1 Endocrine Oncology and Therapeutic Options

1 Structure and Development of the Endocrine System

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Key points

- Endocrine gland normal gross and microscopic structure is well known.
- Peptides and amines are released by exocytosis; steroids are now known to be transported out of cells.
- Mechanisms involved in "constitutive" secretion of hormones remain poorly understood; in tumors, both "regulated" and "constitutive" secretion can occur.
- Understanding of molecular aspects of the development of endocrine tissues has increased dramatically recently. Mutations in the molecular signals identified in developing animal models have, in many cases (e.g., pituitary development), been shown to be associated with the expected human endocrine developmental anomalies, showing that these signalling mechanisms are well conserved in mammals.
- Increased understanding of these molecular aspects could help elucidate the role of dysdifferentiation and stem cells in endocrine tumors. It is possible, for example, that the molecular signal(s) which normally down-regulate(s) gastrin production in the pancreas are themselves switched off when gastrinomas develop.

Introduction

Concepts of what comprises the endocrine system are rapidly expanding. Classically thought of as the discrete endocrine glands and their products, the concept has broadened to include the diffuse endocrine systems of the gut, respiratory tract, heart and endothelium. The term "cytokines" originally covered the products of the immune system but it is now clear that they are produced more widely; similarly, growth factors are chemical signals which are produced by many tissues and generally have local actions, although liver insulin-like growth factor may have much wider effects. Therefore, it is now clear that most if not all tissues produce signal molecules that act over longer or shorter distances. To the original concept of an endocrine action via the bloodstream have thus been added paracrine, juxtacrine, autocrine, and even intracrine modes of action. Indeed any one hormonal signal may act in many of these ways.

Endocrine cells produce, in addition to their principal hormone(s), many different compounds in differing amounts. Some, such as the neurophysins, are produced as part of the hormone precursor; others, such as the chromogranin group of proteins, are produced rather widely in peptide- and amine-secreting cells; both can serve as useful markers of hormone-producing tumors. Some have signalling activity, some do not. Co-secreted molecules which are not part of the main prohormone are usually produced in very much smaller (0.1–1.0%) amounts than the main product; the amounts vary physiologically and their actions are largely autocrine or paracrine. Many molecules produced by endocrine cells are not restricted to one tissue; for example, many "gut" peptides are also found in the brain. Apparent "ectopic" production is even more marked in endocrine tumors.

Endocrine tissues can conveniently be divided into those secreting peptide/protein, amine, steroid and iodothyronine signals. To these can be added the eicosanoids (prostaglandins, thromboxanes, leukotrienes, hydroxyeicosatetraenoic acid), nitric oxide and carbon monoxide; such compounds are produced by many different cell types and will not be considered further here. It is now clear that most steroid- and amine-secreting endocrine cells also produce protein peptide/protein secretory products. It is also clear that some tissues, especially the brain, can produce and/or modify steroid to new active compounds – the neurosteroids.

Whereas the general structure and development of the endocrine system have been well understood for many years, our understanding of molecular and developmental endocrinology has mushroomed [1]. The genes for most hormones and hormone-producing enzymes have been identified and their chromosomal location determined. For this reason, most of the references quoted are to reviews of genetic and molecular aspects of development. Although original concepts of the formation of various glands from ectodermal, endodermal or mesodermal tissues still largely hold true, we now have a much better understanding of the

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diverse contributions made by the neural crest, and are beginning to understand the induction processes and molecular switches that lead to the differentiation of endocrine tissues and thereby determine the tissue-specific production of hormones. Indeed, the concept of dysdifferentiation [2] involving tumor formation by clonal expansion offers perhaps the best explanation of the variety of hormones produced by tumors. Equally, understanding of the receptors and postreceptor mechanisms that lead to hormone release allows insight into how autonomous secretion of hormone can result from defects in these mechanisms.

This basic science, combined with clinical experience of endocrine system dysfunction, now permits a better understanding of the development of endocrine tumors and the products they secrete.

Chemically different hormones: "regulated" and "constitutive" release

The chemically different types of hormone necessarily have different cellular mechanisms for their production and release (Fig. 1.1).

The *peptide/protein/glycoprotein* group of hormones are products of genes that usually code for much larger precursors. The mRNAs are translated by ribosomes and simultaneously sequestered in the rough endoplasmic reticulum where the signal sequence is removed, primary glycosylation occurs and disulfide bridges are formed. The immature products (prohormones) are then translocated to the Golgi apparatus where any terminal

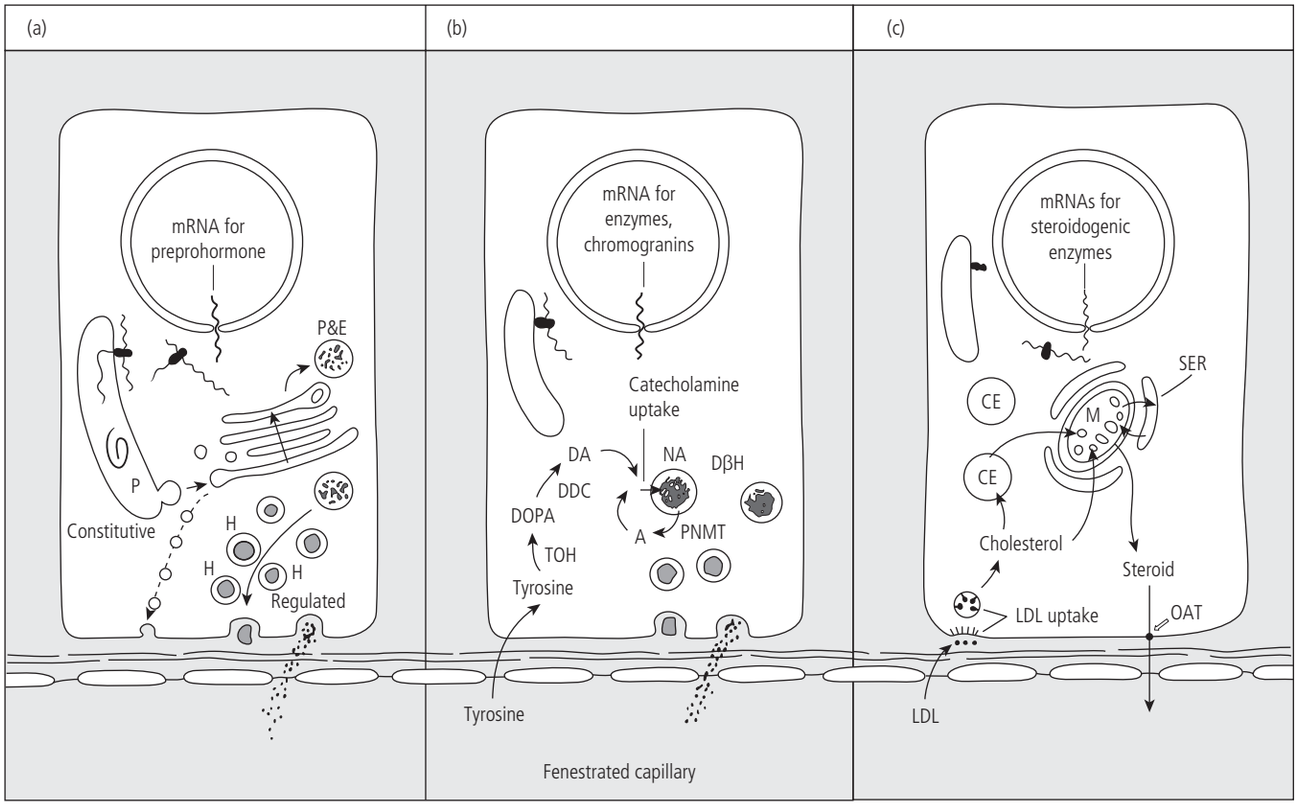

Figure 1.1 Different cellular mechanisms of hormone secretion. (a) Production of peptide, protein and glycoprotein hormones (H). mRNA for the preprohormone and processing enzymes attach first to free ribosomes, then are translated into the rough endoplasmic reticulum. The signal is removed co-translationally to yield the prohormone (P) which may be core-glycosylated in the RER; this is passed to the Golgi where, in the "regulated" pathway, the prohormone is packaged into vesicles in a concentrated form with processing enzymes (E) then transported to the plasma membrane or stored until a secretagogue stimulus is received by the cell which causes exocytosis of the dense-cored vesicles. In the "constitutive" pathway the hormone is packaged apparently unconcentrated into small vesicles which are then immediately transported to the membrane and exocytosed. Constitutive release may predominate in tumors. (b) In catecholamine-secreting cells secretory vesicles are produced in the same way but the DOPA and dopamine (DA) precursors are produced in the cytoplasm by the enzymes tyrosine hydroxylase (TOH) and DOPA decarboxylase (DDC), and pumped into the vesicles where DA is converted to noradrenaline (NA) by dopamine β-hydroxylase (DβH); amine leaks slowly out of the vesicles and, in adrenaline-producing cells, NA is converted by phenylethanolamine-*N*-methyl transferase (PNMT) to adrenaline (A) which is pumped back into vesicles which are stored prior to release. (c) Steroid-producing cells are characterized by distinctive mitochondria (M) surrounded by smooth endoplasmic reticulum (SER). These organelles contain the enzymes which convert cholesterol derived from low-density lipoprotein (LDL) uptake or from stored cholesterol ester (CE) into steroids, which are transported out of the cells by organic anion transporters (OATs).

glycosylation occurs and proteolytic cleavage of the prohormone commences. The Golgi apparatus packages the prohormones and converting enzymes (prohormone convertase; PC1–3) into vesicles which are then transported to the plasma membrane for release by exocytosis. For the "regulated" secretory pathway the prohormones and convertases are concentrated within the vesicles so that the vesicles have an electron-dense core in which cleavage of the prohormone continues. Variable numbers of the dense-cored vesicles are stored in the cytoplasm awaiting the signal for release (or if not released, for lysosomal degradation). The membranes of dense-cored vesicles have a proton pump which maintains an acidic intravesicular pH which stabilizes the peptides near their isoelectric point and is optimal for intravesicular proteolysis.

