Integrated Endocrinology
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John Laycock and Karim Meeran
Both of Imperial College London
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Preface

Both of us have always been inspired by the interest shown by our students in the subject of endocrinology, and we are grateful to them for enhancing our desire to produce a readable textbook covering the main aspects of the subject. Because the understanding of endocrinology is enhanced by a consideration of the clinical conditions associated with diminished or excessive production of individual hormones, we hope that the integration we have attempted between basic and clinical aspects is successful. In addition we are grateful to our wives and families, who have given us their continual support, and to our colleagues, in particular Waljit Dhillon and Gareth Leng who gave us many suggestions for improvement in those chapters which they read for us.

John Laycock and Karim Meeran
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
apparently restored the cock’s comb. Likewise, the appropriate administra-
tion 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 physi-
ological 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 tis-
sue. 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).
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.

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

<table>
<thead>
<tr>
<th></th>
<th>Neurotransmitters</th>
<th>Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission</td>
<td>Across synaptic cleft</td>
<td>By blood (or other fluid)</td>
</tr>
<tr>
<td>Target cells</td>
<td>Direct to specific neurones or other cells</td>
<td>Can be some distance from source cells</td>
</tr>
<tr>
<td>Speed of action</td>
<td>Milliseconds</td>
<td>From seconds up to days</td>
</tr>
</tbody>
</table>

**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
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
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
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 A₂ and is then acted upon by other enzymes called cyclooxygenases (COX1 and COX2) to form intermediate prostaglandins PGG₂ and the unstable intermediate PGH₂. These prostaglandins are rapidly converted to other prostaglandins of the series by specific prostaglandin synthases, forming PGE₂, PGF₂α and so on (Figure 1.6). PGH₂ is also the precursor for prostacyclin (PGI₂) 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 eicosapentanenoic 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.
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:

\[ \text{[free hormone H]} + \text{[plasma protein P]} \leftrightarrow \text{[protein-bound hormone HP]} \]

At equilibrium, the equation reaction can be expressed as:

\[ K_a = \frac{[\text{HP}]}{[\text{H}][\text{P}]} \]

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