Clinical biochemistry

The hypothalamus and pituitary gland

GENERAL PRINCIPLES OF ENDOCRINE DIAGNOSIS

A hormone can be defined as a substance secreted by an endocrine gland that is transported in the blood, thereby

regulating the function of another tissue(s).

Ex.GH,T4,INSULIN and rout of secretion from glands.

- Conversely, trophic hormones from the pituitary gland stimulate target endocrine

Endocrine glands may secrete excessive or deficient amounts of hormone.

Abnormalities of target glands may be primary or secondary to dysfunction of the controlling mechanism, usually located in the hypothalamus or anterior pituitary gland.

Hormone secretion may vary

It is also important to know about the assay's performance, as sometimes heterophilic interfering antibodies may cross-react with various hormones, as can certain immunoglobulins, for example macroprolactin If the results of preliminary tests are definitely abnormal, this may be primary or secondary to a disorder of one of the controlling mechanisms.

if the results be equivocal when considered together with the clinical findings, so-called 'dynamic' tests should be carried out.

dynamic' tests

Suppression tests are used mainly for the differential diagnosis of excessive hormone secretion.

The substance (or an analogue) that normally suppresses secretion by negative feedback is administered and the response is measured.

Failure to suppress implies that secretion is not under normal feedback control (autonomous secretion).

Stimulation tests are used mainly for the differential diagnosis of deficient hormone secretion.

The trophic hormone that normally stimulates secretion is administered and the response is measured.

A normal response excludes an abnormality of the target gland, whereas failure to respond confirms it.

HYPOTHALAMUS AND PITUITARY GLAND

There are two lobes of pituitary gland anterior and posterior lobes.

both depend on hormones synthesized in the hypothalamus for normal function.

The hypothalamus also has extensive neural connections with the rest of the brain.

stress and some psychological disorders affect the secretion of pituitary hormones and of the hormones from other endocrine glands

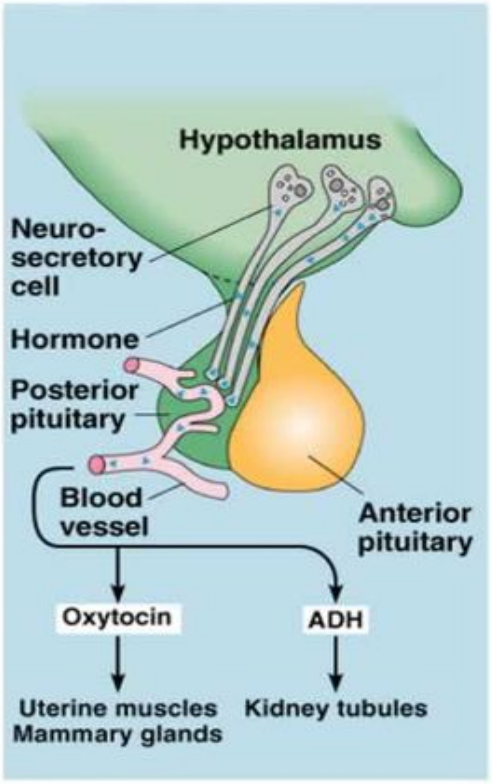
Control of posterior pituitary hormones

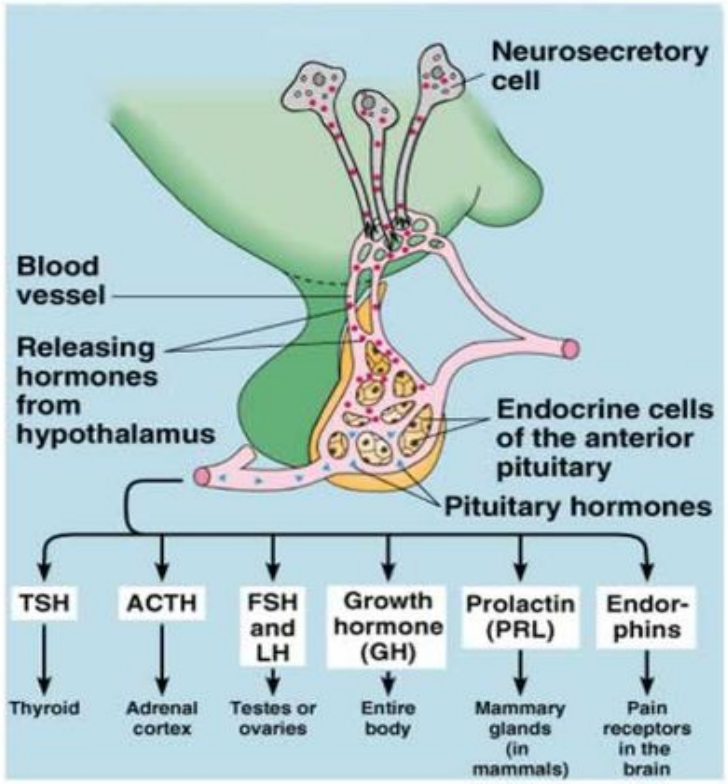
Two structurally similar peptide hormones, antidiuretic hormone (ADH) — and oxytocin, are synthesized in

the hypothalamus and transported to pituitary stalk attached to specific carrier proteins – neurophysins.

Then stored in the posterior pituitary gland and are released into the bloodstream under hypothalamic control, together with neurophysin.

Neurophysin has no apparent biological function and is rapidly cleared from plasma.





Antidiuretic hormone (arginine vasopressin) is mainly synthesized in the supraoptic nuclei of the hypothalamus and enhances water reabsorption from the collecting ducts in the kidneys .

Oxytocin is synthesized in the paraventricular nuclei of the hypothalamus. It controls the ejection of milk from the lactating breast and may have a role in initiating uterine contractions,

It may be used therapeutically to induce labour.

Anterior pituitary hormones

There is no direct neural connection between the hypothalamus and the anterior pituitary gland.

The hypothalamus synthesizes small molecules (regulating hormones or factors) that are carried to the cells of the anterior pituitary lobe by the hypothalamic portal system.