Some peptide hormones, in particular the growth factors and cytokines, are not stored but released immediately via the "constitutive" pathway. The vesicles that transport the peptide to the membrane in the constitutive pathway are not well understood. They are difficult to identify because the peptide is not concentrated and the vesicles are short-lived. In the "regulated" pathway the extracellular concentration of the hormone is controlled by signals that stimulate release from the stored pool of vesicles, usually within seconds of the stimulus; at the same time synthesis is stimulated more slowly to replenish the stores. By contrast, the extracellular concentration of peptides released by the "constitutive" pathway is determined by control of their synthesis and usually takes several hours to reach peak levels.

In cells that normally release hormone by the regulated pathway, the number of hormone-containing vesicles that are stored in the cytoplasm varies depending, in part, on the demand for the acute release of large amounts of hormone. Thus, for hormones controlling parameters that vary only slowly (e.g., parathyroid hormone (PTH) controlling plasma calcium) the store is small and hormone-containing vesicles are sparse; by contrast, where the demand can be large (e.g., insulin) the cytoplasmic dense-cored vesicles are plentiful.

All endocrine cells have the cellular machinery for both the regulated and constitutive secretory pathways and in some normal endocrine cells both pathways may operate. For example, there is evidence that, while luteinizing hormone (LH) is released predominantly by the regulated pathway, most follicle-stimulating hormone (FSH) is released constitutively [3]. It is therefore not surprising that in some endocrine tumors a peptide normally released by the regulated pathway is released constitutively, with virtually no dense-cored vesicles in the tissue. In tumorous endocrine cells that do contain dense-cored vesicles these may be of a different size to the vesicles that characterize the normal tissue (indeed, little is known about how vesicle size is determined). It is also not surprising that the peptide produced may be abnormal in some way so that, even it if is detectable by radioimmunoassay of the plasma, it may not be bioactive. Finally, tumor cells may have defects in their secretory mechanisms such that hormone can be identified within the cells but is not secreted in detectable amounts.

Cells secreting *catecholamine* hormones also contain numerous dense-cored vesicles, which is not surprising considering their role as acute stress hormones. However, the synthetic pathway is very different. Tyrosine is converted in the cytoplasm first to dihydroxyphenylalanine (DOPA) and then to dopamine (DA). The amine is then pumped into Golgi-derived vesicles which already contain proteins such as chromogranin, co-packaged peptides such as corticotropin-releasing hormone (CRH) and enkephalin, the enzyme dopamine β-hydroxylase, together with ATP and calcium accumulated from the cytoplasm. Dopamine is converted to noradrenaline within the vesicles, but the amines leak slowly through the vesicle membrane and, in adrenaline-producing cells, the noradrenaline is converted to adrenaline by the cytoplasmic enzyme phenylethanolamine-*N*-methyl transferase. The amine pump on the membrane of the vesicles ensures that only about 1% of the amine is free in the cytoplasm. The membrane of catecholamine vesicles also has a proton pump which, by protonating the intravesicular amines, slows their diffusion out of the vesicles. Production of mature amine-containing vesicles takes about 20 h; they are released by exocytosis as in the regulated pathway for peptide hormones, immature vesicles tend not to be released. The stores of catecholamine-containing vesicles, although large, can become exhausted if severe stress is prolonged, depriving the body of its ability to respond to shock.

Cells secreting *steroid* hormones are characterized by mitochondria with tubulo-vesicular cristae, surrounded by large amounts of smooth endoplasmic reticulum. These two organelles contain the enzymes of the steroid-synthesizing pathways. Cholesterol, derived from low-density lipoprotein (LDL) uptake or from cholesterol esters stored as lipid droplets in the cytoplasm, is transported into the mitochondria by a steroid acute regulatory (STAR) protein. In the mitochondria the rate-limiting side-chain cleavage enzyme ($P450_{SCC}$) converts the cholesterol to pregnenolone. Which other steroids are produced from the pregnenolone depends on which other enzymes the cells express. Steroids are not stored to any significant extent (though a small amount may be retained in sulfated form) and were thought to diffuse from the cell through the plasma membrane. However, recent evidence indicates that organic anion transporters (OATs) are responsible for releasing the majority of the steroid. Some steroid-producing cells also secrete proteins (some corpus luteum cells secrete both oxytocin and a progesterone-binding protein) which are packaged in dense-cored vesicles. It is therefore likely that in cells secreting both steroid and protein some steroid is associated with hydrophobic regions of the secreted proteins.

The mechanisms involved in the secretion of *iodothyronines* are described in the section on the thyroid gland.

Many hormones of all chemical types are secreted in pulses; the amplitude and frequency of these pulses are often critical for the action of the hormone.

The normal histological characteristics of endocrine cells are given in Table 1.1.

Table 1.1 Cellular characteristics of hormone-secreting cells

Organ	Principal hormones: cell types	Cellular characteristics	Secretory vesicles: diameter, form
Hypothalamus			
Magnocellular (neurohypophysis)	Oxytocin Vasopressin	Large neurons (50 μm), prominent RER, Golgi; both beaded axons and dendrites contain peptide; axonal dilatations (Herring bodies) and nerve terminals in neural lobe	Spherical, 160–200 nm
Parvocellular (median eminence)	CHR (+ AVP) TRH GHRH GnRH Somatostatin Dopamine	Small neurons (15 μm), modest RER, Golgi, both fine beaded axons and dendrites contain peptide; axons terminate on portal capillaries in median eminence	Spherical, 100–110 nm
Pituitary adenohypophysis	Growth hormone: somatotroph (50%)*	Rounded cells (acidophil), often perivascular; perinuclear RER, Golgi	Profuse, spherical, 350–600 nm
	Prolactin: lactotroph (10–25%; proliferate in pregnancy)	Rounded or irregular (acidophil) cells, modest RER, prominent Golgi. Sparsely and profusely granulated types; types I, II, III	Type I, ovoid, 275–600 nm Type II, spherical , 200–300 nm Type III, spherical, 100–200 nm
	TSH, thyrotropin: thyrotroph (<10%)	Small, irregular (basophilic) cells; sparse peripheral secretory vesicles	Spherical, 100–150 nm
	LH, FSH; gonadotropins: gonadotroph (10–15%)	Type 1: large, oval basophilic cells; plentiful RER, Golgi, many large secretory vesicles Type 2: smaller (basophilic) cells; scant RER, Golgi; fewer, smaller secretory vesicles	Type 1: most 300–400 nm, some smaller Type 2: 200 nm
	ACTH; corticotropin (with other POMC products): corticotroph (15–20%)	Medium-sized stellate (basophilic) cells; perinuclear bundles of cytokeratin filaments; peripheral secretory vesicles	Spherical, 250–350 nm
Thyroid	T_4, T_3 (thyroxine, triiodothyronine): thyroid follicular cells	Cuboidal (normal), columnar (active) or flattened (inactive) cells around follicular lumen containing, respectively, modest, little or much colloid. Cells have basal RER, supranuclear Golgi, active apical membrane with microvilli, pino- and phagocytosis of colloid, fusion with lysosomes	Vesicles of thyroglobulin exocytosed at apical membrane into the colloid
	Calcitonin: parafollicular "C" cells; a few also in parathyroids, thymus	Large, ovoid cells between or in base of follicular epithelium; rich in mitochondria and secretory vesicles, which also contain 5-HT	Spherical, 150 nm
Parathyroid	PTH: chief cells	Chief cells: round or polygonal (8–12 μm), glycogen-rich; rather small numbers of peripherally placed secretory vesicles Oxyphil cells (10–18 μm): cytoplasm rich in mitochondria, scanty RER, glycogen	Irregular, 200–400 nm
Pancreatic islets	Insulin (and IAPP): B or β cells (70%)	Large cells with well developed RER and Golgi; numerous vesicles with pale, (immature) or crystalloid (mature) content	300 nm; electron-dense crystalloid core or pale core
	Glucagon: A or α cells (20%)	Similar to B cells; more peripherally placed in islets; eccentric dense-cored secretory vesicles	Spherical, 200 nm, eccentric electron-dense core
	Somatostatin: D or δ cells (15–20%)	Scattered cells, well developed RER, Golgi	Spherical, 350 nm; rather pale core
	Pancreatic polypeptide: F or PP cells (1–2%)	The predominant cell in the uncinate lobe of pancreas; sparse in dorsal pancreas	Spherical, 150–170 nm; dense-cored vesicles with wide halo
	VIP: EC cells	Occasional scattered cells in islets and exocrine tissue	Spherical, 250 nm, variable electron density
	Gastrin: G cells	In fetus and gastrinomas only	
Gastrointestinal tract†	Different peptide and/or amine hormones produced in different parts of the GI tract	Cells stain less than most enterocytes. All contact basal lamina; most ("open") also contact GI tract lumen with receptive microvillous border. Prominent RER and Golgi; granules located basally	
	Gastrin: G cells	Pyramidal cells in neck and middle third of pyloric antral glands; "open". Also numerous in duodenal crypts, villi and Brunner's glands. Also in fetal pancreas	Spherical, 200–400 nm, variable electron density

