormally stimulated by an appropriate chemical (carcinogen) such that the gene becomes 'switched on' inappropriately. The consequence is that some tumours of non-endocrine tissue (e.g. lung) can become abnormal (ectopic) sources of protein hormones. In addition to the direct effects of the tumour growth itself, problems can also arise due to the inappropriate, unregulated and very often exceedingly high circulating levels of the bioactive hormone being produced.

Steroid hormones

The other major group of hormones consists of steroids synthesised from the same initial precursor, cholesterol. Steroids are lipid soluble (lipophilic), so when they are synthesised by an endocrine cell they are immediately capable of moving out of that cell. Until recently it was believed that they simply move out of the cell by diffusion through the lipid membrane, but increasingly there is evidence for specific transporters in the cell membrane which would certainly aid the process. Not surprisingly, very little steroid hormone is found within its cell of synthesis since it would simply pass out of the cell immediately, so it is generally produced on demand (i.e. when the endocrine cell is stimulated appropriately). Consequently, the synthesis process involves the activation of specific intracellular enzymes which

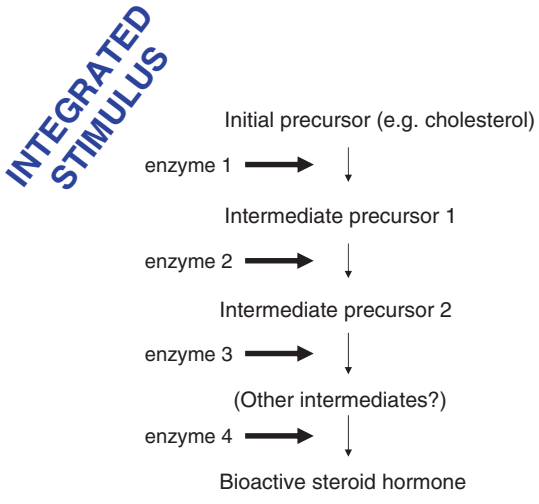

Figure 1.5 Diagram illustrating how the integrated stimulus to the endocrine cell, via intracellular mechanisms, activates enzymes which are involved in the formation of the biologically active steroid hormone from precursor molecules. It is quite possible that more than one bioactive hormone is produced when the synthesis pathway is stimulated.

catalyse chemical conversions such as hydroxylation and aromatisation, from precursors to the final bioactive hormone molecules.

Just as with peptide- and protein-producing endocrine cells, the cells producing steroid hormones receive constant stimulatory and inhibitory signals about the internal environment from a variety of sources, and these are somehow integrated in order to produce the final endocrine response. In these cells the various enzymes converting earlier molecular stages to the final bioactive molecule are the targets for the signalling pathways (Figure 1.5).

Amino acid–derived hormones

A few important hormones, such as those of the thyroid gland (iodothyronines) and the adrenal medulla (catecholamines), have very specific synthesis pathways and these will be considered in detail elsewhere in the relevant chapters of this volume. Essentially, the initial precursor molecule is an amino acid, and this is enzymatically altered to produce the final bioactive molecule. Their general properties vary, and are specific for each type of hormone. Thus, they are usually stored either in the endocrine cells or in follicles, they are transported in the blood either freely or protein bound and they bind to their receptors which are located on the plasma membranes of their target cells.

The eicosanoids: Prostaglandins, thromboxanes, prostacyclins and leukotrienes

There are other non-steroidal molecules which can be considered to be 'hormones' (see section 'What is a hormone?') and which also readily pass through plasma membranes by means of specific transporters, and therefore are only synthesised on receipt of appropriate stimuli. For example, the prostaglandins are lipids derived from 20-carbon essential fatty acids, called eicosanoids, found in most cells in the body. There are three series of prostaglandins, each derived from specific precursors: series 1 derived from gamma-linolenic acid, series 2 from arachidonic acid and series 3 from eicosapentaenoic acid. The best described are the molecules derived from arachidonic acid, which include the thromboxanes, prostacyclins and leukotrienes. All these groups of molecules together form the prostanoids, ubiquitous lipids which have physiological (and pathological) effects within the cells in which they are formed (autocrine) or on adjacent cells (paracrine). They have very short half-lives, are not transported to distant cells and therefore differ from the 'classic' hormones described here.

The precursor substrates are released from the cell membrane phospholipids by the action of specific enzymes. Thus, for example, arachidonic acid is released from cell membranes by the action of phospholipase A_2 and is then acted upon by other enzymes called cyclooxygenases (COX1 and COX2) to form intermediate prostaglandins PGG_2 and the unstable intermediate PGH_2. These prostaglandins are rapidly converted to other prostaglandins of the series by specific prostaglandin synthases, forming PGE_2, $PGF_{2\alpha}$ and so on (Figure 1.6). PGH_2 is also the precursor for prostacyclin (PGI_2) and thromboxanes by the action of yet other enzymes, prostacyclin and thromboxane synthases.

The gaseous molecules

The gaseous molecules that have been shown to act like locally acting hormones are nitric oxide and, it has been suggested, carbon monoxide. These molecules have very short half-lives, measured in seconds, and therefore act only on cells in close proximity to the cells producing them.

The free radical nitric oxide (NO) was first shown to be produced in living cells by Moncada and colleagues in the late 1980s (Palmer *et al.* 1987). They showed that the previously sought endothelium-derived relaxing factor (EDRF) was in fact this essentially toxic NO molecule. It was subsequently identified as having numerous physiological effects in maintaining homeostasis and is synthesised in many tissues in the body. It is particularly important as a regulator of the vasculature. There are three nitric oxide synthases (NOS): inducible (iNOS) and two calcium-dependent constitutive enzymes found in either vascular endothelial cells (eNOS) or nervous tissue (nNOS). In the vasculature, for example, NO

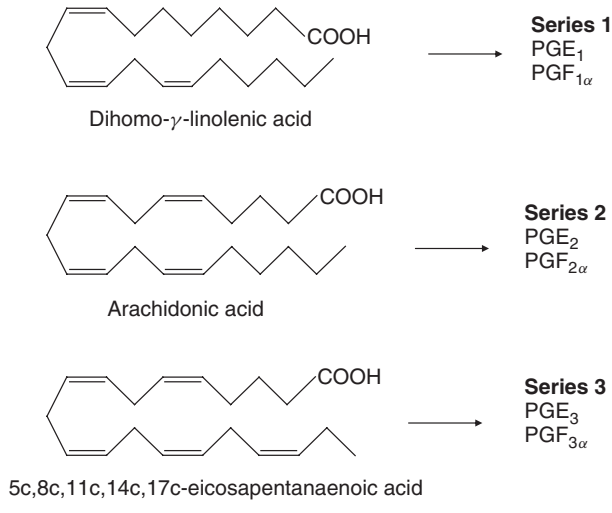

Figure 1.6 The precursor substrates linolenic, arachidonic and eicosapentanaenoic acids, derived from cell phospholipids, for series 1, 2 and 3 prostaglandins PGE and PGF.

is synthesised from the amino acid L-arginine by the action of eNOS or iNOS, as shown in Figure 1.7. The NO molecule diffuses from the endothelial cell into the adjacent outlying vascular smooth muscle cells where it stimulates the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP).

The stimuli for eNOS include a direct effect of shear stress produced by blood flow against the endothelial cells, and a receptor-activated pathway, both of which stimulate the release of calcium ions from intracellular storage sites. The increase in the intracellular free calcium ion concentration results in the stimulation of the constitutive eNOS. The iNOS is activated by a longer term inflammatory response. The production of NO then results in the activation of guanyl cyclase in the adjacent smooth muscle and the subsequent synthesis of cGMP. The cGMP induces muscle relaxation partly by activating potassium channels, inducing membrane hyperpolarisation which results in the inhibition of calcium entry into the cell through voltage-dependent calcium channels. The consequent decrease in the intracellular calcium ion concentration decreases calcium–calmodulin formation which in turn decreases myosin–actin binding (the contractile protein 'machinery') resulting in muscle relaxation. It also stimulates myosin light chain phosphatase which dephosphorylates myosin light chains, contributing to the smooth muscle relaxation.

Nitric oxide has a short half-life of only a few seconds because it is rapidly inactivated by superoxides, these being reactive oxygen species (ROS) produced within the cell.