This network of capillary loops, which, after passing down the pituitary stalk, divide into a second capillary network in the anterior pituitary

The cells of the anterior pituitary lobe can be
classified simply by their staining reactions as acidophils,
basophils or chromophobes.
Acidophils are of two cell types:
lactotrophs, which secrete prolactin,
somatotrophs, which secrete GH (somatotrophin).

Basophils secrete hormones that affect other endocrine glands. The hypothalamic

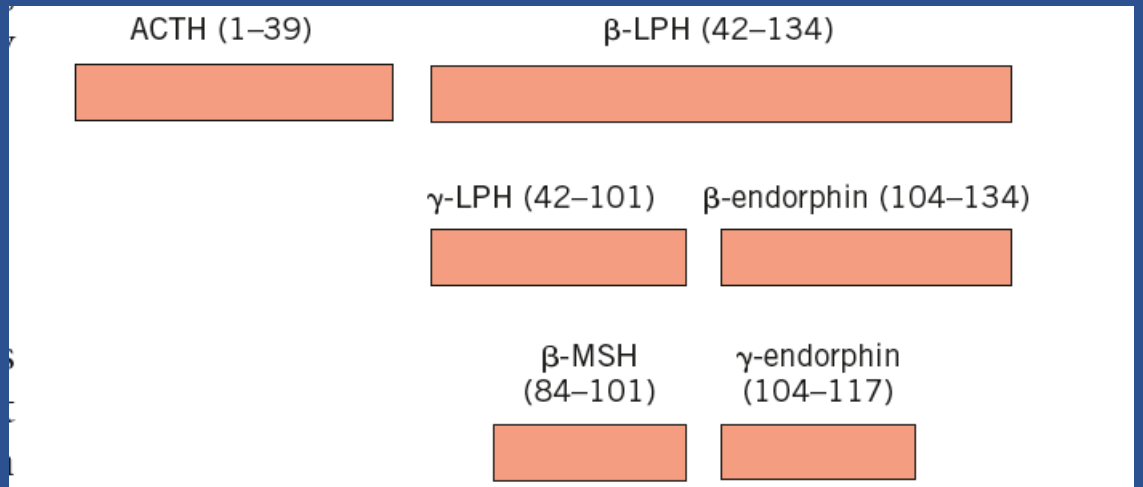


Figure 7.1 The products of pro-opiomelanocortin (POMC): adrenocorticotrophic hormone (ACTH), β-lipotrophin (LPH), γ-LPH, β-melanocyte-stimulating hormone (MSH) and β- and γ-endorphin. The numbers indicate the amino acid sequence in POMC.

Part of the molecule has melanocyte-stimulating activity, and high circulating concentrations of ACTH are often associated with pigmentation.

β-Lipotrophin is inactive until rapidly converted to endorphins. These are neurotransmitters which because they have opiate-like effects, help control pain

. Gonadotrophs secrete the gonadotrophins, folliclestimulating hormone (FSH) and luteinizing hormone (LH), which act on the gonads.

Thyrotrophs secrete TSH (thyrotrophin), which acts on the thyroid gland.

These hormones are structurally similar glycoproteins consisting of two subunits, α and β. The α -subunit is common to all three hormones; the β -subunit is important for receptor recognition.

Chromophobes, once thought to be inactive, do contain secretory granules.