Table 1.1 (*Continued*)

Organ	Principal hormones: cell types	Cellular characteristics	Secretory vesicles: diameter, form
	Secretin: S cells	Scattered in duodenal and jejunal mucosa	Spherical, 200 nm
	Cholecystokinin I cells	Cells in duodenum and jejunum; also in enteric neurons in distal intestine	Spherical or slightly irregular, 250 nm
	GIP: K cells	In duodenum and jejunum, a few in ileum	Irregular, 350 nm
	Enteroglucagon: L cells	In ileum and colon	Spherical, 260 nm
	VIP	In subepithelial neurons of the GI and respiratory tracts	Dense-cored vesicles in nerves; 150 nm
	Somatostatin: D cells	In stomach and small bowel. Fundic D cells are "closed"; antral D cells are "open". Cells often have basal processes which contact other endocrine cells. Also in enteric neurons	Spherical, 260–370 nm, weakly electron-dense
	Serotonin, substance P, motilin: Enterochromaffin cells	EC_1 and EC_2 forms throughout the bowel	EC_1: large, very pleomorphic EC_2: irregular, large
	Histamine, 5-HT: Enterochromaffin cells	Widely distributed through bowel. In stomach, source of histamine	
Adrenal medulla	Epinephrine and norepinephrine (also chromogranins, enkephalins)	Large, polarized columnar cells, moderate RER and Golgi. Cholinergic synapses contact apical pole; secretory vesicles concentrated toward capillary pole. Noradrenaline vesicles more electron-dense than those containing adrenaline	250–300 nm; noradrenaline often ellipsoidal
Adrenal cortex	Adrenal corticosteroids	Variable cells. Characteristic mitochondria with distinctive cristae; abundant SER often encircling mitochondria. "Lipid" droplets of cholesterol ester more prominent in inactive than in active cells	Dense-cored vesicles in some steroid-secreting cells reflect co-secretion of peptides
Zona glomerulosa	Aldosterone	Small polyhedral cells arranged in spherical clusters; dense nuclei, little cytoplasm, few lipid droplets, elongated mitochondria	
Zona fasciculata	Cortisol	Large polyhedral cells arranged in sheets, two cells thick, between vascular sinusoids; spherical mitochondria with tubular cristae; abundant lipid droplets	
Zona reticularis	Androgens (DHEA)	Rounded cells arranged in a network; abundant SER, lysosomes and pigment granules, possibility indicating senescence	
Fetal zone	DHEA-sulfate	The major part of the gland in the fetus	
Testis	Androgens (testosterone) Leydig cells	Angular shaped cells in interstices of seminiferous tubules. Scanty cytoplasm filled with SER, lipid droplets, 20-μm-long crystals of Reinke	
	Inhibin, steroid interconversion Sertoli cells	Variable shape and nuclear configuration, long cytoplasmic processes among developing germ cells. Abundant cytoplasmic organelles including RER, SER, but very few secretory vesicles	
Ovary	Androgens (mainly androstenedione): Theca interna cells	Vascularized layer of spindle-shaped steroid-secreting cells clustered around the follicle basal lamina	Type II cells: 250–450 nm
	Activin, inhibin, follistatin, IGF-1, steroid conversion to estrogen: Granulosa cells	Non-vascularized cells lining basal lamina of follicle and surrounding the oocyte. Regionally variable cells with abundant organelles including RER, SER but few secretory vesicles	
	Progesterone, estrogen, relaxin, oxytocin, progesterone-binding protein: Corpus luteum	Vascularized steroid-secreting cells produced by luteinization of the follicle. Type I: small cells (<25 μm), irregular nuclei, abundant SER, mitochondria, lipid droplets, no dense-cored secretory vesicles. Type II: very large (up to 40 μm) polyhedral cells with abundant SER, variously shaped mitochondria, sparse lipid droplets; extensive RER, Golgi, dense-cored secretory vesicles	

* Proportion of total endocrine cells in the gland. NB this varies in different physiological conditions.
† Only the major gastrointestinal hormones are included, in particular those which give rise to tumors.
ACTH, adrenocorticotropic hormone; AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; DHEA, dehydroepiandrosterone; EC, enterochromaffin; GHRH, growth hormone-releasing hormone; GI, gastrointestinal; GnRH, gonadotropin-releasing hormone; 5-HT, 5-hydroxytryptamine; IAPP, islet-associated polypeptide (amylin); POMC, pro-opiomelanocortin; PTH, parathyroid hormone; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; VIP, vasoactive intestinal polypeptide.

Hypothalamus and pituitary gland

The hypothalamus is that part of the forebrain on either side of the ventral part of the third cerebral ventricle. Its floor comprises the optic chiasm and tract, the pituitary stalk, mamillary bodies and posterior perforated substance; anteriorly it is limited by the lamina terminalis and anterior commissure; posteriorly it blends with the midbrain; superiorly it is continuous with the thalamus and epithalamus; and laterally it blends with the zona incerta and internal capsule. Its neuroendocrine neurons are situated in its medial zone (Fig. 1.2): magnocellular neurons secreting vasopressin or oxytocin as their primary product are located mostly in the supraoptic and paraventricular nuclei; parvocellular neurons secreting the releasing factors that control the anterior pituitary are more widely distributed but many are located in the ventromedial part of the paraventricular nucleus (CRH, thyrotropin-releasing hormone (TRH)), in the arcuate nucleus (dopamine, growth hormone-releasing hormone (GHRH)), in the adjacent medial basal hypothalamus (gonadotropin-releasing hormone (GnRH)) and in the periventricular zone (somatostatin). Parts of the hypothalamus, notably the arcuate nucleus and lateral hypothalamus, contain the cells producing neuropeptide Y (NPY) and agouti-related protein (AgRP), α-melanocyte-stimulating hormone (α-MSH), ghrelin and orexin peptides, all of which are involved in the control of appetite. Vasopressin and oxytocin are released into the systemic circulation from the axons of the magnocellular neurons which form the bulk of the posterior pituitary; the releasing factors are secreted from axons of parvocellular neurons into the portal capillary plexus in the median eminence. Central branches of the internal carotid artery enter the ventral surface of the hypothalamus to supply it; superior hypophysial branches form a capillary plexus in the neurohemal contact zone of the median eminence from which the hypophysial portal veins pass down the pituitary stalk to perfuse the anterior pituitary. It is now clear, however, that many neurosecretory peptides can be released, not only from the axon terminals of the neurosecretory neurons, but also from their dendrites into the extracellular fluid of the brain [4] and the same may be true for peptidergic neurons in general. They are therefore capable of diffusing over considerable distances (perhaps via the cerebrospinal fluid) in the CNS and producing organized behaviors such as nest-building (oxytocin), and appetitive behavior (NPY), in a way quite