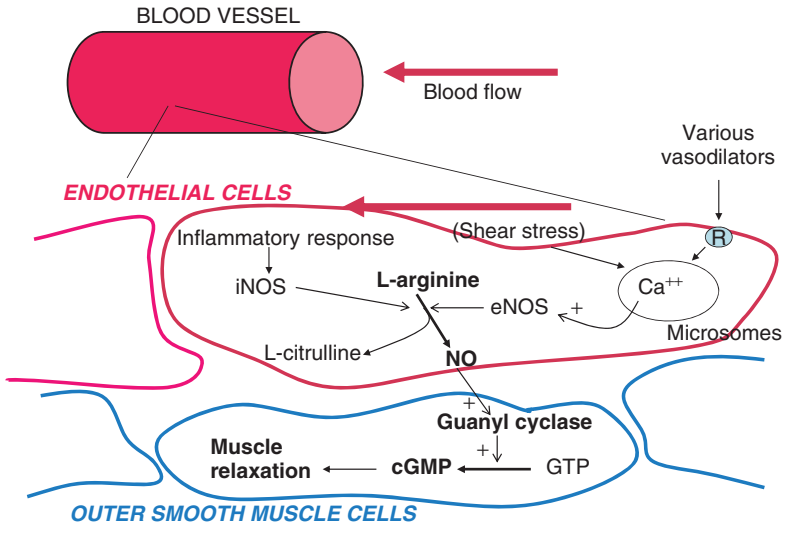

Figure 1.7 Diagram illustrating how the generation of nitric oxide (NO) by vascular endothelial cells results in the relaxation of the underlying smooth muscle cells by cGMP. R = receptor.

Storage of hormones

The chemical structure of a hormone will play an important part in determining whether a hormone is stored prior to its release or not. As with the synthesis pathways which differentiate protein and polypeptide hormones from the others, there is also a general difference between these two main groups of hormones with respect to whether or not they are stored.

Protein and polypeptide hormones

Proteins and polypeptides are generally stored in intracellular vesicles (see section 'Polypeptide and Protein Hormones'), these being formed as part of their synthesis pathway. Thus, when a protein or polypeptide hormone-producing cell is stimulated, not only will the genomic synthesis pathway be activated, but also the vesicles containing stored hormone can be mobilised and shifted to the cell's plasma membrane. This is important to appreciate: that the endocrine cell can be stimulated to not only synthesise new protein and polypeptide molecules for longer term use, but also release previously synthesised hormone molecules stored in the vesicles. Differing intracellular (second messenger) pathways for each of these activities can be induced separately or together, depending on the incoming stimulus.

However, another source of preformed protein and polypeptide hormones can be available within the blood. Some of these hormones when

released bind to plasma proteins, and then circulate in a bound form which is in dynamic equilibrium with any free (unbound) hormone. One example is growth hormone (somatotrophin) which has a number of binding proteins in the blood. The binding of protein and polypeptide hormones to plasma proteins is not the norm, however. In general, these hormones are stored in intracellular vesicles, to be released when the endocrine cell is stimulated appropriately. Other possible sources of stored protein and polypeptide hormones are cellular components in the blood, such as platelets. For example, the polypeptide hormone vasopressin is present within platelets at quite high concentrations, so it can be released from these circulating 'containers' when they are disrupted. This may well be physiologically appropriate in certain circumstances such as at a site of haemorrhage, since one action of this hormone is to cause vasoconstriction.

Amino acid–derived hormones

These hormones, such as the iodothyronines and catecholamines, are present in the endocrine glands in storage form. The catecholamines adrenaline and noradrenaline are stored in vesicles like the larger polypeptide and protein hormones, and are similarly released by exocytosis. The iodothyronines are stored in a more unusual, extracellular form within follicles lined by the thyroid follicular cells which synthesise them. When the follicular cells are stimulated appropriately, the iodothyronines are taken back into the cells and ultimately released into the general circulation. While the catecholamines are transported in the free unbound state, the iodothyronines are transported mostly bound to plasma proteins. For further details about the catecholamines and iodothyronines, see Chapters 11 and 14 respectively.

Steroid hormones

Steroids, being lipophilic, are not generally stored within the endocrine cells in bioactive form, but are mainly synthesised on demand. All cells will contain some cholesterol (e.g. in the form of esters), so since cholesterol is the parent precursor molecule for steroid hormones, one could just about get away with saying that there is a tiny amount that could be considered to be present in storage form as the precursor.

Does this mean that such hormones are not present in the circulation until the endocrine cells are stimulated to synthesise and release them? The answer is no, because steroid hormones are transported in the blood mainly bound to plasma proteins. A dynamic equilibrium exists between free (biologically active) hormone molecules and those bound to plasma proteins. In probably simplistic terms, this dynamic equilibrium between free and bound forms of hormone ensures that much of the hormone at any moment is 'stored' in the blood as the inactive, bound component.

The equilibrium can be expressed in terms of concentrations of the three components:

[free hormone H] + [plasma protein P] ↔ [protein-bound hormone HP]

At equilibrium, the equation reaction can be expressed as:

$$K_a = [HP]/[H][P]$$

where K_a is the association constant.

Not only would this equilibrium reaction provide a constant source of hormone in the circulation in 'storage form' as the protein-bound hormone component, but also it means that if the equilibrium is upset, then the components automatically rearrange themselves in order to restore that equilibrium. In theory, at least, in regions of the body where there may be an increased presence of hormone receptors, for instance, then the bioactive free component would bind to its receptors (having the higher affinity for binding) resulting in a local disequilibrium state. The physical forces in operation would result in an unloading of hormone from the protein-bound to the free hormone state in order to restore the balance (Figure 1.8). The same equilibrium reaction can also be used to describe the relationship between the hormone and its (protein) receptor.

The plasma proteins involved in the transport of hormones fall into two main categories: those that are carriers for specific hormones, and those that are not particularly specific but are present in high concentrations and can 'mop up' large amounts of hormones. The defining characteristic for a plasma protein is whether it has a high or a low affinity for binding hormones, and what capacity it has for binding to them. Those plasma proteins which have a high affinity for specific hormones, such as for glucocorticoids (e.g. cortisol), can transport relatively large amounts of these hormones. They are usually globulins (e.g. cortisol-binding globulin). Because these globulins are not present in particularly high concentrations in the blood, they may have a high affinity for specific hormones, but they have a low capacity for hormones generally. In contrast, albumins form the largest plasma protein component of the blood. While this fraction does not have a high affinity for any hormone in particular, because there is so much of it, it will have a relatively high capacity for hormone transport and can carry a significant proportion of many hormones in the circulation.

While the globulin proteins in the blood are relatively specific regarding which hormones they can bind, there is some overlap between them. For instance, sex hormone–binding globulin as its name suggests can bind not only androgens such as testosterone but also oestrogens, while cortisol-binding globulin (or transcortin) binds not only the glucocorticoid cortisol

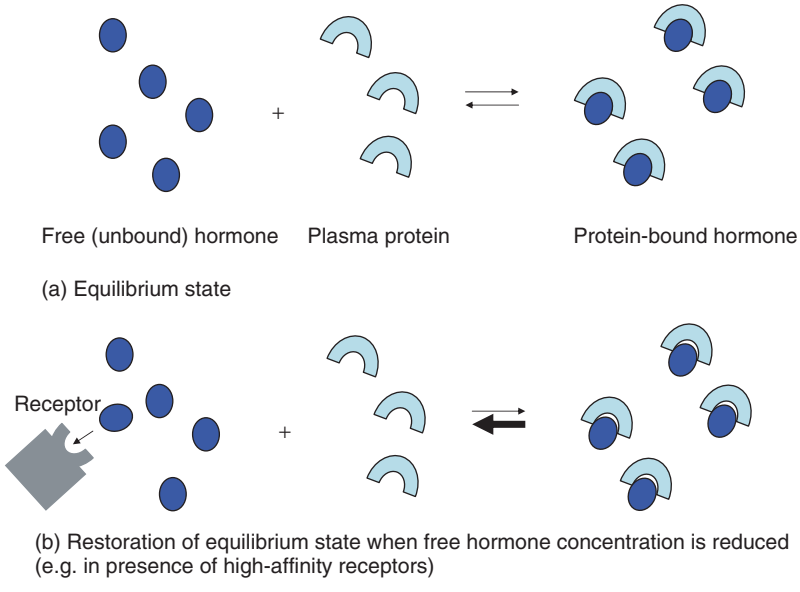

Figure 1.8 Diagram illustrating (a) the equilibrium state existing between the free and bound hormone concentrations and the concentration of relevant binding plasma proteins, and (b) the theoretical unloading of hormone from the bound component whenever there is a decrease in free hormone as exemplified by the presence of receptors with a greater affinity for the hormone than the plasma protein molecules.

but also the mineralocorticoid aldosterone, both being steroid hormones produced by the adrenal cortex.