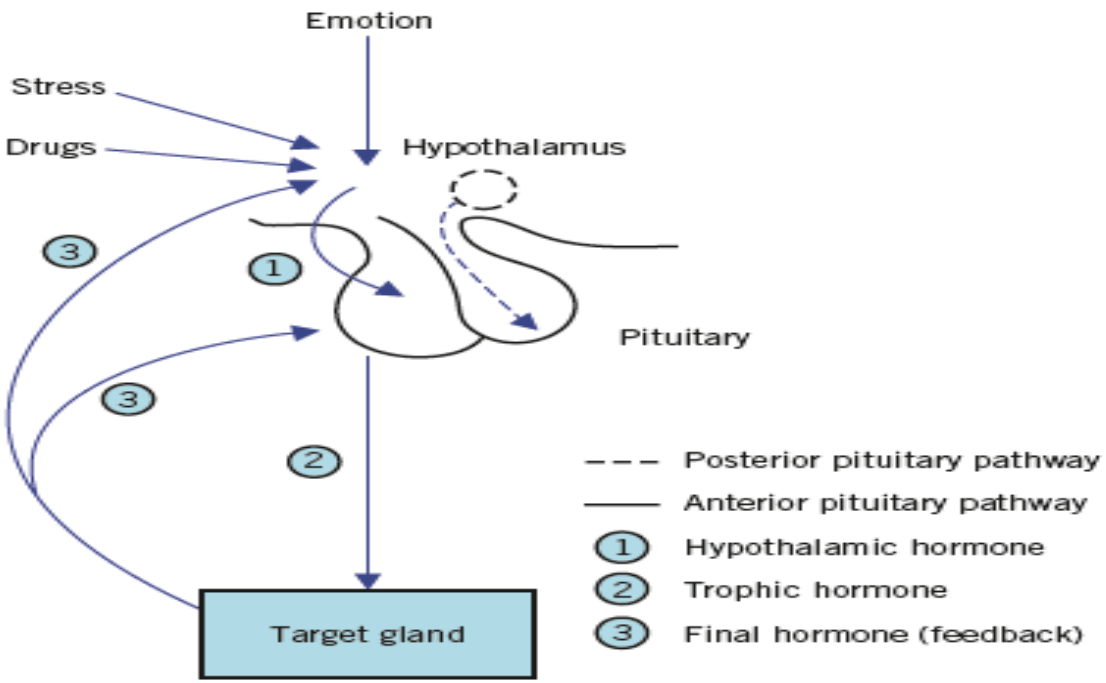
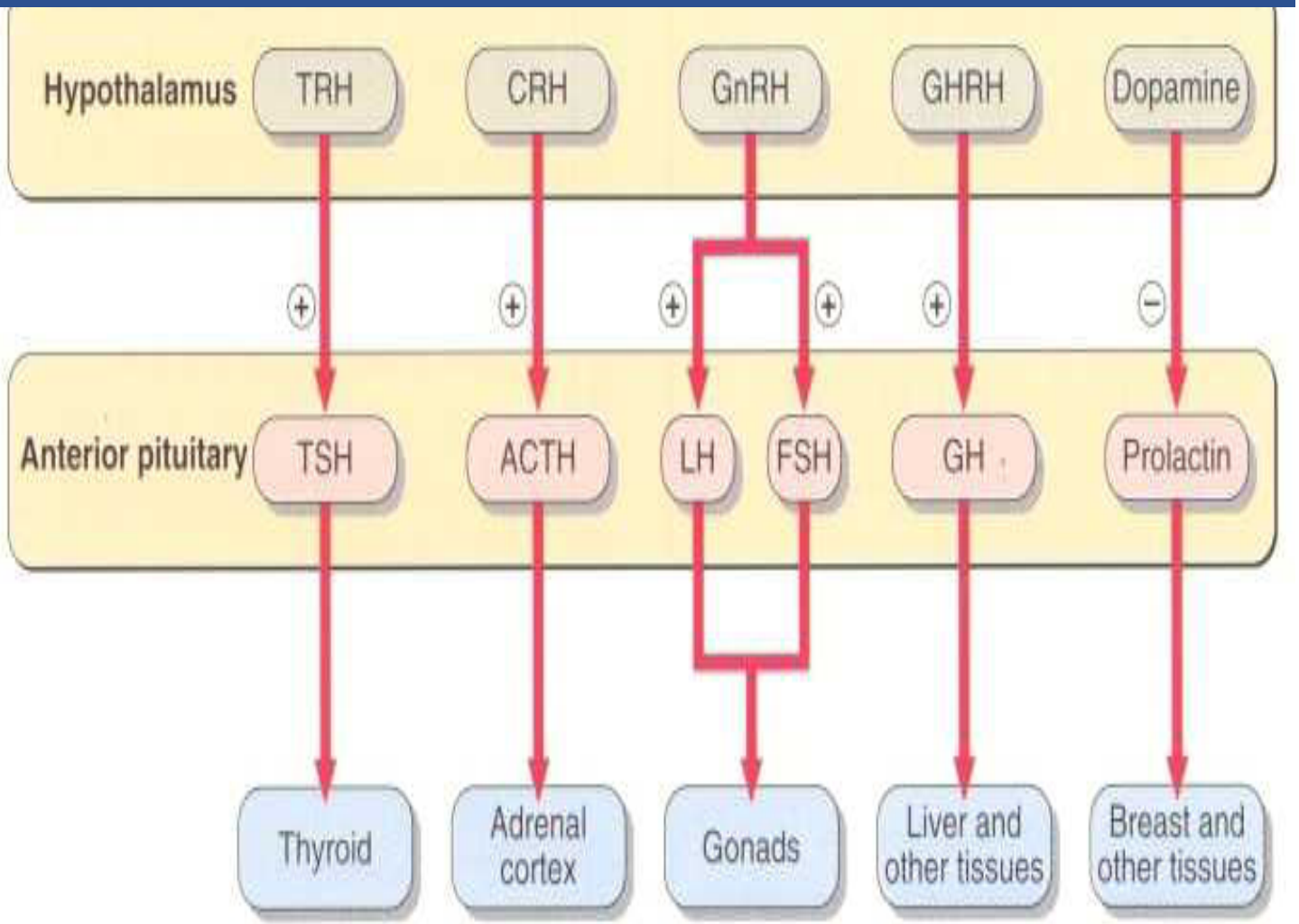


Figure 7.2 Control of pituitary hormone secretion.


Hypothalamus
TRH
CRH
GnRH
GHRH
Dopamine
+
+
+
+
+
−
Anterior pituitary
TSH
ACTH
LH
FSH
GH
Prolactin
Thyroid
Adrenal cortex
Gonads
Liver and other tissues
Breast and other tissues