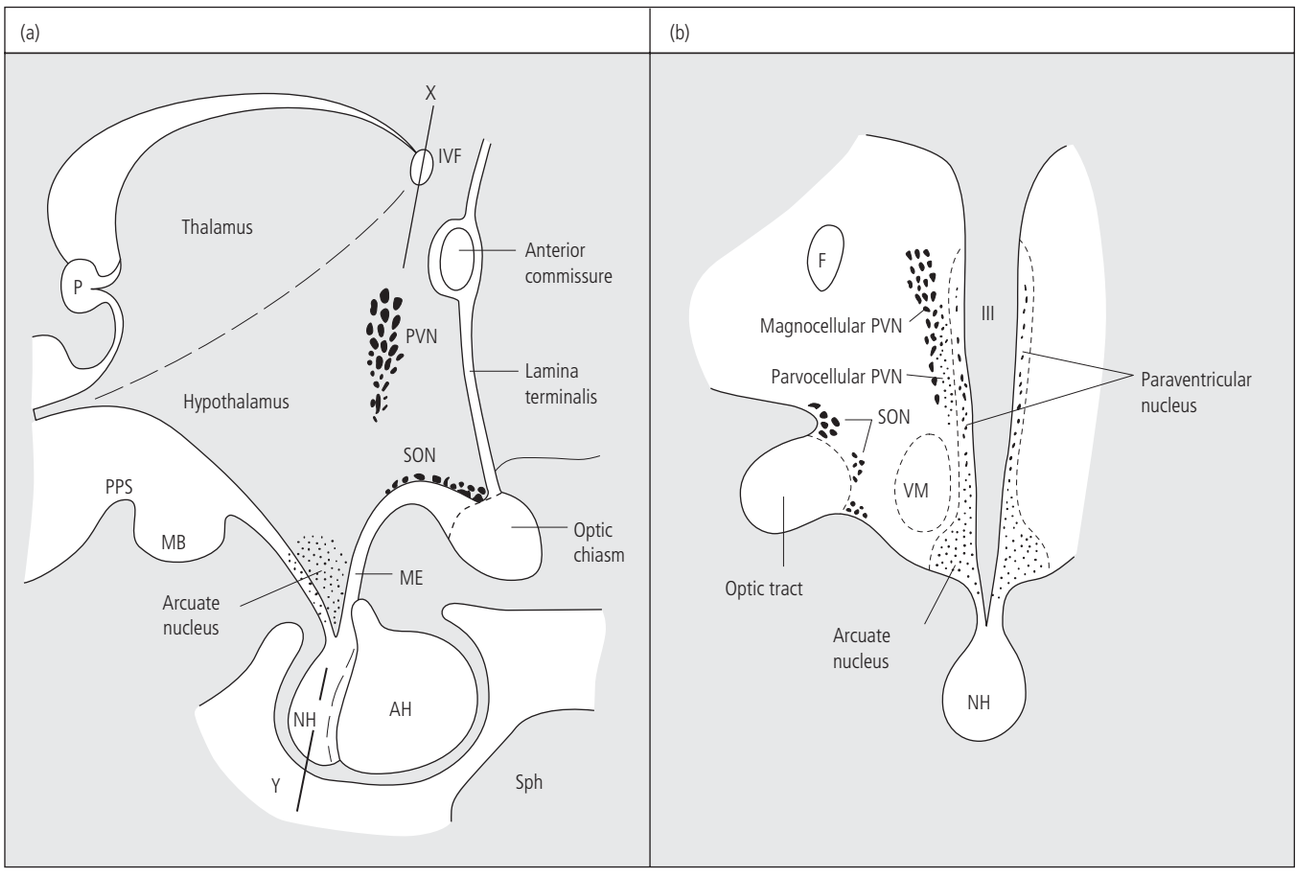

Figure 1.2 Location of cells producing hypothalamic hormones. (a) Midline and (b) near coronal (along plane X–Y in (a)) sections through the human diencephalon. PVN, paraventricular nucleus; SON, supraoptic nucleus; AH, adenohypophysis; F, fornix; IVF, interventricular foramen; MB, mamillary body; ME, median eminence (infundibulum); NH, neurohypophysis; P, pineal; PPS, posterior perforated substance; Sph, sphenoid air sinus; III, third ventricle; VM, ventromedial nucleus.

different from the cellular responses resulting from activation of postsynaptic target cell membranes [5] but analogous to paracrine or even endocrine actions in the peripheral endocrine systems.

The pituitary gland is situated in the pituitary fossa of the sphenoid. Below is the sphenoid air sinus, on either side the internal carotid artery, abducent nerve and cavernous sinus, and above the posterior pituitary is continuous with the pituitary stalk of the basal hypothalamus which, with the pituitary portal veins, passes down through the dura mater which roofs the pituitary fossa. The pituitary gland is composed of two very different types of endocrine tissue: the individual endocrine cells of the adenohypophysis; and the axonal terminals of magnocellular hypothalamic neurons which, with the glial pituicytes, form the neurohypophysis. The posterior pituitary is supplied by inferior hypophysial branches of the internal carotid artery; the anterior pituitary by the hypothalamo-hypophysial portal veins. Both parts drain into the internal jugular venous system.

The hypothalamus is formed from the floor of the diencephalic forebrain vesicle (Fig. 1.3) in the region of prosomeres P4–P6. Expression of fibroblast growth factor 8 (FGF-8) by the anterior neural ridge induces expression of brain factor-1 (BF-1) which regulates the development of the forebrain. Sonic hedgehog induces and organizes the ventral forebrain. Steroidogenic factor 1 (SF-1) is important in the development of the hypothalamic ventromedial nucleus. GnRH neurons, however, do not originate in the neural tube, but in the nasal placode; they migrate centrally into the hypothalamus controlled by glycoprotein neural adhesion molecule anosmin-1 coded for by the *KAL1* gene on the X chromosome (failure of this migration is the origin of the hypogonadism of Kallmann syndrome). The neurohypophysis develops from the neuroectodermal floor of the forebrain vesicle which, at 3.5 weeks' development, lies adjacent to the roof of the ectodermal stomodeum, just anterior to the oropharyngeal membrane. By about 3 weeks' gestation, the forebrain induces this ectoderm (which originally lay just anterior to the neural plate but was carried beneath it by embryonic folding) to form an adenohypophysial (Rathke's) pouch which then forms a vesicle that separates from the roof of the developing mouth. The anterior wall of the vesicle forms trabeculae of proto-endocrine cells which interact with the surrounding mesoderm to form the pars distalis, an extension of which grows up around the developing neurohypophysial stalk as the pars tuberalis; the posterior wall of the pouch apposed to the developing neurohypophysis remains small and free of blood vessels to form the pars intermedia; and the original cavity of the vesicle becomes the pituitary cleft. Axonal terminals of the hypothalamic magnocellular neurons

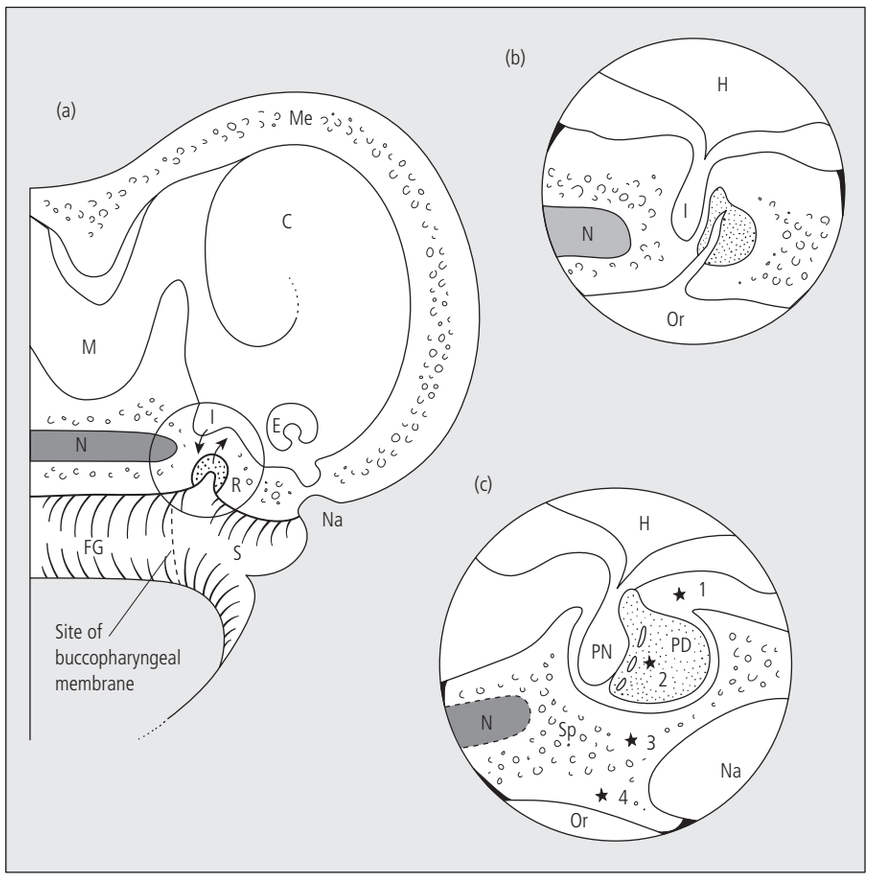

Figure 1.3 Development of the pituitary gland and sites of associated tumors. Diagrams showing the progressive development of the infundibular process (I) downward from the floor of the forebrain vesicle and of Rathke's pouch (R) upward from the roof of the ectodermal stomodeum (S) to form, respectively, the pars nervosa (LPN) and pars distalis (PD) or the pituitary gland. (a) A low power view of early stage; (b, c) area circled in (a) at progressively later stages. Developing structures: C, cerebral cortex; E, eye; F, foregut; H, hypothalamus; M, midbrain; Me, mesenchyme destined to form skull; N, notochord; Na, nasal cavity; Or, oral cavity; Sp, sphenoid bone. Asterisks mark the position of (1) suprasellar, (2) intrasellar, (3) intrasphenoid and (4) palatal cysts or tumors attributed to Rathke's pouch remnants.

grow to contact systemic capillaries in the posterior pituitary part of the neurohypophysis. This is continuous with a similar neurohemal contact zone in the base of the hypothalamus, where parvocellular neurosecretory axon terminals, which secrete the various releasing factors and thereby control the adenohypophysial cells, end on portal capillaries that descend the stalk to perfuse the adenohypophysis. The anterior pituitary and neurosecretory systems become active around the middle of prenatal life.