Another point to bear in mind clinically is that plasma proteins are synthesised by the liver, and their synthesis can be influenced by specific hormones such as oestrogens, so conditions which affect liver function or the production of oestrogens can influence the protein-binding capacity of the blood.

Eicosanoids and gaseous hormones

These hormones tend to be lipophilic and are not stored to any significant extent either within their cells of production or elsewhere. They have short half-lives, so they are synthesised only on demand (i.e. on receipt of specific stimuli) and rapidly removed.

Release of hormones

The release of a hormone is yet another stage at which hormone production can be regulated. As will be already appreciated, different categories of hormone are either released from a pre-stored form or released on demand.

Protein and polypeptide hormones

Since protein and polypeptide hormones are generally stored in vesicles within the cells of their synthesis, their release requires that (i) the vesicles be moved to the outer plasma membrane, and (ii) the vesicle membranes fuse with that plasma membrane, allowing the vesicle contents to be expelled to the exterior of the cell. The latter process is called exocytosis.

Within each eukaryotic cell exists a dynamic structure called the cytoskeleton, which consists of a continually changing lattice-like framework of protein filaments which not only provide structural support to cell organelles but are also involved in various intracellular activities such as cell division and the movement of vesicles to the outer plasma membrane. The protein filaments which comprise this cytoskeletal network are actin filaments, intermediate filaments and microtubules. These filaments are held together and linked to the other intracellular structures by a variety of accessory proteins.

Actin is a globulin protein (G-actin) which can polymerise to a filamentous form (F-actin) known as a microfilament, in the presence of adenosine triphosphate (ATP). Each 8 nm long microfilament can link an intracellular protein to the plasma membrane. The actin filaments are particularly concentrated underneath the plasma membrane. Intermediate filaments, so called because their length is intermediate between the actin and microtubule at approximately 10 nm, consist of various proteins with specific characteristics, generally providing a supporting network within the cell. Microtubules are made up of the protein tubulin which is a dimer of α- and β-tubulin polypeptide molecules which bind to guanosine triphosphate (GTP). Each microtubule is a dynamic hollow structure made of polymers of tubulin, with a length of approximately 25 nm. Hydrolysis of the GTP bound to β-tubulin destabilises the microtubule allowing it to depolymerise, which accounts for the dynamic nature of its structure. Microtubules have various functions within the cell, including an involvement in regulating the movement of vesicles from within the cytoplasm towards the plasma membrane.

The movement of vesicles from the Golgi complex to the outer plasma membrane is sometimes called vesicle 'trafficking'. This process is believed to involve the movement of vesicles along microtubules towards the plasma membrane, with the process of exocytosis occurring only when the actin filaments below the membrane depolymerise, allowing the vesicles to penetrate the actin layer and reach the membrane (Figure 1.9). Various other molecules, such as recognition and anchoring proteins, play an important role in the intracellular transport process of vesicles. Clathrin is a surface protein on the vesicle which 'recognises' specific molecules to be

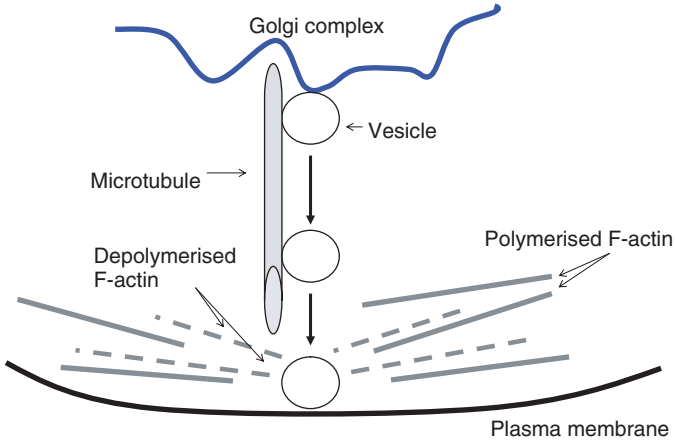

Figure 1.9 Diagram illustrating the current view that the vesicle containing hormone molecules is 'trafficked' down the microtubule towards the plasma membrane which is reached following the depolymerisation of the F-actin mesh lying underneath it.

transported within the vesicle, while other proteins are important for the docking of vesicles to the target membranes (e.g. the plasma membrane). These are called soluble NSF attachment protein receptors (SNAREs, where NSF is *N*-ethylmaleimide-sensitive fusion protein). Clearly, there are various signalling pathways which activate the various components of the intracellular vesicle-trafficking system within the cell. Our current knowledge of this process is still relatively rudimentary, but more details can be found in standard cell biology textbooks.

The actual process of exocytosis culminates in the fusion of the vesicle membrane with the plasma membrane resulting in expulsion of vesicle contents to the exterior of the cell. Exocytosis of hormones expressly results in the hormone molecules entering the bloodstream through the endothelial lining of the blood vessels (capillaries) vascularising the endocrine gland. This may be transcellular or through the gaps between the endothelial cells.

Amino acid–derived hormones

As indicated throughout this chapter, the hormones which fall within this category have similarities and differences with the protein and polypeptide hormones. Catecholamines are stored in vesicles within the cells of the adrenal medulla, so the secretory process is similar to the one described here. The synthesis, storage and release of the iodothyronines from the thyroid are all processes which differ somewhat from the standard, and will be discussed further in Chapter 14.

Steroids and other lipophilic hormones

These hormones, being lipophilic, are not stored in vesicles but are synthesised on demand. Their release into the bloodstream has generally been considered to be the consequence of the passive process of diffusion through the lipid component of the cell membrane. However, there is the possibility that at least some of these molecules may actually be secreted by a process involving membrane transporters, but at present this is generally speculative.

Transport of hormones in the circulation

It should already be apparent that a hormone, once released into the general circulation, can be transported free (i.e. unbound), be bound to a plasma protein or indeed be transported within a cell (e.g. an erythrocyte or leukocyte) or cell derivative (e.g. platelet) in the blood. It is generally believed that it is the free, unbound hormone which represents the bioactive fraction. The relationship between free and bound components has already been considered in section 'Steroid hormones', and while the importance of plasma proteins is particularly relevant for steroids and other small hormones, it should be noted that protein and polypeptide hormones can also be transported partly within the protein-bound component. Growth hormone, also known as somatotrophin (see Chapter 3), is one such hormone: there are a number of binding proteins for this hormone, with one at least being derived from the extracellular component of the growth hormone receptor found in target cell membranes.

Transport of hormones within the cells and cellular components of blood may also occur. For example, the gaseous hormone nitric oxide can interact with haemoglobin within erythrocytes, and it is feasible that preservation of the molecule within the red blood cell could allow it to be transported for release elsewhere in the circulation. Also, a significant fraction of the polypeptide hormone vasopressin in the circulation is transported within the platelets.

Hormone receptors

Once a hormone reaches its target cell, it must first of all recognise it (and be recognised), and then influence it in some way by a process called signal transduction. The first step is therefore the recognition process between hormone and target cell, and this requires the presence of some specific molecule associated with that target cell to which the hormone can bind. This molecule is known as the receptor. It can be located in the target cell's plasma membrane with an extracellular domain projecting outside

the membrane into the circulating medium, or it may be located within the cell, usually in the cytoplasm or nucleus. Hundreds of receptor proteins have so far been identified. In principle, a receptor should be specific for a given hormone molecule. In practice, the receptor usually is capable of actually binding to other chemically similar molecules, or ligands, in much the same way as a 'specific' globulin in the blood can actually bind more than one chemically similar hormone (see section 'Steroid hormones').

There are five specific points to appreciate about hormones and their receptors:

1. Any one hormone may actually have a number of different receptors to which it can bind. The distribution of those subtypes of receptors can be tissue specific, allowing that hormone to have varying effects on different target cells and tissues.
2. The converse is also true: that any receptor is not necessarily specific for only one hormone, but can bind other ligands with differing affinities.
3. Receptor subtypes are likely to be linked to different intracellular second messenger pathways which, when activated, can produce varied responses in different target cells.
4. A very important concept in endocrinology is that a hormone's effect in a target tissue can be influenced not only by the concentration of bioactive hormone in the close vicinity of the target cell, but also by the presence of varying numbers of receptors. Thus an important feature of endocrinology will be the relationship between hormone concentration and receptor number. It also becomes apparent that a clinical disorder arising from the loss of a hormone's activity can be caused not only by a deficiency in its production but also by a deficiency (or abnormality) of its receptors.
5. If a hormone remained bound to its receptor, then that particular stimulated intracellular pathway could remain active indefinitely. It is clearly important to have cellular regulatory mechanisms which disrupt the hormone receptor interaction and inactivate the hormone (see the following section).