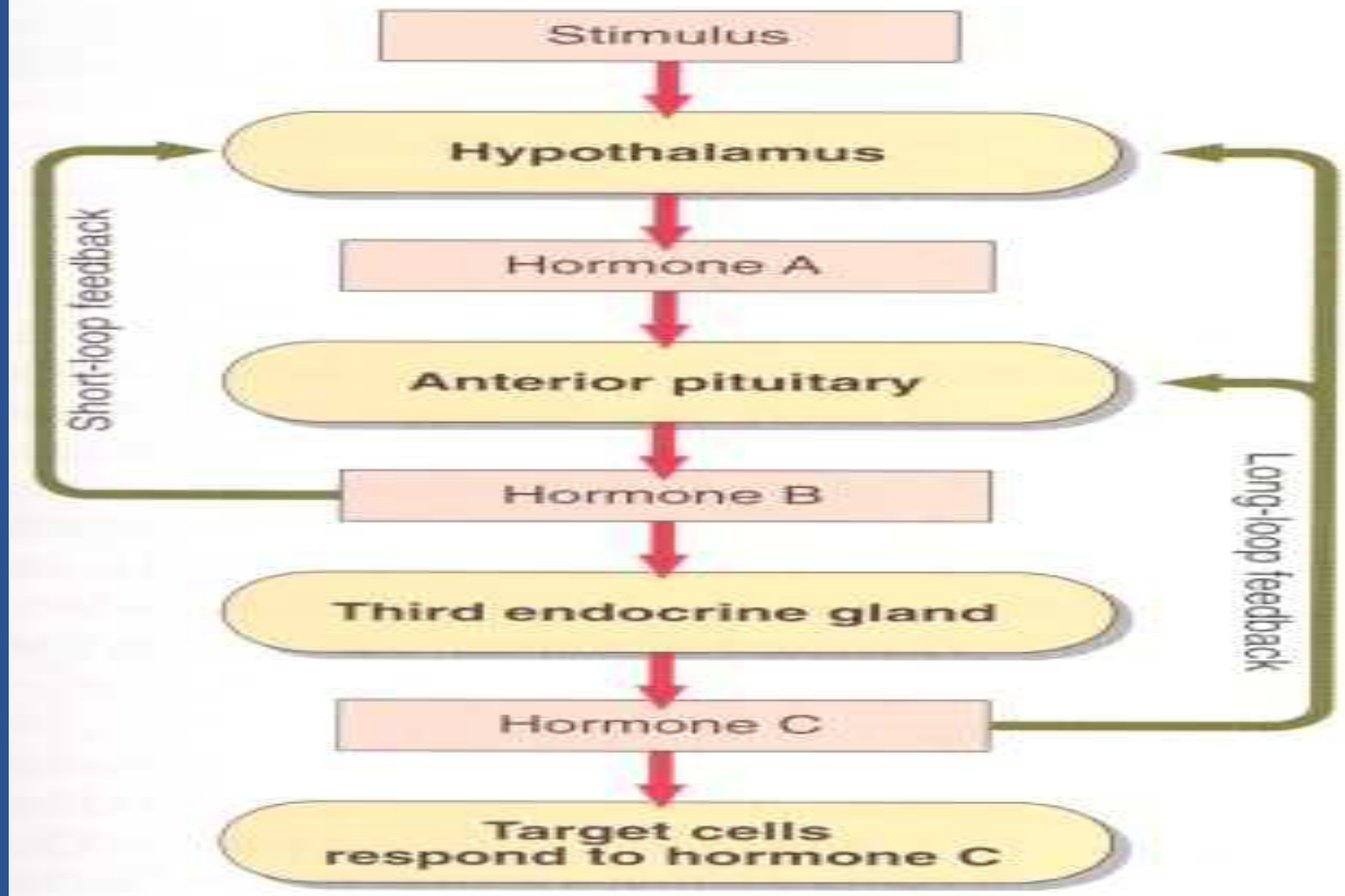


Fig. 2 **Feedback interactions in a hypothalamic-pituitary-endocrine gland system.**

Extra hypothalamic neural stimuli modify, and at times over-ride, other control mechanisms.

Physical or emotional stress and mental illness may give similar findings to, and even precipitate, endocrine disease.

The stress caused by insulin-induced hypoglycaemia is used to test anterior pituitary function.

Stress may also stimulate the secretion of ADH from the posterior pituitary.

Feedback control is mediated by the concentrations of

circulating target-cell hormones; a rising concentration

usually suppresses trophic hormone secretion.

This negative feedback may directly suppress hypothalamic hormone secretion or may modify its effect on pituitary cells (long feedback loop).

The secretion of hypothalamic hormones may

2-Inherent rhythms: hypothalamic, and consequently

pituitary, hormones are released intermittently, either

in pulses or in a regular circadian rhythm.

Disturbances of such rhythms may be of diagnostic value.

3-Drugs may also stimulate or block the action of neurotransmitters, such as catecholamines,

- Certain neuroleptic drugs, such as chlorpromazine and haloperidol, interfere with the action of dopamine. This results in reduced GH secretion

(reduced effect of releasing factor) and increased prolactin secretion (reduced inhibition).

- Bromocriptine, which has a dopamine-like action, and levodopa, which is converted to dopamine, have the opposite effect in normal subjects.
- Bromocriptine causes a paradoxical suppression of excessive GH secretion in

Evaluation of anterior pituitary function

The diagnosis of suspected hypopituitarism is best excluded by the direct measurement of pituitary hormones after stimulation or by demonstrating target gland hyposecretion after the administration of the relevant trophic hormone.

However, prolonged hypopituitarism may result in secondary failure of the target gland with diminished response to stimulation.

Laboratory tests establish only the presence or absence of hypopituitarism, and the cause must be sought by other clinical means such as radiological imaging

DISORDERS OF ANTERIOR PITUITARY HORMONE SECRETION

The main clinical syndromes associated with excessive or deficient anterior pituitary hormone secretion are shown in Table 7.1.

Excessive secretion usually involves a single hormone, but deficiencies are often multiple. However, many pituitary tumours are non-secretory and may present clinically with eye signs or headaches.

Table 7.1 Disorders associated with primary abnormalities of anterior pituitary hormone secretion

Hormone	Excess	Deficiency
Growth hormone	Acromegaly or gigantism	Short stature
Prolactin	Amenorrhoea Infertility Galactorrhoea Osteopenia	Lactation failure
Adrenocorticotrophic hormone (corticotrophin)	Cushing's disease	Secondary adrenal hypofunction
Thyroid-stimulating hormone	Hyperthyroidism (very rare)	Secondary hypothyroidism
Luteinizing hormone/ follicle-stimulating hormone	Precocious puberty	Secondary hypogonadism Infertility

Growth hormone

Growth hormone secretion from the anterior pituitary gland is mainly controlled by hypothalamic GH releasing hormone (GHRH).

After synthesis by the hypothalamus, this is transported via the hypothalamic portal system to the somatotrophs of the anterior pituitary.

Secretion of GHRH, and therefore of GH, is pulsatile, occurring about seven or eight times a day, usually associated with:

- exercise,
- onset of deep sleep,
- in response to the falling plasma glucose concentration about an hour after meals.

At other times, plasma concentrations are usually very low or undetectable, especially in children.

Growth hormone release is inhibited in a negative feedback pathway by another

Somatostatin is found not only in the hypothalamus and elsewhere in the brain, but also in the gastrointestinal tract and pancreatic islet cells, where it inhibits the secretion of many gastrointestinal hormones.

Insulin like growth factor 1 (IGF-1) acts by feedback to inhibit GHRH action.