Our understanding of the molecular signals which transform the anterior ectoderm into the different cell types of the anterior pituitary has increased markedly in recent years. Formation of Rathke's pouch involves the homeodomain genes Rpx (Hesx1) and Pitx (three different transcription factors); mutations in Rpx are associated with septo-optic dysplasia, isolated pituitary hypoplasia and with holoprosencephaly; mutations in the equivalent of Pitx2 (RIEG) are associated with Rieger syndrome, which includes pituitary anomalies. Animal studies suggest that expression of a fibroblast growth factor (FGF-8) and a bone morphogenetic protein (BMP-4) control the initial derivation of Pit-1-independent thyrotrophs from ectodermal stem cells. Where FGF influence declines definitive Pit-1-independent thyrotrophs are formed but disappear by birth. The continuing presence of FGF maintains the progenitor state. Corticotroph differentiation requires expression of NeuroD1 and the T box factor Tpit which, with Pitx1, activate POMC transcription. Pitx1 (with LIM homeobox genes *Lhx3* and *Lhx4*) directly activates the promoter of the α-subunit gene of the glycoprotein hormones. Humans with mutations in *Lhx3* completely lack GH, PRL, TSH, and gonadotropins. Proper development of gonadotrophs, thyrotrophs, lactotrophs and somatotrophs also requires the transcription factor Prop-1, which is restricted to the anterior pituitary. Formation of gonadotrophs also involves steroidogenic factor 1 (SF-1) and GATA2. Tissue-specific expression of growth hormone (GH) prolactin (PRL) and thyroid-stimulating hormone (TSH) in definitive anterior pituitary cells and proliferation of these cells is controlled by the POU-domain transcription factor Pit-1 (POUF1) [6]. Humans with mutations in the Pit-1 gene have a syndrome of postnatal growth retardation, PRL deficiency, and congenital hypothyroidism. Somatotrophs and prolactotrophs develop from a common stem cell and differentiate to form the two "acidophil" cell types and somatomammotrophs which express both GH and PRL. It is therefore not surprising that many acidophil tumors produce both GH and PRL. Other pituitary tumors produce TSH, gonadotropins, pro-opiomelanocortin (POMC) products and peptides with no apparent endocrine effects, and not infrequently produce more than one of these. It is thought that most pituitary tumors are monoclonal [7] though it is possible that a few arise as a result of hyperstimulation from the hypothalamus but, once tumorous, secretion of hormone becomes autonomous.

Asymptomatic cystic remnants of Rathke's pouch are found in 13–23% of autopsies, and traces of the oral end of the pouch may be found at the junction of the nasal septum and palate. Tumors (craniopharyngiomas) containing epithelial cells and a brown fluid rich in cholesterol have been thought to derive from such remnants. However, the fact that >75% occur in a suprasellar position rather than within the pituitary fossa (sella) casts doubt on this origin. Like many adenohypophysial tumors, Rathke's pouch tumors signal their presence by pressure on the optic chiasm or hypothalamus. The tip of the notochord lies near the developing pituitary; chordomas can therefore occur in the region of the pituitary.

The cells secreting the releasing factors and the neurohypophysial hormones oxytocin and vasopressin are non-dividing hypothalamic neurons, so tumors in the hypothalamus and posterior pituitary are derived from glia (gliomas), ependymal cells (ependymomas), or pituicytes (pituicytomas). However, tumors in other sites can secrete releasing factors (e.g. GHRH in pancreatic tumor) and lung tumors not infrequently secrete vasopressin and neurophysin, causing the Schwartz–Bartter syndrome.

Pineal gland

The pineal is a small midline gland projecting backward from the epithalamus above the posterior commissure and midbrain. It is derived from cells in the roof of the forebrain (3rd) ventricle. It comprises two types of cell: pinealocytes (derived from primordial photoreceptive cells) and interstitial glial-like cells. The pineal synthesizes melatonin from tryptophan and secretes it in the dark phase. It responds to light by an indirect route which involves a special class of photoreceptive retinal ganglion cells which innervate the suprachiasmatic nucleus (the intrinsic body clock) which in turn innervates cells in the paraventricular nucleus of the hypothalamus that project to the sympathetic preganglionic neurons in the upper thoracic cord. The preganglionic neurons synapse on neurons of the superior cervical ganglion which innervate the pineal (the only part of the CNS to receive autonomic innervation). The melatonin is released into both the CSF and bloodstream. It acts on melatonin receptors in the suprachiasmatic nucleus, providing a feedback loop which explains its ability to reset the internal body clock in jet-lag, in "free-running" blind subjects and in circadian-based sleep disorders. It also acts on the tuberal part of the pituitary and exerts powerful regulatory actions on the reproductive axis of many animals, in particular seasonal breeders, but any role on human reproduction is uncertain. With increasing age calcified deposits appear in the pineal but these have little effect on its function.

Despite the uncertain relation between melatonin and human reproduction, pineal tumors (which are rare) can cause precocious puberty as well as local neurological abnormalities such as Parinaud syndrome. However, the precocious puberty is probably also the result of local effects of the tumor on the hypothalamus, or of secretion of human chorionic gonadotropin from pineal choriocarcinomas. Tumors of pinealocytes are primitive neuroectodermal tumors of variable differentiation, but immunopositive for the neuronal marker synaptophysin. Pineoblastomas are basically neuroblastomas. Germinomas also occur in the pineal.

Thyroid gland and parafollicular C-cells

The thyroid gland is normally situated in the neck and comprises a midline isthmus which lies anterior to the second and third tracheal rings, and two lateral lobes that extend upward over the lower half of each thyroid cartilage lamina. The gland lies deep to the strap muscles of the neck, enclosed in pretracheal fascia which anchors it to the trachea, so that the thyroid moves upward on swallowing. Its lateral lobes receive the superior thyroid branches of the external carotid artery and inferior thyroid branches from the thyrocervical trunk of the subclavian artery; a small thyroidea ima artery from the arch of the aorta may pass up to the isthmus. Superior and middle thyroid veins follow, respectively, the superior and inferior thyroid arteries; inferior thyroid veins drain downward within the pretracheal fascia to the left brachiocephalic vein. Histologically the thyroid is a unique endocrine gland because its epithelial cells form follicles. The cells secrete a protein (thyroglobulin) into the follicular lumen where certain of its tyrosine residues are complexed with iodine produced by the apical membrane peroxidase from iodide that is pumped into the cells by the basal sodium-iodide transporter. The peroxidase also catalyses the linkage of iodotyrosyl residues in the thyroglobulin to form "colloid." The iodothyronines (principally T_4 if sufficient iodide is available) are produced by proteolytic (lysosomal) cleavage of endocytosed iodinated colloid. The system contains huge reserves of iodinated colloid and secretion of iodothyronines is slow, starting only about 30 minutes after stimulation by TSH. Release of T_4, once thought to be by diffusion, is probably due to a specific transporter. After release the T_4 is converted by peripheral tissues (mainly the liver) to the metabolically active T_3 or to inactive (reverse) T_3.

The human thyroid gland starts to develop at 3 weeks' gestation from thickened endoderm in the midline of the floor of the pharynx between pharyngeal arches 1 and 2 (Fig. 1.4). Its cells form a bilobed mass connected to the pharynx by a stalk (thyroglossal duct) which, at 6 weeks, migrates caudally through the mesoderm anterior to (sometimes through) the developing hyoid bone, thyrohyoid membrane, thyroid and cricoid cartilages to reach its adult position by the 7th week. During development its posterior aspect becomes associated with the developing parathyroid glands (and sometimes thymic tissue), and parafollicular (C) cells derived from neural crest cells in the ultimobranchial body become incorporated into its substance. In the 7th week the epithelial cells begin to organize themselves into follicles; colloid formation starts one week later and iodothyronines are formed at 10–12 weeks.

The thyroid gland or remnants may be found at any position along this route, i.e. in the tongue (lingual thyroid), in relation to the hyoid, as a pyramidal lobe (thyroglossal duct remnant), or it can descend too far and reach the superior mediastinum. The thyroid may fail to form (congenital cretinism), a thyroglossal cyst may form along the duct, and the duct may persist to form a thyroglossal fistula that develops an opening in the midline of the neck. Accessory thyroid tissue may be found elsewhere in the neck, presumably as a result of anomalous attachment to adjacent developing organs, though it must also be borne in mind that apparently ectopic thyroid tissue is usually a secondary deposit of a well-differentiated thyroid tumor. For unknown reasons, thyroid tissue may rarely also develop in the ovary.

The thyroid transcription factors TTF-1 and -2 and the paired domain factor PAX8 are expressed in thyroid follicular cells from the start of their differentiation. Mice in which TTF-1 is deleted lack both follicular and C-cells; heterozygous TTF-1 mutations in humans produce only mild thyroid dysfunction. Homozygous mutations in TTF-2 prevent thyroid formation, but heterozygous parents are euthyroid; heterozygous PAX-8 mutations cause thyroid hypoplasia. Upstream genes controlling these three transcription factors and commitment to a thyroid cell lineage are presently unknown [8, 9]. Mutations in the TSH receptor gene also cause thyroid hypoplasia with associated TSH resistance and congenital hypothyroidism.

Hyperplasia and benign or malignant tumors can develop in thyroid tissue at any location. In the neck there is usually plenty of room for enlargement (goiter). However, if thyroid tissue expands behind the hyoid or manubrium, it will compress the larynx or trachea. Hyperplastic adenomatous nodules are organized like normal thyroid follicles. They may secrete autonomously and cause thyrotoxicosis but never become malignant. Their distinction from true adenomas is difficult, as is the distinction between true adenomas (which are of a number of different morphological subtypes) and well-differentiated follicular carcinomas. Invasion of the capsule or blood vessels distinguishes the carcinomas. Follicular and papillary carcinomas obviously originate from malignant change in thyroid epithelial cells (possibly stem cells) [10] and the epithelial markers low molecular weight (cyto)keratin and lactoferrin are usually expressed. The very aggressive, undifferentiated anaplastic thyroid carcinomas probably share the same origin as they may express the same markers and anaplastic areas can be found in otherwise well-differentiated carcinomas; also, about 50% produce iodothyronines and thyroglobulin. The so-called "small cell carcinomas" are not of thyroid origin but probably represent either thyroid lymphomas or occasionally thyroid metastases of small cell lung carcinomas [11].