Protein and polypeptide (and certain other) hormone receptors

Not surprisingly, protein and polypeptide hormones being lipophobic are unlikely to penetrate target cell membranes, certainly without the assistance of specific transport systems to get them across. Consequently, it is not surprising to find that these hormones generally have their receptors embedded in the plasma membranes of their target cells. Some eicosanoids such as prostaglandins also have cell surface receptors.

A typical receptor of this type would be a transmembrane protein having an extracellular domain to which the hormone molecule can attach, a

section of the receptor spanning the membrane and finally an intracellular domain which would be associated with a specific transduction process. One large, ubiquitous family of such receptors is the seven-transmembrane receptor group, so named because all the receptors in this group have seven membrane-spanning helices connecting the extracellular and intracellular domains. The intracellular domain is connected to a specific intracellular guanine nucleotide-binding protein, called a G protein, which is the initial part of the intracellular transducing mechanism activating the cell in a specific fashion. These receptors are therefore called G protein–coupled receptors (GPCRs). It is clear that these GPCRs have complex regulatory mechanisms, and at least 30 intracellular molecules which can influence them have so far been identified, called regulators of G protein signalling (RGSs). Furthermore, other novel intracellular molecules which can activate the G proteins have been identified, and they are called activators of G protein signalling (AGS). Clearly, the intricate intracellular regulation of the G proteins adds a whole new dimension to the cellular transduction of the initial hormone-induced process.

Some membrane receptors may have a single strand spanning the membrane, with an extracellular domain with hormone-binding capability and an intracellular domain having intrinsic enzyme activity when activated. Such receptors are identified by the nature of the enzymes on their intracellular domains (e.g. tyrosine kinase receptors). A variant of this type of receptor would be the homodimer which is essentially a double single-spanning receptor (see Figure 1.10). There is also evidence to suggest that heterodimers can exist also, where two different receptors can link together. These receptors do not have to be single-spanning membrane receptors but can be GPCRs. This suggests some intriguing possibilities, particularly when each receptor is linked to different intracellular second messenger systems. (The molecules which transduce the hormone's response within the cell are called the 'second messengers'.)

When the hormone, or other ligand, binds to the extracellular domain the receptor conformation changes, resulting in activation of the enzyme associated with the intracellular domain or G protein. Activation of this intracellular enzyme is associated with the transduction of the hormone's 'message' to the interior of the target cell.

Hormone receptor removal

The binding of the hormone to its 'recognition' site leads to the desensitisation of the receptor in response to a further hormonal challenge by a series of mechanisms that include a mere decrease in its affinity for the agonist, an internalisation with or without recycling to the membrane, an increased proteolytic degradation or ultimately a blockade of its synthesis at a pre- or post-transcriptional level. Internalisation refers to the loss of

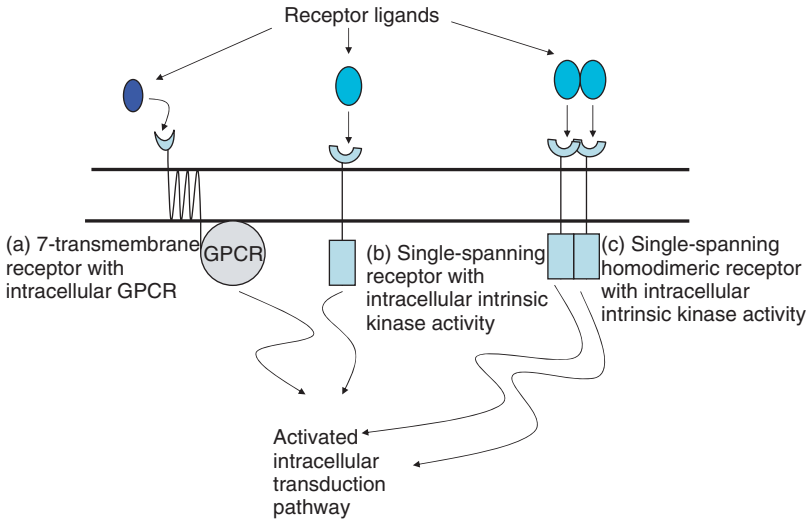

Figure 1.10 Diagram illustrating types of membrane-spanning receptors: (a) the seven-transmembrane receptor with the extracellular domain to which the ligand (e.g. hormone) binds, the seven transmembrane helices and the intracellular domain linked to the G protein–coupled receptor (GPCR); (b) the single membrane-spanning receptor with the extracellular ligand-binding domain and the intracellular domain with intrinsic enzyme (kinase) activity and (c) the same as for (b) but in a homodimeric form.

membrane receptors and results from the combined effects of receptor uptake by endocytosis, and recycling.

As mentioned earlier, much of the magnitude of a hormone's action is due to the concentration of specific receptors in its target cells. The down-regulation of receptors is one mechanism by which the prolonged effect of a specific hormone is reduced. It can be a global phenomenon, where the ligand-induced internalisation process is compounded with irreversible sequestration and intracellular proteolysis. The mechanisms responsible for these events involve two main families of proteins: the G protein–coupled receptor kinases (GRKs), and the arrestins. After binding the hormone, the receptor undergoes a conformational change and binds to a GRK which then phosphorylates it on serine or threonine residues located on the intracellular loops or on the C-terminal tail. This phosphorylation diminishes the receptor's affinity for the ligand and mainly leads to its subsequent binding to an arrestin molecule. This physically prevents coupling of the receptor to its G protein and therefore to the transduction of the signal. Phosphorylation of the receptor can also be caused by kinases other than the GRKs, such as protein kinases A and C (PKA and PKC, respectively), thus sustaining the desensitisation process. In other cases, the tightly bound arrestin dissociates from the receptor and accompanies it

into the cell where the complex stays for extended periods of time within endosomal vesicles before being directed to lysosomes or being recycled.

The reuptake of receptors is by the process of endocytosis, mediated by various proteins including clathrin. Clathrin can be recruited to membranes by a variety of proteins. One of these, AP180 (an adaptor protein), is a very efficient recruitment protein that binds to phosphoinositol 4,5 bisphosphate (PIP2) in the plasma membrane. It also induces the polymerisation of clathrin into a lattice within the cytoplasm in the region of the cell membrane. Clathrin plays a key role in the formation of vesicles for internalisation, along with other proteins. The arrestins target the areas of the cell membrane where clathrin is located, called clathrin-coated pits. The arrestins can behave as adaptor proteins facilitating the recruitment of receptors to the plasma membrane domains where the clathrin-coated pits develop, prior to the endocytosis process. Arrestins play this role not by binding to clathrin itself but by interacting with other endocytic elements, including the adaptor protein AP2 (Figure 1.11).

Steroid hormone receptors

Receptors for steroids are also proteins, but because these lipophilic hormones (and a few others) can enter their target cells with ease, their receptors are found intracellularly, most commonly in the cytoplasm as well as in the nucleus. As with protein and polypeptide hormones, while each receptor may recognise specific molecules, they are not usually

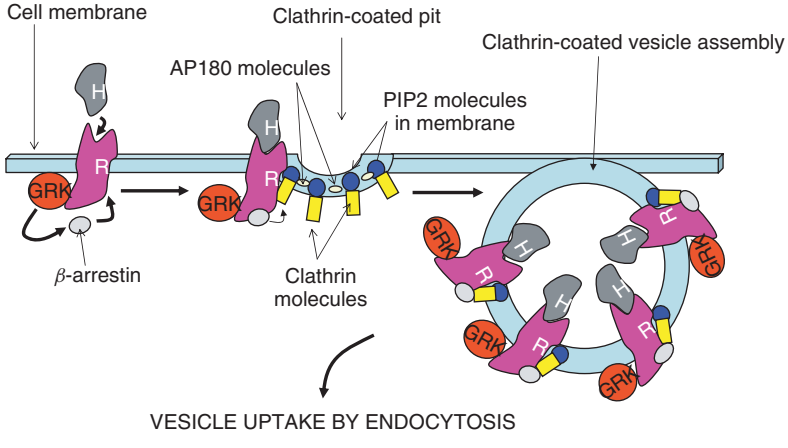

Figure 1.11 Diagram illustrating the uptake of a hormone receptor complex by endocytosis. This process is initiated when the hormone molecule first binds to its receptor in the cell membrane. It elicits the transduction of the hormonal effect via induction of its second messenger system and, at the same time, the GRK associated with the receptor is activated. See section 'Hormone receptor removal' for further details.

totally specific for the one hormone and can bind other chemically similar molecules with varying affinities. Once bound to their receptors, these hormone receptor complexes can influence the target cell genome to induce changes in protein synthesis. These new proteins, often enzymes, then mediate the actions of the hormone within the target cells (see the following section). Furthermore, there is some evidence (mainly indirect) suggesting that some steroids may also exert rapid non-genomic effects, perhaps through cell membrane receptors. This remains a controversial issue and an area of much research interest.