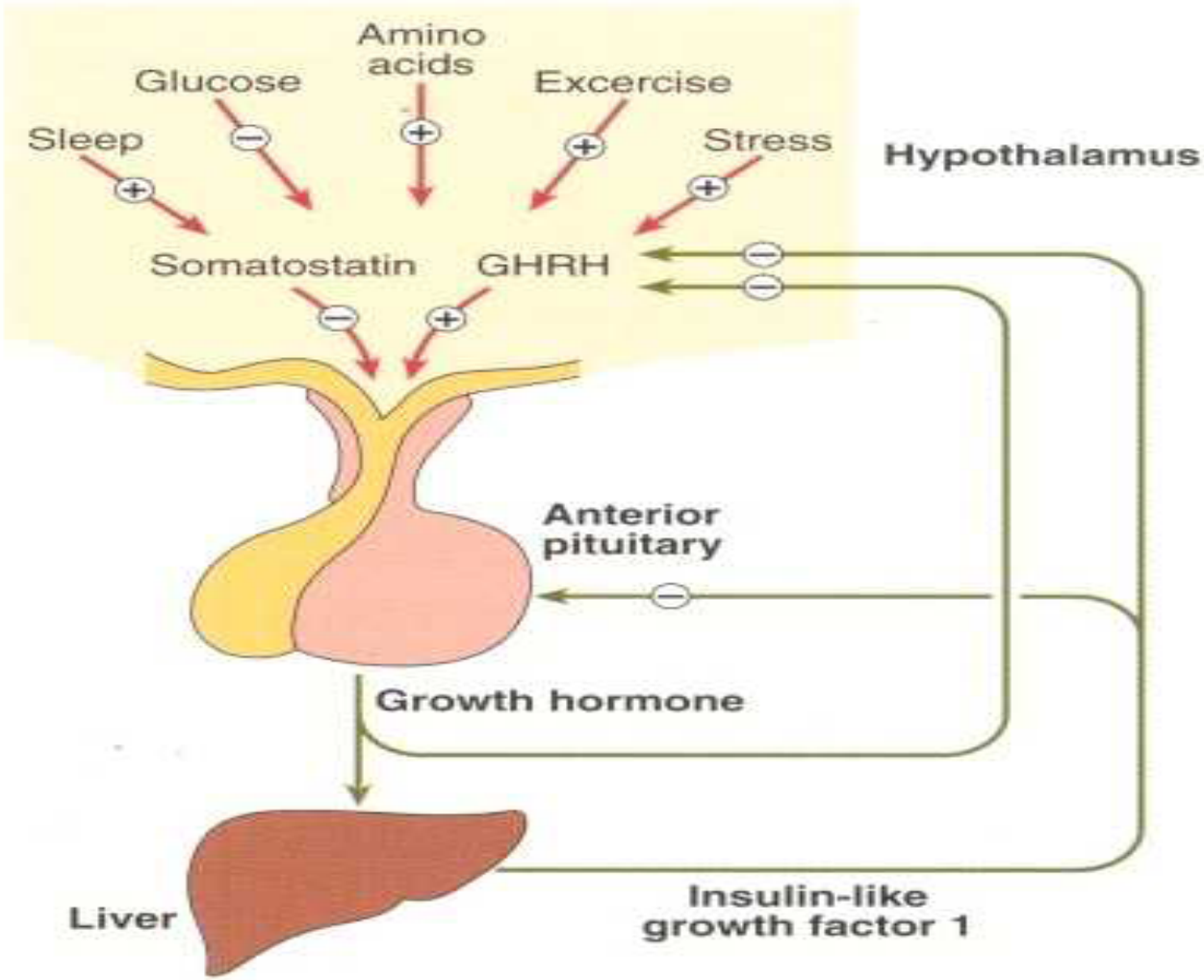


Fig. 2 **The normal regulation of GH secretion.**

Growth hormone secretion may be stimulated by:

- stress, one cause of which is hypoglycaemia,
- glucagon,
- some amino acids, for example arginine,
- drugs such as levodopa and clonidine.

All these stimuli have been used to assess GH secretory capacity, which may also be impaired in obese patients, in hypothyroidism and hypogonadism, in some cases of Cushing's syndrome and in patients receiving large doses of

Actions of growth hormone

action is primarily mediated by IGFs, polypeptides that are synthesized in many tissues, where they act locally.

Plasma concentrations of one of these, IGF-1 (also known as somatomedin C), correlate with GH secretion.

Carbohydrate metabolism is affected by GH: GH antagonizes the insulin-mediated cell uptake of glucose,

and excess secretion may produce glucose intolerance.

The production of IGF-1 is also influenced by other

factors, the most important of which is nutritional status.

In undernutrition, plasma concentrations are

low, whereas GH concentrations are elevated, suggesting that plasma IGF-1 may influence GH secretion by negative feedback.

Other factors, such as adequate nutrition and T4, are also needed for normal growth.

Growth hormone excess: gigantism and acromegaly

Growth hormone excess causes gigantism during childhood and acromegaly in adults.

Most patients with GH excess have acidophil adenomas of the anterior pituitary gland, which may be secondary to excessive hypothalamic stimulation.

Rarely, malignant tumours may release GH or GHRH.

- Acromegaly is sometimes one of the manifestations of multiple endocrine neoplasia (MEN).
- The clinical manifestations of GH excess depend on whether the condition develops before or after fusion of the bony epiphyses.
- Gigantism is caused by excess GH secretion in childhood before fusion of the epiphyseal plates, which may be delayed by accompanying hypogonadism.

The features of acromegaly may include the following;

- An increase in the bulk of bone and soft tissues with enlargement of, for example, the hands, tongue, jaw and heart.

Changes in facial appearance , due to the increasing size of the jaw and sinuses;

Excessive hair growth, hyperhidrosis and sebaceous gland secretion are common.

- Menstrual disturbances are common in females.
- Impaired glucose tolerance is present in about25 per cent of patients, about half of whom develop symptomatic diabetes mellitus. In most cases the pancreas can secrete enough insulin to overcome the antagonistic

- Hyperphosphataemia, hypercalcaemia and hypertriglyceridaemia may also be present.
- Many of these features are due to the action of IGF-1, which acts as a general growth factor.
- Compression of the optic chiasma may cause visual field defects such as bitemporal hemianopsia.

- If destruction of the gland progresses, other anterior pituitary hormones such as ACTH, LH, FSH and TSH may become deficient .
- Plasma prolactin concentrations may, however, be raised as prolactin differs from all other pituitary hormones in its method of control.
- Secretion is inhibited, not stimulated, by dopamine; therefore, impairment of hypothalamic control causes hyperprolactinaemia.

Diagnosis

- The diagnosis of GH excess is suggested by the clinical presentation, biochemical tests and radiological findings of the pituitary.

- Magnetic resonance imaging (MRI) is more sensitive than computerized tomography (CT) scanning.