The parafollicular C-cells form isolated or small groups of cells at the periphery of thyroid follicles. They secrete calcitonin. They are derived from neural crest cells which become incorporated into the ultimobranchial body of the caudal pharyngeal complex (Fig. 1.4) and thus into the thyroid gland in which they disperse, principally in the upper two-thirds of the lateral lobes. Tumors of parafollicular cells (medullary thyroid carcinoma) can occur sporadically or as part of multiple endocrine neoplasia (MEN) 2a or 2b associated with a mutation in chromosome 10. The unregulated secretion of calcitonin does not disturb calcium homeostasis partly because osteoclasts down-regulate their calcitonin receptor mechanisms ("calcitonin escape") and partly because of the overriding control by parathyroid hormone.

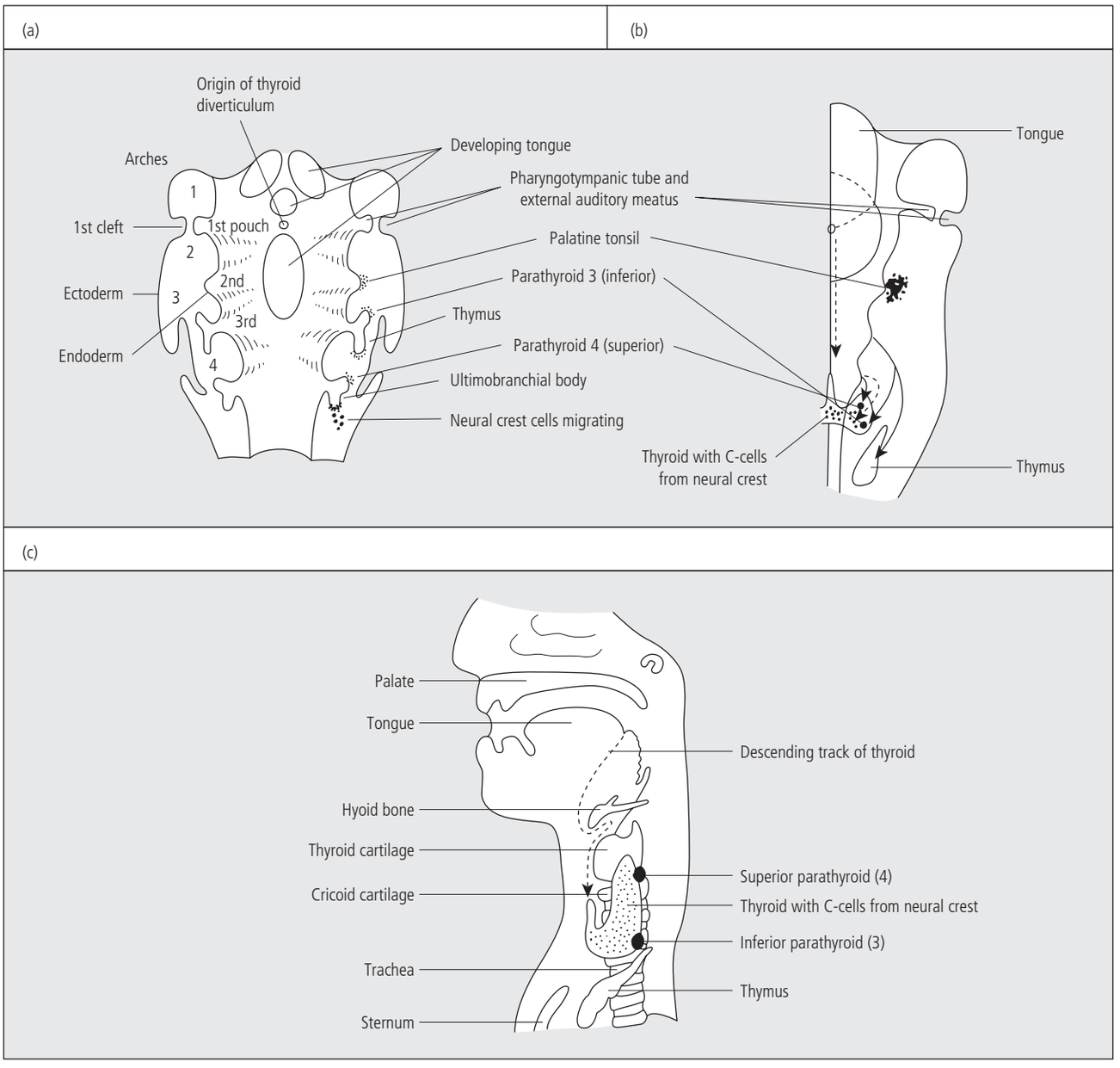

Figure 1.4 Development of the thyroid, parathyroid and thymus. (a) Floor of the mouth showing the first four branchial arches (1–4) and the corresponding endodermal pouches. Note that the 4th and more caudal pouches open as a single complex. (b) Right side of the floor of the mouth at a later stage of development. The thymus has migrated caudally carrying parathyroid 3 caudal to parathyroid 4. Neural crest cells have entered the thyroid gland, which has also migrated caudally, where they will produce C-cells. (c) Lateral view to show the course of descent of the thyroid gland. Ectopic thyroid and tumors can appear anywhere along the tract; the thymus and parathyroid 3 are most likely to be ectopic and can be either more cranially or more caudally situated.

Parathyroid glands and thymus

There are usually four parathyroid glands (two superior and two inferior) embedded in the posterior aspect of the lateral lobes of the thyroid close to the anastomotic vessel linking the superior and inferior thyroid arteries, all of which can supply the glands. Each gland is a ball of cells: principally cells which secrete parathyroid hormone (PTH), and oxyphil cells of unknown function.

The adult thymus gland comprises two more or less joined lobes of lymphoid tissue situated in the upper part of the anterior mediastinum above the pericardium and usually extending into the neck. In addition to its key role in the development and maintenance of cell-mediated immune responses, the thymus also

produces a number of associated hormones (e.g. thymosin, thymopoietin). The thymus grows in size until puberty but thereafter atrophies progressively. This age-associated involution is accelerated by glucocorticoids.

The parathyroid glands develop in the region of the 3rd and 4th pharyngeal pouches (Fig. 1.4). They are usually said to arise from the pouch endoderm but more recently it has been suggested that they arise from the adjacent cleft ectoderm. Parathyroid 3 arises as a cellular mass from the dorsal and the thymus from the ventral part of the 3rd pharyngeal pouch. As the thymus descends into the anterior mediastinum parathyroid 3 is carried part-way with it so that it forms the inferior parathyroid gland. It is much more variable in position than parathyroid 4 which becomes the superior parathyroid. The number of parathyroids can vary from 2 to 6; they frequently contain cysts. In the DiGeorge syndrome, the thymus and parathyroids are absent, apparently as a result of agenesis of the 3rd and 4th pharyngeal pouches. The absence of the parathyroids causes hypocalcemia; absence of the thymus immunological incompetence. The frequently associated aortic arch and facial defects are of neural crest origin (see below).

The master gene regulating parathyroid development appears to be (glial cell missing) *GCMB* and *GCMB* expression is increased in parathyroid adenomas [12] but mouse knockouts suggest that both *Six* and (eyes absent) *Eya1* genes are required for the development of not only parathyroid but other pharyngeal pouch tissue such as thymus [13].

Tumors of the parathyroids may be restricted to the parathyroids or be associated with tumors of the pancreatic islets and pituitary (MEN 1; autosomal dominant, chromosome 11) or with medullary carcinoma of the thyroid and phaeochromocytoma (MEN 2a; chromosome 10). In MEN 1 two mutations are involved: the first leads to hyperplasia, the second to tumor formation. Of the tumor-prone tissues in MEN 2, the C-cells and adrenal medulla derive from the neural crest, whereas the parathyroids are apparently of endodermal origin. However, occipital neural crest cells migrate through the 3rd and 4th arches and all three tissues express the *ret* (receptor-type tyrosine kinase) proto-oncogene.

Parathyroid hormone-related protein

This molecule, which acts like PTH on the PTH receptor, is produced by a wide variety of tumors (particularly lung and breast) and is the cause of the hypercalcemia of malignancy. It is also secreted constitutively by a similarly wide variety of fetal and adult tissues. Its physiological autocrine and paracrine function in these tissues has yet to be determined. In the placenta it is suggested to help activate the placental calcium pump.

Adrenal glands

The adrenal glands are situated on the medial aspect of the upper pole of each kidney. Each comprises a steroid-secreting cortex and a catecholamine-secreting medulla. They receive a profuse blood supply from the adjacent aorta, phrenic and renal arteries and drain by a large central vein into the inferior vena cava (right) or left renal vein (left). Each also receives a profuse innervation largely composed of preganglionic cholinergic sympathetic fibers from the thoracic splanchnic nerves and celiac plexus; some postganglionic fibers also enter the gland. Many of the preganglionic fibers synapse on the chromaffin cells of the medulla to control the secretion of catecholamines. It is now clear that splanchnic nerves also modulate the secretion from and sensitivity of the adrenal cortex, at least in part by controlling blood flow through the gland. In sympathetic arousal, when most of the splanchnic vascular bed is constricted, blood flow through the adrenals is increased.