Mechanisms of action

Once a hormone has bound to its receptor on a target cell, the 'message' of that hormone molecule needs to be somehow passed on within the target cell so that it can respond in such a way that the body's general homeostasis is ultimately restored. The process by which that message is passed on to the target cell is known as transduction. One important feature of this process is that one hormone molecule, by binding to its receptor, can 'switch on' that cell in such a way that the message is multiplied intracellularly by the production of a whole cascade of molecules. Thus the signal provided by any one hormone molecule binding to its receptor can be amplified many times. Another feature, discussed in a previous section, is the establishment of an internal regulatory process whereby hormone receptor activation can be terminated.

Protein and polypeptide hormones

As explained elsewhere, the hormone molecules released into the bloodstream act as (first) messengers carrying information from one part of the body to another in order to induce a specific response. In general, once the hormone binds to its receptor, it can instigate the activation of specific pathways involving other intracellular (second) messenger molecules. For proteins and polypeptides, and other hormones which bind to the extracellular binding sites of their receptors in the plasma membranes of target cells, there are various second messengers which can be produced, each one associated with one or more specific intracellular pathways. These pathways are usually cascades of activated enzymes, in a sequence culminating in the production of the appropriate response to the initial stimulus, which can vary from the opening of an ion channel to the synthesis of a new protein molecule.

The mechanisms of action are related in part to the types of membrane receptor to which the hormones bind. The two main receptor types relevant to most hormones are the enzyme-coupled receptors and the more numerous G protein–coupled receptors.

Mechanisms linked to ligand-gated channel receptors

Some receptors are part of the chemical structure of ion channels in the plasma membrane. They are often referred to as ligand-gated channels to distinguish them from other ion channels such as voltage-gated and stretch-activated channels. The best known examples are neurotransmitters such as acetylcholine and gamma aminobutyric acid (GABA). When the messenger molecule binds to the extracellular receptor portion of the ion channel, it can open to specific ions, allowing them to flow down electrochemical gradients across the cell membrane.

Mechanisms linked to enzyme-coupled receptors

There may be ligand-linked receptors in the cell membrane which combine target cell recognition to a specific, and direct, response. For example, some transmembrane receptors have an intracellular enzyme as part of their structure, so that when the hormone binds to such a receptor, then the intracellular enzyme component is activated. Examples would include receptor tyrosine kinases, receptor serine and threonine kinases and receptor guanyl cyclases.

Once activated by the binding of ligand to an extracellular binding site on the transmembrane receptor, the intracellular enzyme component becomes capable of activating specific amino acids in associated proteins, by phosphorylating them. Some of these receptors are single-strand transmembrane proteins, while others may be paired to form homo- (or hetero-) dimers with adjacent receptor molecules. For example, growth factors activate tyrosine kinase–coupled receptors (TKCR) resulting in adjacent identical receptors forming homodimers. Consequently the activated tyrosine kinases phosphorylate tyrosyl residues in the intracellular (tail) components of the TKCRs and can result in (i) autophosphorylation, prolonging and magnifying the activity associated with the activation of the receptors, and/or (ii) activation of tyrosyl residues in other, intracellular, proteins (Figure 1.12).

There are many intracellular proteins which can be activated by phosphorylation. The result is that the activation of different intracellular ‘second messenger’ pathways involving various intracellular proteins can occur, culminating in more than one biological response to the original hormonal signal. Furthermore, the activation of a single receptor kinase can result in the phosphorylation of a stream of intracellular proteins, providing an internal amplification of the initial hormonal stimulus.

Hormones such as insulin and various growth factors bind to tyrosine kinase receptors of this type.

Mechanisms linked to G protein–coupled receptors (GPCR)

This is a very large group of transmembrane receptors with a common general structure, comprising an extracellular ligand-binding domain, seven

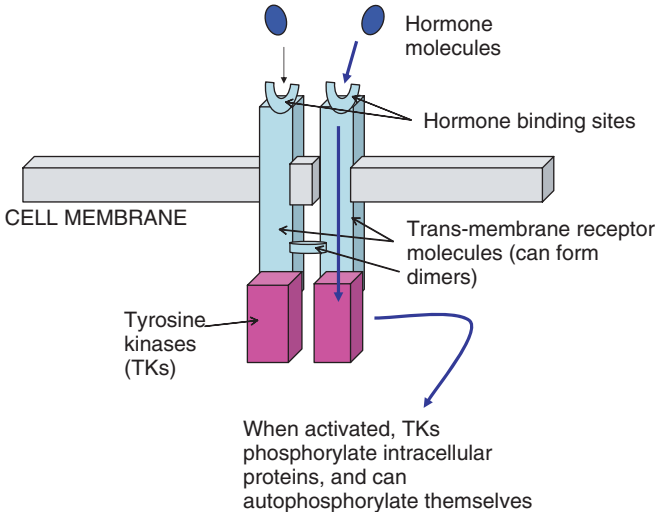

Figure 1.12 Diagram illustrating a membrane tyrosine kinase receptor, here shown as a homodimer. Binding of the hormone to the extracellular domain activates the receptor proper, resulting in the activation of tyrosine kinases present in the intracellular component. The activation of these tyrosine kinases can result in autophosphorylation of the receptor itself, or the phosphorylation of nearby receptors. However, the main effect will be to phosphorylate intracellular tyrosyl-containing proteins which can then act as intracellular second messengers, transducing the initial hormonal signal ultimately into the cellular biological response.

intramembrane loops and an intracellular enzyme-linked region which is activated when the ligand (hormone) binds to the extracellular domain. Many hormones bind to receptors belonging to this group, including adrenaline, glucagon, vasopressin and parathormone.

The intracellular domain of a GPCR is indeed a guanine nucleotide protein which is comprised of a triad of subunits called α, β and γ. Both α and γ subunits are linked to the cell membrane. Initially, the unstimulated Gα unit is inhibited by the Gβγ subunits, and is linked to a guanosine diphosphate (GDP) molecule. When a hormone molecule binds to the extracellular domain of its receptor, the subsequent conformational change of the receptor activates the Gα subunit which then preferentially binds to intracellular guanosine triphosphate (GTP) instead of GDP. Binding of GTP to Gα overcomes the inhibitory influence of the Gβγ subunits which then dissociate from it. The Gα–GTP activates a specific intracellular enzyme, which then initiates formation of a second messenger. The intracellular second messenger can then, in turn, induce a cascade of intracellular steps which culminate in the production of the appropriate cellular response to the initial stimulus. The α subunit has GTPase activity, so it then hydrolyses GTP back to GDP restoring the G protein to its initial state. There are various

Gα subunits: for example, one (Gαs) can stimulate a membrane-bound enzyme, adenyl cyclase, while another (Gαi) can inhibit it.

Adenyl cyclase is an enzyme which is part of one of the principal intracellular second messenger systems in cells. When activated, this enzyme catalyses the conversion of adenosine triphosphate (ATP) to the intracellular second messenger cyclic adenosine monophosphate (cAMP) with the release of two phosphates. There are 10 known isomers of adenyl cyclase, each one having distinguishing properties which identify specific cAMP-activated pathways. An important intracellular protein which is activated by cAMP is PKA which then phosphorylates other intracellular proteins.

Another membrane enzyme which, when activated, catalyses the formation of another important intracellular second messenger system is phospholipase C which is located in the plasma cell membrane. When stimulated, it catalyses the breakdown of a membrane phospholipid called phosphatidylinositol-4,5-bisphosphate (PIP2) into two components, both of which act as intracellular second messengers: inositol triphosphate (IP3) and diacylglycerol (DAG). These second messengers, in turn, switch on cascades of further intracellular events. IP3 readily diffuses into the cytosol and, through its binding to calcium channels on the endoplasmic reticulum, allows the release of calcium from this intracellular storage site into the cytoplasm. DAG, in the presence of calcium, directly activates PKC which then phosphorylates a series of protein substrates which may, or may not, differ from those of PKA. DAG can also be cleaved into arachidonic acid, the precursor for prostaglandin synthesis (see Figure 1.13).