- Random GH measurements are often not diagnostic owing to episodic secretion and a

- The diagnosis is confirmed by demonstrating a raised plasma GH concentration that is not suppressed by a rise in plasma glucose concentration.
- In normal subjects, plasma GH concentrations fall to very low levels – to below 1 mg/L after a 75 g oral glucose load.
- In acromegaly, the secretion of GH is autonomous and this fall may not occur or be only slight, or there may even be a paradoxical rise.

Glucose suppression test for suspected acromegaly

- Procedure

After an overnight fast,insert IV canula, After at least 30 min why?, take basal samples for plasma glucose and GH estimation.

The patient should drink 75 g of glucose dissolved in about 300 mL of water, or an equivalent glucose load.

Take samples for glucose and GH assays at 30, 60, 90 and 120 min after the glucose load has been taken.

interpretation

In normal subjects, plasma GH concentrations fall to undetectable levels.

Although failure to suppress suggests acromegaly or gigantism, it may be found in some patients with severe liver or renal disease, in heroin addicts or in those taking levodopa.

The plasma glucose concentrations may demonstrate impaired glucose tolerance or diabetes mellitus in acromegaly.

- If acromegaly is confirmed, it is wise to

-
- Plasma IGF-1 has a long half-life and may be used in screening for acromegaly.

- Plasma concentrations correlate with the activity of the disease.
- Measurement of the plasma concentrations of GH, or of IGF-1, may be used to monitor the efficacy of treatment.
- Remember that pregnancy increases IGF-1 concentration, and starvation, obesity and diabetes mellitus decrease it

- Insulin-like growth-factor-binding protein-3 is the main binding protein for IGF-1 and its concentration is also increased in acromegaly.
- Sometimes plasma GHRH concentrations are useful and can be raised where there is an ectopic source or may be suppressed in pituitary disease.
- Computerized tomography or MRI body scanning may help to find an ectopic source of GH or GHRH.

Treatment

- 1-surgery to remove the adenoma,
- 2-medical therapy, usually with either bromocriptine or cabergoline (dopamine receptor agonists)
- or somatostatin analogues (somatostatin itself has too short a half-life for effective therapeutic use).
- Octreotide or lanreotide, which bind to somatostatin receptors, can be used or pegvisomant (GH receptor antagonist).

Growth hormone deficiency

- In adults, GH deficiency may cause clinical symptoms, such as tiredness, dyslipidaemia and increased cardiovascular disease.
- Growth hormone deficiency can cause short stature in children.
- It is important to investigate children with reduced growth rate to identify those who may benefit from recombinant human GH replacement treatment.
- Isolated GH deficiency is most commonly secondary to idiopathic deficiency of

Diagnosis

- It is, important to exclude hypothyroidism, chronic diseases and malabsorption states, poor nutritional state and failure to thrive.
- Clinical examination should assess for obvious syndromes, pubertal status, bone age, growth or growth velocity .
- Karyotyping may be indicated if a chromosomal disorder such as Turner's syndrome (45,X0) is suspected.
- There is a physiological reduction in GH secretion at the end of pre-puberty. Thus, in

- Plasma GH concentrations in normal children are often low and assays under basal conditions rarely exclude the diagnosis.
- A low plasma IGF-1 concentration may be a useful screening test.
- Urinary GH excretion, either in 24-h collections or timed overnight, may offer a relatively safe screening test.

- If blood is taken at a time when

- If GH deficiency is not excluded by the above measurements, it is necessary to perform one or more stimulation tests.
- The response of GH to insulin may be the most reliable to detect GH deficiency, but it is not without the risk of fatal hypoglycaemia.
- Glucagon could also be used as an alternative (see Glucagon stimulation test of the hypothalamus–pituitary axis).
- A GH absolute response of greater than 20 mU/L (7 μg/L) makes GH deficiency unlikely.