The adult adrenal cortex comprises an outer zone (about 5%) of balls of cells, the zona glomerulosa; a large mid-zone (about 65%) of radially arranged sheets of cells interspersed with blood vessels, the zona fasciculata; and an inner network of cells (about 5%), the zona reticulata. Cells of the zona glomerulosa express the enzyme 18-hydroxylase and secrete aldosterone; cells of the zona fasciculata secrete largely cortisol in humans; and cells of the zona reticulata secrete weak androgens, principally dehydroepiandrosterone (DHEA). The medulla is composed of the adrenaline- and noradrenaline-secreting (epinephrine, norepinephrine) chromaffin cells and their associated cholinergic innervation; adrenaline-secreting cells predominate. Chromaffin cells also secrete numerous peptides including met-enkephalin, CRH and chromogranins.

The cortex and medulla develop from quite different primordia (Fig. 1.5). The cortical cells develop from the intermediate mesoderm which forms the celomic epithelium between the mesonephros and dorsal mesogastrium. Cords of endocrine cells form between the developing vascular sinuses. The first cells to develop form that large *fetal zone* of the cortex; this then becomes surrounded by cells that will form the definitive cortex. The fetal cortex cells do not express 3-β-hydroxysteroid oxidoreductase and therefore produce largely DHEA (which is sulfated). At birth the adrenal glands are relatively large (about 30% of the size of the kidneys); this is because the fetal zone is very large but the definitive zone and medulla are both small. The fetal cortex decreases in size during the first 2–3 postnatal months by a non-inflammatory involution process; the definitive cortex increases rapidly at first, then more slowly up to 20 years of age. The chromaffin cells of the medulla are derived from migrating neural crest cells (and are the equivalent of sympathetic ganglion cells without neuritic processes). At 5–6 weeks' development, neural crest cells migrate into the developing adrenal cortex and eventually become surrounded by cortical tissue. The cortical cells probably induce the neural crest cells to form chromaffin cells; also cortisol stimulates the expression of phenylethanolamine-*N*-methyl transferase (PNMT) which converts noradrenaline to adrenaline. Other neural crest cells migrate further to form the midline sympathetic ganglia, the ganglion cells of the enteric nervous system, and the chromaffin cells of the organ of Zuckerkandl, which is thought to be the major source of catecholamines in the first postnatal year.

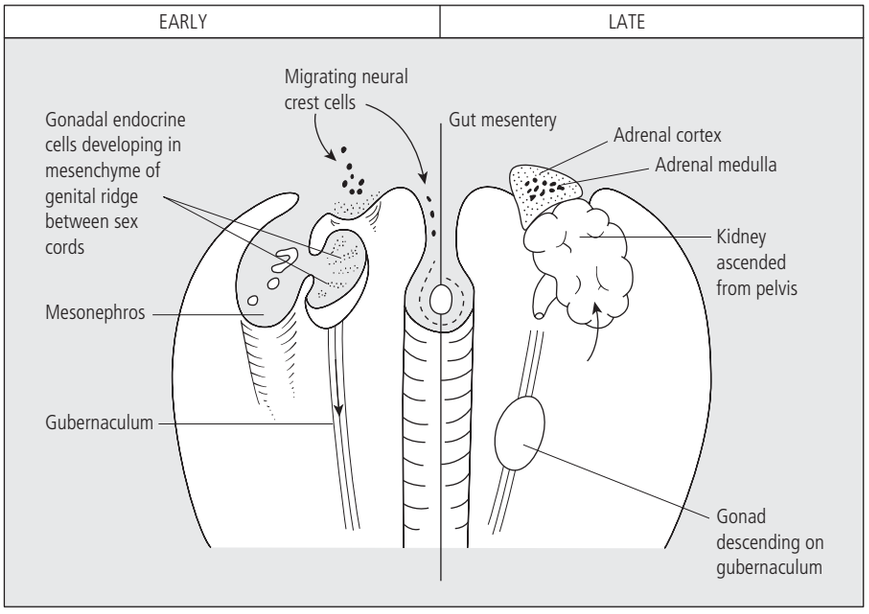

Figure 1.5 Development of the suprarenals and gonads. Diagrammatic views of early (left) and later (right) stages in the development of the adrenal glands and gonads. Celomic epithelium between the mesonephros and the gut mesentery develops to form adrenal cortical tissue which comes to surround neural crest cells which have migrated into the region and which will form the adrenal medulla. Other neural crest cells migrate into the gut mesentery where they form sympathetic ganglion and enteric nervous system cells. Endocrine cells of the gonads develop in the mesenchyme of the genital ridge between the sex cords or primordial follicles.

The development of the intermediate mesoderm which forms the adrenals and gonads depends on a number of genes including (Wilms tumor) WT1, (steroidogenic factor) SF1, LIM1 and EMX2. Formation of the primordial adrenal requires the continued presence of SF1 which regulates a number of genes involved in steroidogenesis. Growth and zonation of the adrenal cortex and regression of the fetal adrenal require (dosage-sensitive sex-reversal, adrenal hypoplasia) DAX1 which interacts with SF1 (see also development of gonads) and insulin-like growth factor-2 (IGF-2) and its receptor. Mutations in SF1 are associated with adrenal insufficiency or failure; high expression of IGF-2 occurs in human fetal adrenal and 85% of adrenocortical tumors and alterations in genomic imprinting appear to be partly responsible for the overexpression of IGF-2 [14, 15].

Considering the extent to which neural crest cells migrate it is not surprising that ectopic clusters of chromaffin cells can be found outside the adrenal glands, along the abdominal aorta and almost anywhere that sympathetic ganglion cells are located. Accessory cortical tissue is also common near the kidneys and along the track of the descending gonads (all of which form from intermediate mesoderm). Abdominal accessory chromaffin and cortical tissue often coexists. Very occasionally suprarenal (cortex and medulla) tissue is found intracranially – the cause for this is unknown.

Developmental defects occur in the expression of most of the steroid-processing enzymes of the adrenal cortex. The most common – an autosomal recessive defect in 21-hydroxylase – leads to the failure of cortisol and aldosterone production. The lack of corticosteroid feedback causes increased ACTH secretion and thus congenital adrenal hyperplasia; the shunting of precursors into androgen production causes the associated virilization of female fetuses.

Tumors of cortical tissue can secrete aldosterone (Conn syndrome), cortisol (Cushing syndrome) or sex steroids, and steroid intermediates. Well-differentiated tumors usually secrete only one major steroid; carcinomas frequently secrete multiple steroids. Tumors of adrenal medullary or ectopic chromaffin tissue (pheochromocytomas) secrete catecholamines (mostly epinephrine) and a wide variety of normally co-secreted peptides (e.g. met-enkephalin, ACTH, vasoactive intestinal polypeptide [VIP]), neural crest-associated peptides (e.g. calcitonin, substance P), and unexpected peptides (e.g. gastrin). Chromaffin tumors may be isolated or part of the MEN 2a (medullary thyroid carcinoma, parathyroid tumors, pheochromocytomas) or MEN 2b (medullary thyroid carcinoma, pheochromocytoma, mucosal neuromas) syndromes. Ganglioneuromas (usually well differentiated) and neuroblastomas (malignant) are tumors of neural crest-derived sympathetic ganglion or more primitive adrenal neuroblast cells; dopamine is the major catecholamine secreted.

Endocrine tissue of the gonads

In the adult male, each testis is an ovoid organ normally located in a pouch of peritoneum within the scrotum. Each consists of a mass of seminiferous tubules comprising the germ cell series and the sustentacular (Sertoli) cells and connected to the rete testis, epididymis and vas deferens. Around the tubules are myoid cells which produce local controlling signals. Between the seminiferous tubules are the interstitial (Leydig) cells and blood and lymph vessels derived from the spermatic cord. Leydig cells secrete androgens, particularly testosterone; Sertoli cells secrete inhibin and Müllerian-inhibiting factor (MIF), and convert testosterone to both dihydrotestosterone and estrogen.

The testes develop at about 6 weeks from indifferent gonadal primordia formed after primordial germ cells (which are first specified in the epiblast) migrate from the yolk sac wall, via the gut mesentery into the thickened celomic epithelium on the medial aspect of the intermediate mesoderm forming the urogenital ridge (Fig. 1.5). Formation of the urogenital ridge involves the autosomal Wilms' tumor suppressor gene WT1, the gene for the "orphan receptor" steroidogenic factor 1 (SF1), LIM1, LX9 and probably GATA4 [16]. Lack of any of these results in failure of formation of gonads of either sex. If the DNA-binding protein coded for by the Y-specific (SRY) gene for the testis-determining factor is expressed, the celomic epithelium proliferates markedly and becomes organized around the germ cells to form primary testicular cords which separate from the surface epithelium, the mesodermal cells becoming differentiated to form pre-Sertoli cells and, later, Leydig cells. It is clear from the insertion of SRY into mouse XX oocytes that SRY can switch the bipotential tissue into forming a testis, but mutations in SRY only account for a minority of human XY sex reversal. Other factors must therefore be involved, but progress in understanding the mechanism of SRY action has been disappointingly slow. As the sex cords form, vascular endothelial cells migrate from the mesonephros to surround the cords and form a testis-specific vasculature. Shortly after this SRY expression declines and the homeobox gene SOX9 assumes control. SOX9 is initially expressed in both XX and XY gonads, but is upregulated in XY gonads and downregulated in XX gonads [17]. An appropriate expression of the orphan nuclear receptor DAX1, which was originally proposed to act as an "anti-testis" factor, is now known to be necessary for proper testis development, and in mice DAX1 deficiency disrupts developmental events between expression of SRY and SOX9. In humans DAX1 mutations cause (in addition to adrenal hypoplasia) hypogonadism which may result from gonadal dysgenesis [18, 19]. SOX9 induces the production of MIF from the Sertoli cells. This directs the involution of the paramesonephric ducts and assists testicular descent in the male; later the Sertoli cells also produce inhibin and androgen-converting enzymes. Mesonephric tubules in the hilus of the developing testis form the rete testis tissue but do not fuse with the developing seminiferous tubules till mid-gestation. Various growth factors and their receptors, including fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin and IGF, WNT, and transforming growth factor-β (TGF-β), are also crucial to correct gonadal development [17]. The interstitial Leydig cells develop in two waves. The first forms at 8 weeks' gestation when testosterone secretion starts and promotes the development of the mesonephric duct system into the epididymis and vas deferens; later most of these early Leydig cells degenerate, but a second wave of Leydig cell formation gives rise to the definitive interstitial cells of the postnatal male. Desert hedgehog (DHH) is required for the differentiation and multiplication of Leydig cells; mutations in human DHH in 46,XY individuals are associated with partial or complete pure gonadal dysgenesis.