One important intracellular event which can stimulate various subsequent reactions within the cell involves second messenger–induced mechanisms which raise the intracellular free calcium ion concentration. Calcium ions play an important role in triggering various intracellular chemical reactions, so regulation of their intracellular (cytoplasmic) concentration is often an important component in controlling a cell's response to the initial stimulus. The cytoplasmic free calcium ion concentration can be regulated through two different mechanisms: by controlling (i) calcium ion channels in plasma cell membranes, which link the intracellular compartment to the exterior of the cell, and (ii) calcium ion channels in the membranes of internal organelles, such as the endoplasmic reticulum, the sarcoplasmic reticulum (in muscle) and microsomes, which act as intracellular calcium stores. Some hormones can directly influence calcium ion channels located in the plasma membrane (see ligand-gated receptors in earlier section), while others do so by initiating the formation of second messenger transduction pathways which then influence calcium channels either in the plasma cell membrane or in the membranes of intracellular organelles (see Figure 1.13). The intracellular calcium ion concentration

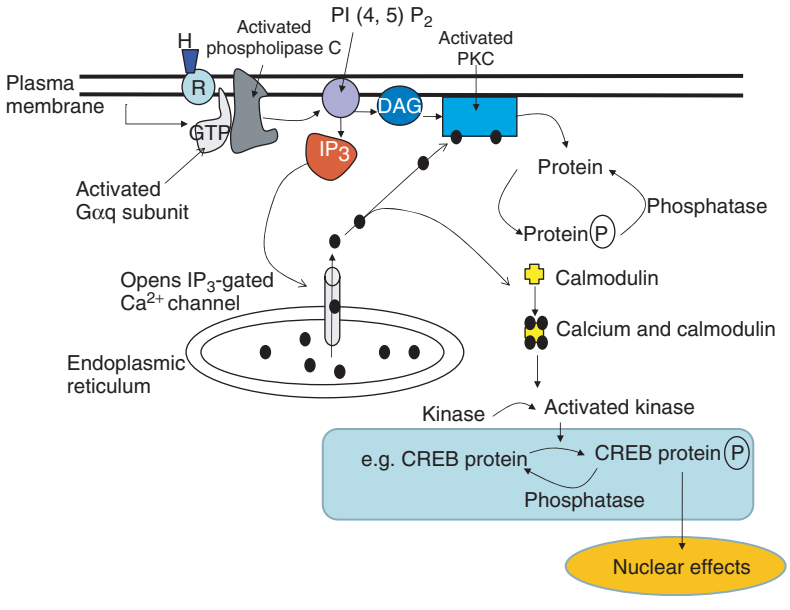

Figure 1.13 Diagram illustrating how a hormone (H) binding to the extracellular domain of its transmembrane receptor (R) can activate the α subunit of the associated G protein, resulting in the activation of the membrane-located enzyme phospholipase C. This enzyme then catalyses the formation of two different second messengers, diacylglycerol (DAG) and inositol triphosphate (IP3), which in turn switch on cascades of intracellular events which can include the release of calcium ions from intracellular stores such as the endoplasmic reticulum, or the activation of protein kinases such as protein kinase C (PKC). Longer term nuclear effects can also be induced by the activation of cyclic adenosine monophosphate (cAMP) response element binding protein (CREB).

is considerably lower (10 000-fold) than its extracellular concentration (approximately 1.15 mM of free ions), so the opening of cell membrane calcium channels results in an inward movement of these ions into the cytoplasm, temporarily increasing its intracellular concentration. Likewise, the opening of calcium channels in intracellular organelle membranes such as in the sarcoplasmic or endoplasmic reticulum will also result in the movement of these ions from the intracellular storage site (with their higher concentration) to the cytoplasm. The concentration of free cytoplasmic calcium ions is normally very low (approximately 0.1μM), so any increase in its concentration or in the frequency of its oscillations can have a marked signalling impact. In particular, calcium can bind to calmodulin and activate the calcium- and calmodulin-dependent kinases which interestingly can also phosphorylate cAMP response element binding protein (CREB) which acts as a DNA transcription factor (see Figure 1.13).

Thus, a ligand (e.g. hormone) can bind to its GPCR and influence cytoplasmic events such as the direct regulation of different enzymes and/or the intracellular free calcium ion concentration, and these actions will tend to produce rapid effects within seconds to minutes. However, that same ligand could also influence longer term (hours or days) events such as the synthesis of new proteins, these being either new intracellular enzymes or peptides and proteins for export from the cell. As can be seen from Figure 1.14, cAMP molecules cause the dissociation of inhibitory regulatory units attached to PKA, thus activating the PKA molecules which then traverse the nuclear membrane through nuclear pores (Figure 1.14).

Steroid hormones

The intracellular hormone receptor complex acts as a transcription factor, binding to a location along the target chromosome acting as the DNA-binding site known as the promoter, which is likely to be a protein such as a histone associated with the DNA. Indeed, it is known that the beaded appearance of the chromosome is due to the presence of nucleosomes

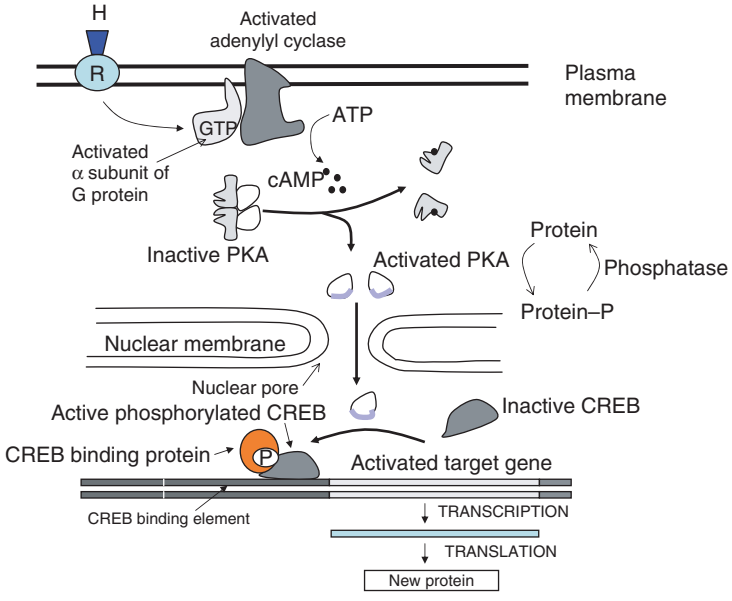

Figure 1.14 Diagram illustrating how a hormone (H) can bind to the extracellular domain of its transmembrane receptor (R) and activate a second messenger pathway such as is indicated here, involving cyclic adenosine monophosphate (cAMP). This second messenger, in turn, activates a protein kinase (here, PKA) which can traverse the nuclear membrane of the cell where it phosphorylates a cAMP response element binding protein (CREB). This molecule then acts as a transcription factor, binding to a CREB binding element (site) on the target gene and switching it on or off.

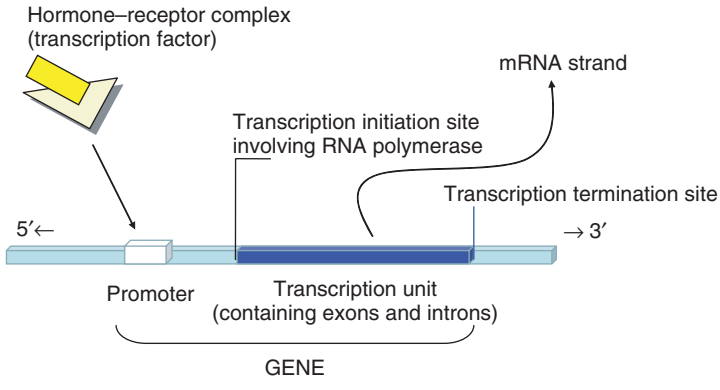

Figure 1.15 Diagram illustrating how the hormone receptor complex, acting as a transcription factor, influences the transcription of a gene's DNA into the complementary mRNA strand which then gets translated into a new protein.

which are tightly wound regions of DNA around a variety of proteins such as histones. The actual transcription unit, which is the DNA segment which carries the genetic information which will be transcribed into the mRNA for the end product protein, consists of introns and exons of which only the exons are transcribed (see section 'Polypeptide and protein hormones'). There will also be the promoter (or regulatory) region located up-stream to the transcription unit in the 5′ direction of the DNA strand, to which the transcription factor (in this case the hormone receptor complex) will bind, and a specific transcription termination site which determines where that particular gene transcription ends (see Figure 1.15).