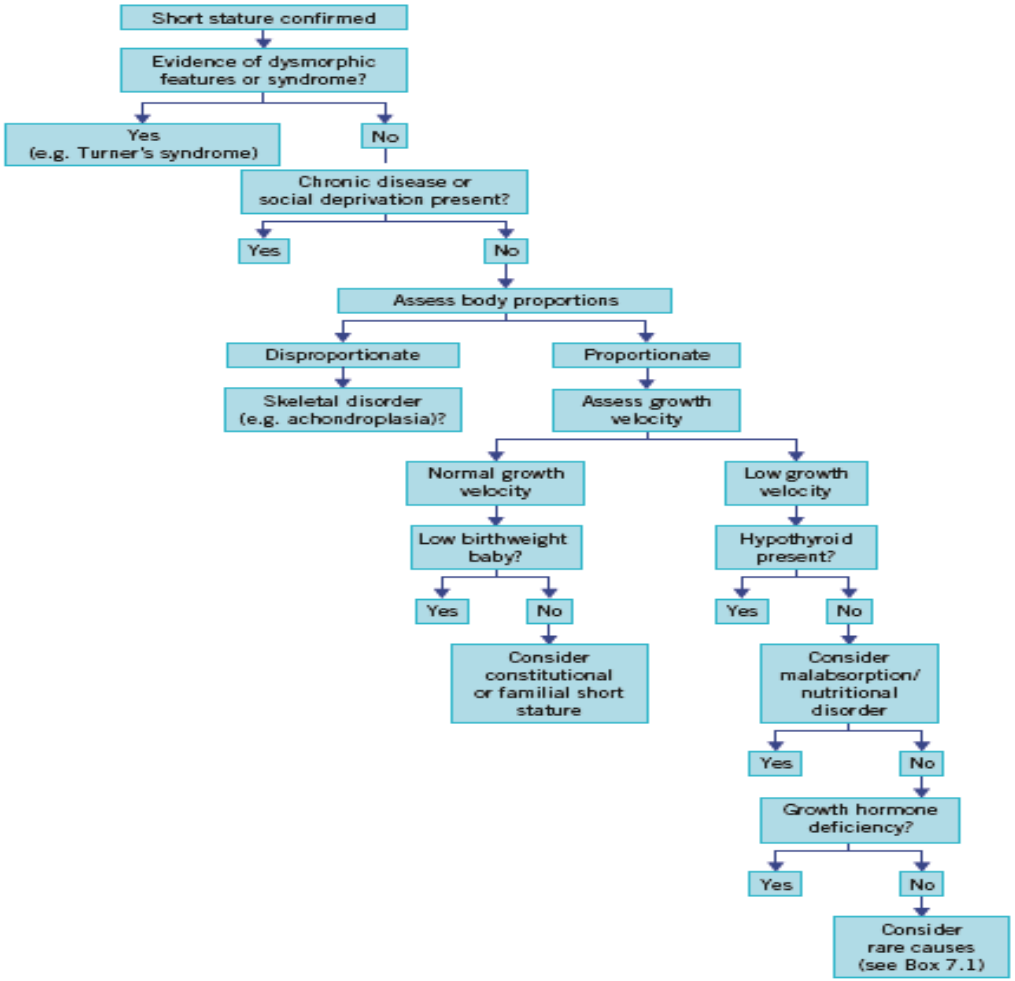


Figure 7.5 Algorithm for the investigation of short stature.

Box 7.1 Some causes of growth retardation and short stature

Familial short stature
Social/emotional deprivation
Undernutrition and chronic disease
- Coeliac disease
- Rickets
- Chronic kidney disease

Endocrine disorders
- Growth hormone deficiency, congenital or acquired
- Hypothyroidism
- Cushing's syndrome
- Congenital adrenal hyperplasia

Chromosomal abnormalities
- Turner's syndrome (45,X0)

Skeletal disorders
- Achondroplasia,

Laron-type dwarfism and Pygmies

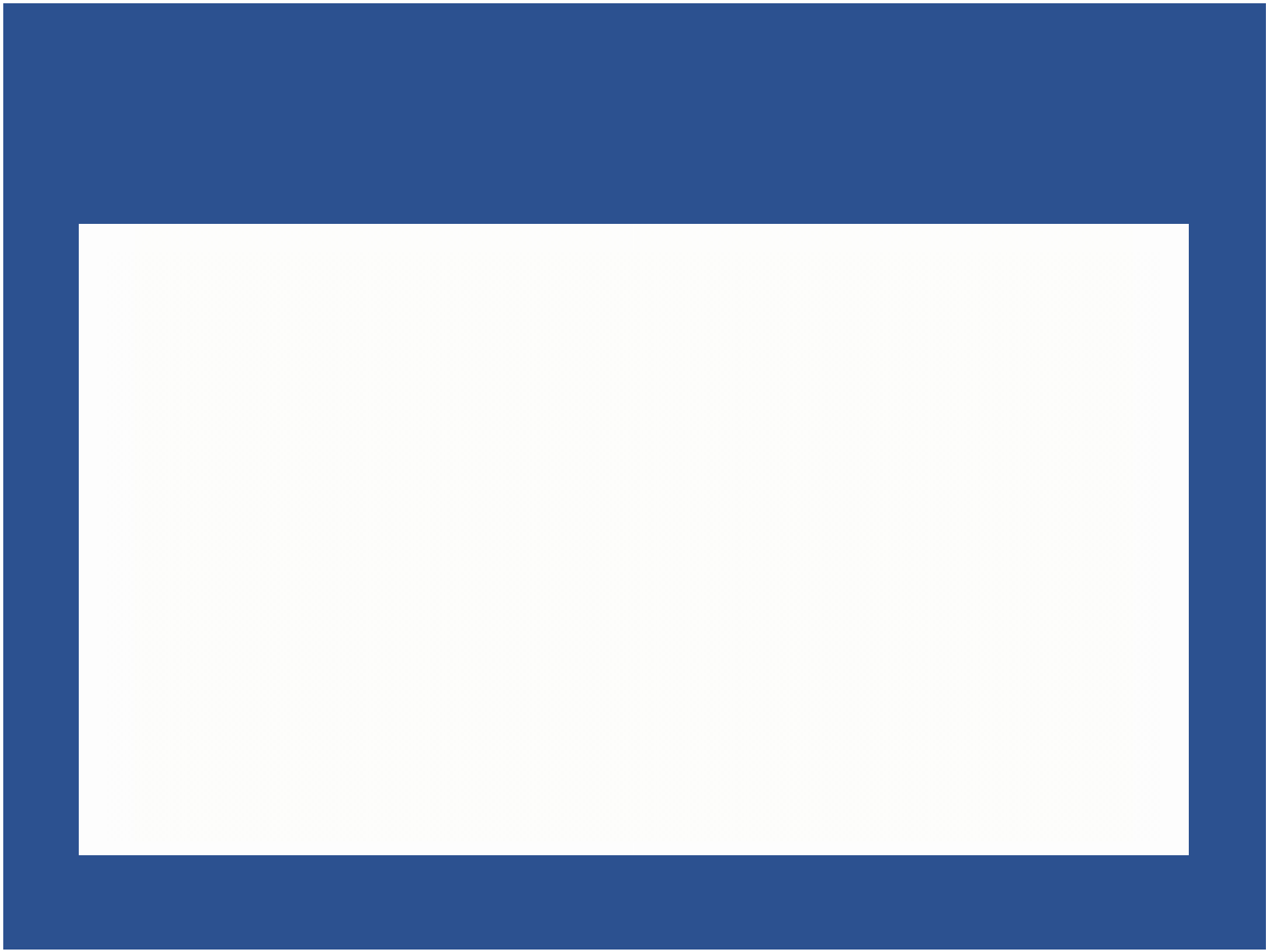
DISORDERS OF POSTERIOR PITUITARY HORMONE SECRETION

- Disorders of the posterior pituitary are rare compared with those of the anterior pituitary.
- Deficiency of ADH in diabetes insipidus may present as polyuria.
- In the syndrome of inappropriate ADH, hyponatraemia due to water excess occurs.

HYPOPITUITARISM

- Hypopituitarism is a syndrome of deficiency of pituitary hormone production that may result from disorders of the hypothalamus, pituitary or surrounding structures.

- Clinical features of deficiency are usually absent until about 70 % of the gland has been destroyed, unless there is associated hyperprolactinaemia, when amenorrhoea and infertility may be early symptoms.