Male differentiation of the external genitalia (at 9–11 weeks' gestation) is dependent on 5α-dihydrotestosterone (DHT) produced by 5α-reductase in those tissues. Testicular descent down the gubernaculum on the posterior abdominal wall and into the scrotum is partly the product of differential growth; descent from the pelvic brim and into the scrotum requires androgens and MIF. The current increase in undescended testes is thought to be due to estrogenic chemicals in the environment. Genetic defects causing the absence of any of these genes, hormones, receptors or enzymes result in defects of the masculinization process.

The ovaries in the adult are small ovoid organs lying on the posterior aspect of the broad ligament in the recto-uterine pouch, close to the fimbriated opening of the Fallopian tube. The ovarian artery, pampiniform plexus of veins, and some nerves reach the ovary in the "suspensory ligament" which descends over the pelvic brim. The ovary is attached to the uterotubal junction by the ovarian ligament component of the gubernaculum; the rest of the gubernaculum forms the round ligament which passes out of the abdomen through the inguinal canal to end in the labia majora.

The initial development of the ovary also involves the migration of primordial germ cells from the yolk sac wall into the thickened celomic epithelium of the intermediate mesoderm (the indifferent gonadal primordium). If SRY is not expressed, the celomic epithelial cells do not separate from the surface to form primary sex cords but, somewhat later, differentiate to form pregranulosa cells which surround the germ cells to form primordial ovarian follicles in which the germ cells enter and arrest in prophase 1 [17]. Thereafter obvious morphological differentiation of the ovary occurs at later stages. The intermediate mesoderm forms the interstitial thecal tissue which remains undifferentiated until puberty. Formation of an ovary rather than a testis requires the expression of the X-associated DAX1 and WNT4 genes. WNT4 appears to control expression of (bone morphogenetic protein) BMP2 and (follistatin) FST, both of which are expressed specifically in XX gonads. The pregranulosa cells appear to be of two types which either induce or inhibit meiosis of the germ cells. Female internal and external genitalia develop independently of any ovarian hormone production, provided that MIF and testosterone are not present. Estrogen secretion starts at about 8 weeks' gestation but in very small amounts and its cellular origin is unclear. The arrest of meiosis in the primordial germ cells in early fetal life involves local signals; thereafter unknown mechanisms regularly cause a few primordial follicles to start their developmental trajectory, but until puberty all are destined to become atretic. Mutations in the forkhead gene *FOXL2* are associated with premature ovarian failure in women, and in mice *FOXL2* is expressed early in and is necessary for the function of pregranulosa cells [20].

At puberty, when pulsatile GnRH and gonadotroph secretion has become established, androgen-secreting theca interna cells become organized around developing follicles and the growing definitive granulosa cells of the follicles produce activins, inhibins, follistatin, growth factors such as IGF-1, and aromatase to convert androgens from the theca to estradiol. After ovulation, cells of both thecal and granulosa origin contribute to the formation of the corpus luteum.

In males, tumors can form from both Leydig and Sertoli cells. Leydig cell tumors can produce androgens, estrogens and progestins; the very rare Sertoli cell tumors usually cause feminization, presumably because of increased aromatase activity. In females, tumors can likewise form from granulosa or thecal cells and also from hilar cells; these can produce androgens (especially hilar cell tumors), estrogens or progestins.

Endocrine tissues of the gut and pancreas

Endocrine cells are found scattered throughout the epithelium of the gut from the stomach to the colon, and are collected as islets of Langerhans in the pancreas.

The islets of Langerhans comprise about 2×10^6 roughly spherical balls of endocrine cells surrounded by a capsule of glia-like cells; they are distributed widely throughout the pancreas. Those in the body and tail of the pancreas contain ~60% insulin-producing B cells located mainly centrally, 15% glucagon-secreting A cells located peripherally, and 10% somatostatin-secreting D cells scattered between. Islets in the uncinate process, which develops from the ventral pancreatic rudiment, also contain pancreatic polypeptide-secreting F cells. Each islet has a rich blood supply which enters it centrally and a rich innervation from both sympathetic and parasympathetic nerves.

The endocrine cells of the gut epithelium are mostly located within the crypts. In the body of the stomach enterochromaffin (ECL) cells produce the histamine that controls gastric acid production and both ghrelin (appetite-stimulating) and adipostatin (appetite-suppressing) are produced from the same gene expressed by the acid-secreting parietal cells; gastrin-secreting (G) cells are located in the pyloric antrum and duodenum. In the small bowel, secretin (S) cells occur from duodenum to distal ileum, cholecystokinin (I) cells and GIP (glucose-dependent insulinotropic peptide/gastric inhibitory peptide) (K) cells are located in duodenum and jejunum, glucagon-like peptide (GLP) in the duodenum, and neurotensin (N) cells in distal ileum. In the colon enteroglucagon is produced by L cells (also by gastric A cells). Somatostatin (D) cells, motilin cells and VIP (H) cells are distributed throughout the tract. Enterochromaffin cells secreting serotonin are also distributed throughout the tract and are the largest single endocrine cell group; they also produce a variety of peptides (e.g. substance P, motilin).

It was at one time thought that gut and pancreatic endocrine cells originated from the neural crest, but transplantation studies in animal fetuses have shown that they originate from endodermal cells of the developing gut. The pancreas develops from two different primordia: a smaller ventral pancreas which forms the lower part of the head and uncinate process from a bud which also forms the hepatic diverticulum; and a larger dorsal pancreas which forms from dorsal gut endoderm under the influence of activin and FGF-2 signals from the adjacent notochord and which represses sonic hedgehog expression in the underlying endoderm to permit the expression of transcription factors Pdx-1 (pancreatic-duodenal-homeobox) and Hlxb-9 which are necessary for pancreatic differentiation. Endocrine cells of the islets of Langerhans differentiate early as cells budding off the branching endodermal tubules that will form the exocrine ducts and secretory acini of the pancreas, and it would appear that the ducts can continue to provide a source of islet cells throughout life. In the endocrine progenitor cells Notch signaling is inactivated and transcription factors neurogenin 3 and Isl-1 are expressed. These then give rise to two groups of endocrine cells: glucagon- and pancreatic polypeptide-producing cells differentiate early under the influence of Pax6; insulin- and somatostatin-producing cells a little later under the influence of Pax4 [21, 22]. It is therefore not surprising that exo-endocrine cells with characteristics of both acinar and endocrine cells can be found. Co-expression of different hormones is also common in early stages. During fetal life activin A- and gastrin-producing cells are prominent in the islets, but these disappear after birth. Abnormal multifocal proliferation of islet cells during development (nesidioblastosis) causes profound uncontrolled hypoglycemia in infants.

The gut endocrine cells develop from multipotential progenitors – common stem cells in the crypt compartment of the intestine. The enteroendocrine cells differentiate as the cells migrate up the crypt–villus axis, turning over every 3–4 days. Unlike endocrine cells in many other glands that differentiate early on and turn over only very slowly, gut endocrine cells are formed throughout life from a stem cell reservoir [23]. As in the pancreas, Notch signaling blocks endocrine differentiation in gut stem cells. Deletion in mice of a gene controlled by Notch increased the number of endocrine cells in the stomach and small bowel and increased expression of pro-endocrine beta helix-loop-helix (bHLH) proteins Math1, NGN3 and Beta2. Deletion of Math1 prevents the development of all gut endocrine cells; deletion of NGN3 prevents their formation in the small intestine but not the stomach. Further downstream, Beta2(NeuroD1) is necessary for the development of secretin- and cholecystokinin-secreting cells; Pax6 for glucagon (GLP-1 and GLP-6), gastrin and somatostatin in the antrum and GIP in the duodenum; Pax4-null mice lack serotonin and somatostatin cells in the antrum and most endocrine cells in the proximal small intestine. Clearly, much remains to be discovered about how the individual endocrine cell types become differentiated [24].

Most, if not all these endocrine cells can form tumors. Interestingly, most gastrinomas arise in the pancreas, not the stomach. Also, the original discovery of GHRH in a pancreatic tumor emphasizes the apparent ectopic expression that can occur in tumors. Tumors secreting somatostatin, enteroglucagon, VIP and serotonin all produce characteristic syndromes. Pancreatic tumors occur in MEN 1; insulinomas produce characteristic hypoglycemic episodes.

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