As mentioned earlier, as with all protein synthesis the transcribed mRNA will ultimately be translated into the new protein which then mediates the cellular response to the hormone. It is also notable that lipophobic hormones such as proteins and polypeptides can have indirect genomic effects (as discussed earlier).

Feedback control

If a released hormone is allowed to act in an uncontrolled manner, then the effect that is generated can be prolonged to a point when that effect becomes disadvantageous to the organism. Indeed, some disorders are actually caused by control systems malfunctioning, an obvious example being tumours which can be defined as abnormal (i.e. uncontrolled) growths of cells, where the abnormality is usually genetic.

Not surprisingly, therefore, control systems exist in order to regulate endocrine function and thus maintain homeostasis. When the integrated

signal to an endocrine cell is such that more hormone is required by the body, then that hormone is released in increased amounts (and maybe synthesised for longer term use) until the necessary action of that hormone has reduced the necessity for further increased production of it as indicated by a reduction in stimulus strength, upon which the endocrine cell returns to its basal activity state. This control mechanism is called negative feedback.

Negative feedback

Direct negative feedback

By far the most common form of feedback regulating an endocrine cell is direct, relating the endocrine cell to the function being controlled. An example of such a direct negative feedback is provided by the regulation of insulin and a function it controls, the blood glucose concentration (Figure 1.16).

When the internal environment is disturbed in a particular manner, that disturbance acts as a stimulus which activates an endocrine cell to release its hormone into the general circulation. The hormone will be transported to its target tissues where it will exert its physiological effect in order to reduce that stimulus and restore the body's internal environment to its undisturbed state. Using the β-cells of the pancreatic islets of Langerhans as an example of an endocrine gland, imagine that the blood glucose

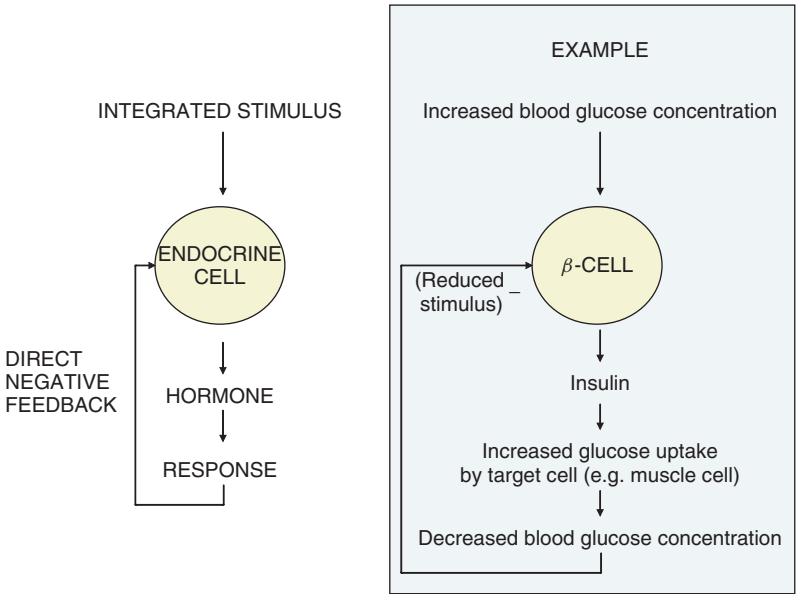

Figure 1.16 Diagram illustrating (left) the general principle of a direct negative feedback loop, and (right) an example provided by the hormone insulin.

concentration rises (e.g. following a meal). The internal environment is disturbed, so that the increased blood glucose concentration acts as a powerful stimulus on the β-cells and causes them to release their hormone, insulin. Insulin is then transported by the general circulation to its target cells (e.g. in muscle) where it stimulates the uptake of glucose. The stimulus acting on the β-cells is consequently reduced, the β-cells are no longer stimulated, insulin release is reduced and the internal environment is restored to its initial undisturbed state. Thus the 'response' to the hormone has exerted a direct negative feedback on the hormone's site of production, the β-cell. See Figure 1.16 and also Chapter 15 for further details.

Interestingly, some endocrine glands when stimulated exert their effects on target cells which may themselves also be endocrine cells. In this case, the production of hormone *b* from endocrine gland *B* acts on endocrine gland *C* to produce hormone *c*. In this case hormone *c* can exert a direct negative feedback on gland *B* (see Figure 1.17). The best examples of this situation are provided by hormones produced by cells of the anterior pituitary gland. Corticotrophin (hormone *b*) is produced by the anterior pituitary (endocrine gland *B*). It acts on the adrenal cortex (endocrine gland *C*) to produce cortisol (hormone *c*). Cortisol exerts a direct negative feedback on the anterior pituitary's production of corticotrophin (also known as ACTH; see Chapter 3).

Indirect negative feedback

The anterior pituitary gland provides us with another level of complexity in terms of negative feedback. In addition to the direct negative feedback loops shown in Figure 1.17, between the anterior pituitary (endocrine gland *B*) and other endocrine glands (e.g. adrenal cortex, C), indirect negative feedback loops can also be present. This is because the anterior pituitary is also influenced by the CNS, via a particular region of the brain called the hypothalamus. While this is described more fully elsewhere (see Chapters 2 and 3), we can stick with our example relating the anterior pituitary to the adrenal cortex in order to demonstrate indirect negative feedback involving the CNS. As will be discussed in Chapter 2, the hypothalamus, which is made up of nerve cells, or neurones, actually has an endocrine function in addition to its other roles. Various groups of neurones, called hypothalamic nuclei, send their axons down towards the pituitary gland which is attached to the base of the hypothalamus, and their neurosecretions are actually released into a special vascular system which links that region of the hypothalamus to the anterior lobe of the pituitary. Thus, the hypothalamus can in this regard be considered as another endocrine gland in its own right.

In this case, there will be not only the direct negative feedback loop operating between endocrine gland *C* (in the example, the adrenal cortex

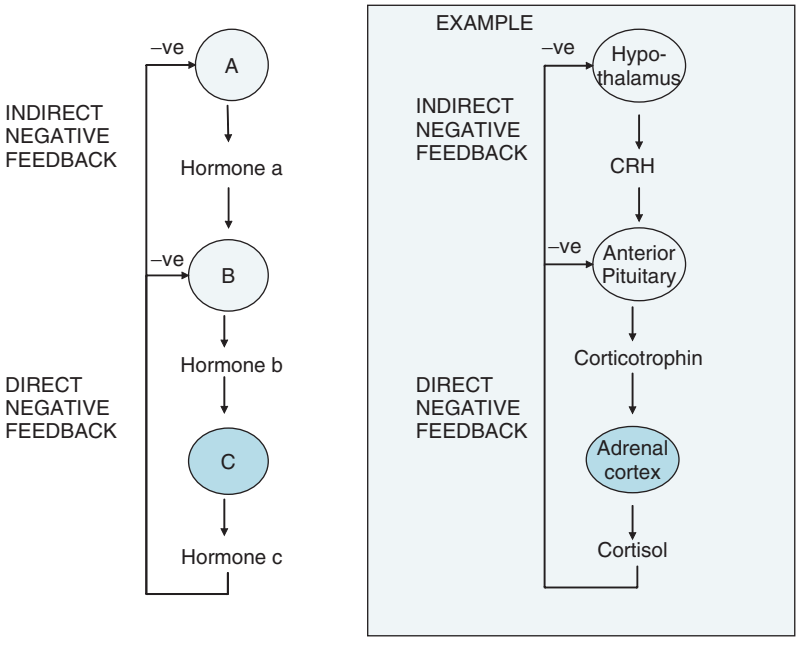

Figure 1.17 Diagram illustrating (left) the general principle of a direct negative feedback loop involving two endocrine glands (*C* and *B*) together with their hormones (*c* and *b*), and (right) an example provided by the adrenal cortex and the anterior pituitary and their hormones (cortisol and corticotrophin, respectively).

producing cortisol) and endocrine gland *B* (the anterior pituitary, influencing the production of corticotrophin) but also an indirect negative feedback loop operating back on endocrine gland *A* (the hypothalamus, influencing the production of corticotrophin-releasing hormone, or CRH). Other examples of direct and indirect negative feedback pathways include the regulation of the iodothyronines from the thyroid (Chapter 13), and the gonadal steroids from the testes or ovaries in males and females respectively (see Chapters 6 and 7). The indirect negative feedback in these cases links the peripheral endocrine gland (*C*) to the CNS (hypothalamus, *A*).