- Panhypopituitarism with the full clinical picture described below is uncommon.
- Suspicion of anterior pituitary hypofunction usually arises in patients presenting with various features such as clinical and radiological evidence of a pituitary or localized brain tumour,
- hypogonadism, adrenocortical insufficiency, short stature caused by GH deficiency, and hypothyroidism.
- Gonadotrophins are often the first to decrease in hypopituitarism and it is unusual for the

Consequences of pituitary hormone deficiencies

- Progressive pituitary damage usually presents with evidence of deficiencies of gonadotrophins and GH.

- Plasma ACTH and/or TSH concentrations may remain normal, or become deficient months or even years later.

The clinical and biochemical consequences of the target-gland failure include the following:

- *Growth retardation in children* This may be due to deficiency of GH; deficiency of TSH, and therefore of thyroid hormone, may contribute.
- *Secondary hypogonadism* This is due to gonadotrophin deficiency, presenting as amenorrhoea, infertility and atrophy of secondary sexual characteristics with loss of axillary and pubic hair and impotence or loss of libido. Puberty is delayed in children.
- *Secondary adrenocortical hypofunction*

- However, cortisol is needed for normal free water excretion, and consequently there may be a dilutional hyponatraemia due to cortisol deficiency.
- Cortisol is also necessary for the maintenance of normal blood pressure. Hypotension may be associated with ACTH deficiency.
- Cortisol and/or GH deficiency may cause increased insulin sensitivity with fasting hypoglycaemia.

- *Secondary hypothyroidism* (TSH deficiency) This may sometimes be clinically indistinguishable from primary hypothyroidism.
- *Prolactin deficiency* Associated with failure to lactate, this may occur after post-partum pituitary infarction (Sheehan's syndrome).
- However, in hypopituitarism due to a tumour, plasma prolactin concentrations are often raised and may cause galactorrhoea (secretion of breast fluid)

Pituitary tumours

- The clinical presentation of pituitary tumours depends on the type of cells involved and on the size of the tumour (microadenomas less than 10 mm and macroadenomas more than 10 mm).
- Tumours of secretory cells may produce the clinical effects of excess hormone secretion:
- excess prolactin causes infertility, amenorrhoea and varying degrees of galactorrhoea .

- Non-secreting tumours are difficult to diagnose using biochemical tests, although the combined pituitary stimulation test may indicate subclinical impairment of function.
- Hyperprolactinaemia, which may be asymptomatic, is a valuable biochemical marker of the presence of a pituitary tumour.
- Prolactin may be secreted by the tumour cells or by unaffected lactotrophs if tumour growth interferes with the normal inhibition of prolactin secretion

Investigation of suspected hypopituitarism

- Deficiency of pituitary hormones causes hypofunction of the target endocrine glands.
- Investigation aims to confirm such deficiency, to exclude disease of the target gland and then to test pituitary hormone secretion after maximal stimulation of the gland.
- Measurement should be made of the plasma concentrations of:
- LH, FSH and oestradiol (female) or testosterone (male),

- If the plasma concentration of the target gland hormone is low and the concentration of trophic hormone is raised, the affected target gland should be investigated.
- Conversely, if the plasma concentrations of both the target gland and trophic hormones are low or low-normal, consider a pituitary stimulation test .
- Investigation of the pituitary region using radiological techniques such as CT or MRI scanning may help elucidate a cause of the

Insulin tolerance or insulin stimulation test

- This test is potentially dangerous and must be done under direct medical supervision due to severe hypoglycaemia and the test should be carried out only in specialist units by experienced staff.
- It is contraindicated in the following patient groups: the elderly, patients with ischaemic heart disease, epilepsy or severe panhypopituitarism, and patients in whom plasma cortisol at 09.00 h is less than 100 nmol/L.

- A resting electrocardiogram should be normal.
- Hypothyroidism should be treated before hand as this can impair the cortisol and GH responses.
- However, note that treatment with thyroxine can precipitate an adrenal crisis in such patients and thus corticosteroid replacement is also necessary.
- Indications of the insulin stimulation test may include:

Procedure

- After an overnight fast, fter at least 30 min, take basal samples at time 0 min for cortisol, GH and glucose.
- Inject soluble insulin in a dose sufficient to lower plasma glucose concentrations to less than 2.5 mmol/L and evoke symptomatic hypoglycaemia.
- The recommended dose of insulin must be adjusted for the patient's body weight and for

Interpretation

- Interpretation is not possible if hypoglycaemia is not attained, and the dose of insulin can cautiously be repeated if this is not attained in the 45-min blood sample.
- If hypoglycaemia has been adequate, plasma cortisol concentrations should rise by more than 200 nmol/L and exceed 580 nmol/L, and an adequate GH response occurs with an absolute response of greater than 20 mU/L (7 μg/L).
- In Cushing's syndrome, neither plasma

Glucagon stimulation test of the hypothalamus–pituitary axis

- This test is useful if the insulin hypoglycaemic test is contraindicated.
- However, it is essential that the test is carried out in a specialist unit by experienced staff.
- The basic principle is that glucagon stimulates GH and ACTH release probably via a hypothalamic route.
- The test is contraindicated if there is severe adrenal failure, for example if cortisol at 09.00 h is less than 100 nmol/L or in

- ***Procedure***
- Patients should fast overnight, although they can drink water.
- For adults, 1 mg of glucagon is injected subcutaneously at 09.00 h.
- Blood samples are taken at 0, 90, 120, 150, 180, 210 and 240 min for cortisol and GH.

- ***Interpretation***
- Plasma cortisol should normally rise by at least 200 nmol/L to more than 580 nmol/L, and an adequate GH response occurs with an absolute response of greater than 20 mU/L (7 μg/L).

- **Treatment of hypopituitarism**
- This consists of specific therapy depending on its cause and may include surgical removal of a large adenoma.
- If the ACTH axis is impaired, it is essential to prescribe a glucocorticoid, for example hydrocortisone in the acute situation or prednisolone for maintenance.
- Secondary hypothyroidism will need thyroid replacement. Adrenal replacement should