Short (or auto) negative feedback

When one examines the negative feedback loops which operate for various endocrine systems involving the hypothalamo-pituitary axis as indicated in Figure 1.17, it becomes apparent that the production of the anterior pituitary hormones is at least partly in response to the drive provided by specific hypothalamic hormones. Thus it would be quite feasible for the anterior pituitary hormones to influence their own production by feedback loops to the hypothalamic level. Indeed, not surprisingly, there

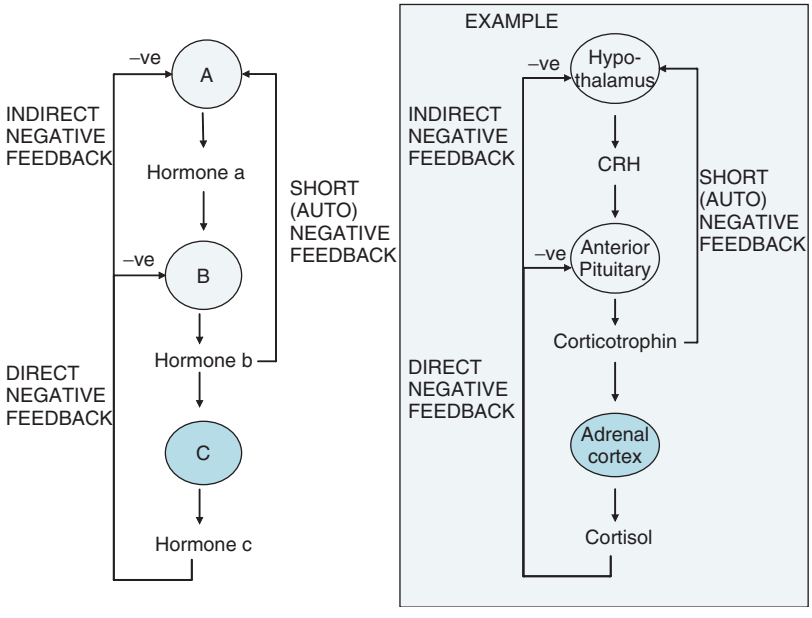

Figure 1.18 Diagram illustrating (left) the general principle of direct, indirect and short (or auto) negative feedback loops involving three endocrine glands (*A*, *B* and *C*) together with their hormones (*a*, *b* and *c*), and (right) an example provided by the hypothalamus, the anterior pituitary gland and the adrenal cortex and their hormones (corticotrophin-releasing hormone (CRH), corticotrophin and cortisol, respectively).

is plenty of evidence in support of this form of feedback control, which is called short (or auto) negative feedback (see Figure 1.18).

In the example given in Figure 1.18, the hypothalamus receives an indirect negative feedback from circulating cortisol and a short (auto) negative feedback from corticotrophin, while the anterior pituitary simply receives a direct negative feedback influence from cortisol. In fact, the situation relating the hormones from the hypothalamo-pituitary-adrenal axis described here is more complex than this, with other hormones and factors also exerting regulatory influences upon it. The point to appreciate is that the control exerted on any endocrine system is multifactorial, and can be at a number of different levels as described here. This allows for a quite sensitive, specific and precise degree of control over these important physiological regulatory systems.

Positive feedback

While it may seem counter-intuitive to learn that positive feedback loops do occur in nature, perhaps we should not be too surprised at their existence. Certainly, under general circumstances positive feedback

per se would lead ultimately to chaos and dysfunction because of the loss of control over a physiological system. However, when different signals influence that system, then a positive feedback loop can arise but only under certain specific conditions. When those conditions are altered, then the positive feedback is no longer capable of being maintained and the initial status quo is restored. Various positive feedback effects are known to exist in nature, and in mammalian physiology there are various examples, which can be either between different parts of the body (different cells) or within cells. In all cases, under normal conditions, the induced change in environment is such that conditions allowing the positive feedback to develop in the first place are ultimately cancelled, and the effect is lost.

The best example of a positive feedback influence in endocrinology is the one which develops during the female menstrual cycle between a steroid hormone from the ovaries called oestradiol (more precisely,

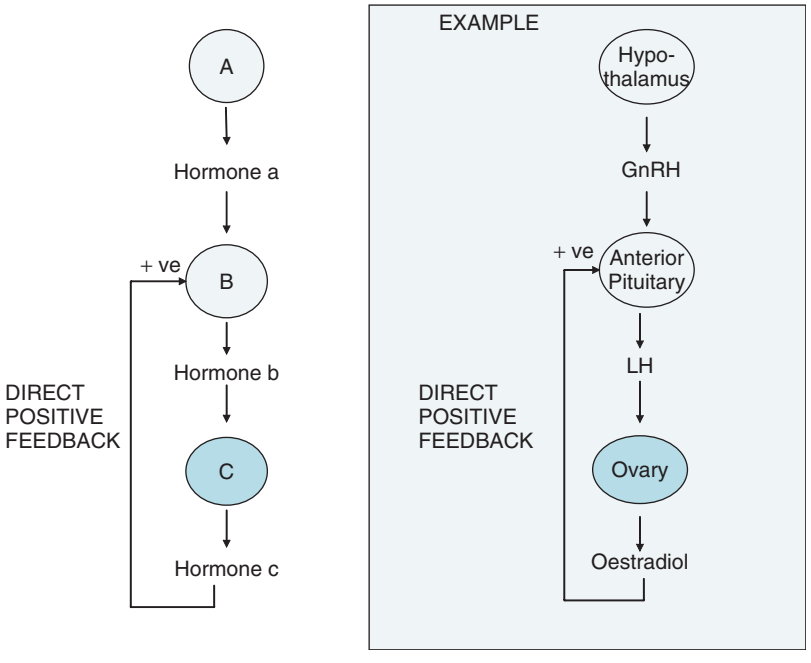

Figure 1.19 Diagram illustrating (left) the principle of positive feedback between two endocrine glands (*C* on *B*) by which hormone *c* sensitises endocrine gland *B* to the effect of stimulatory hormone *a* from endocrine gland *A*, and (right) the example of positive feedback by ovarian oestradiol on the anterior pituitary. The oestradiol effect is one of sensitising the anterior pituitary cells which produce luteinising hormone (LH) to the stimulatory effect of gonadotrophin-releasing hormone (GnRH) from the hypothalamus. The effect of the positive feedback is to stimulate a surge in LH which promotes the process of ovulation. The endocrine background is altered, the conditions for positive feedback are lost and the hypothalamo-pituitary-ovarian axis returns to its 'normal', or basal, state once again by negative feedback.

17β-oestradiol), and the hypothalamo-pituitary axis which plays a vital role in regulating the release of a ripe egg, or ovum, a process called ovulation (see Figure 1.19). For a more detailed discussion of ovulation, see Chapter 7.

Internal feedback

In addition to the control loops linking 'effect' to hormone via the general circulation, there is considerable regulation within the cells of an endocrine gland. We have already seen how the endocrine cell itself acts as an integrator of probably many different signals arriving at that cell at any given moment. This implies considerable intracellular regulation at various levels, including synthesis, storage, removal or release of the hormone. Furthermore, the target cell has many ways of regulating its response to the hormone, including receptor synthesis, recycling or removal, as well as the intracellular control of the activated second messenger systems.

Summary

1. Endocrinology is the study of endocrine glands which are composed of specialised cells synthesising hormones, and releasing them into the general circulation which transports them to their target cells.
2. Hormones are important not only because of their communication role regulating metabolic and other activities in response to perturbations to the body's homeostasis, but also because it is now appreciated that they are integrated with other 'systems' in the body such as the nervous and immune systems.
3. Hormones can be generally classified according to their chemical structures. For example, the two main groups (proteins and polypeptides as well as steroids) differ in their lipid solubilities making them either lipophilic or lipophobic, and this can define processes such as their synthesis, storage, release, receptor site and mechanism of action.
4. Hormones bind to receptor molecules in their target cells and induce actions either directly on cellular processes, or indirectly via the mediation of intracellular second messenger systems.
5. Simple and complex mechanisms exist to closely regulate each endocrine system, relating hormone production to the hormone-induced effect, in order to maintain homeostasis.

Conclusion

The field of endocrinology has widened considerably since the concept was first proposed, and the definitions are no longer as clear-cut as they

once were. Indeed, the study of the various endocrine systems, and the roles they play, is now an essential and integral component in our current understanding of how the body functions.

Reference

Palmer, R.M., Ferrige, A.G. & Moncada, S. (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, 327 (6122), 524–6.