ENDOCRINE PHYSIOLOGY

DR. ABDULMAJEED AL-DREES

OBJECTIVES

- By the end of this lecture, students should be able to describe :
- Hormones
  - Definition
  - Chemical structure
  - Paracrine and autocrine
- Secretion and clearance of hormones
- Mechanism of action of hormones
  - Hormone receptors, down-regulation and up-regulation
  - Intracellular signaling
  - Second messenger mechanism (cAMP, IP_3)

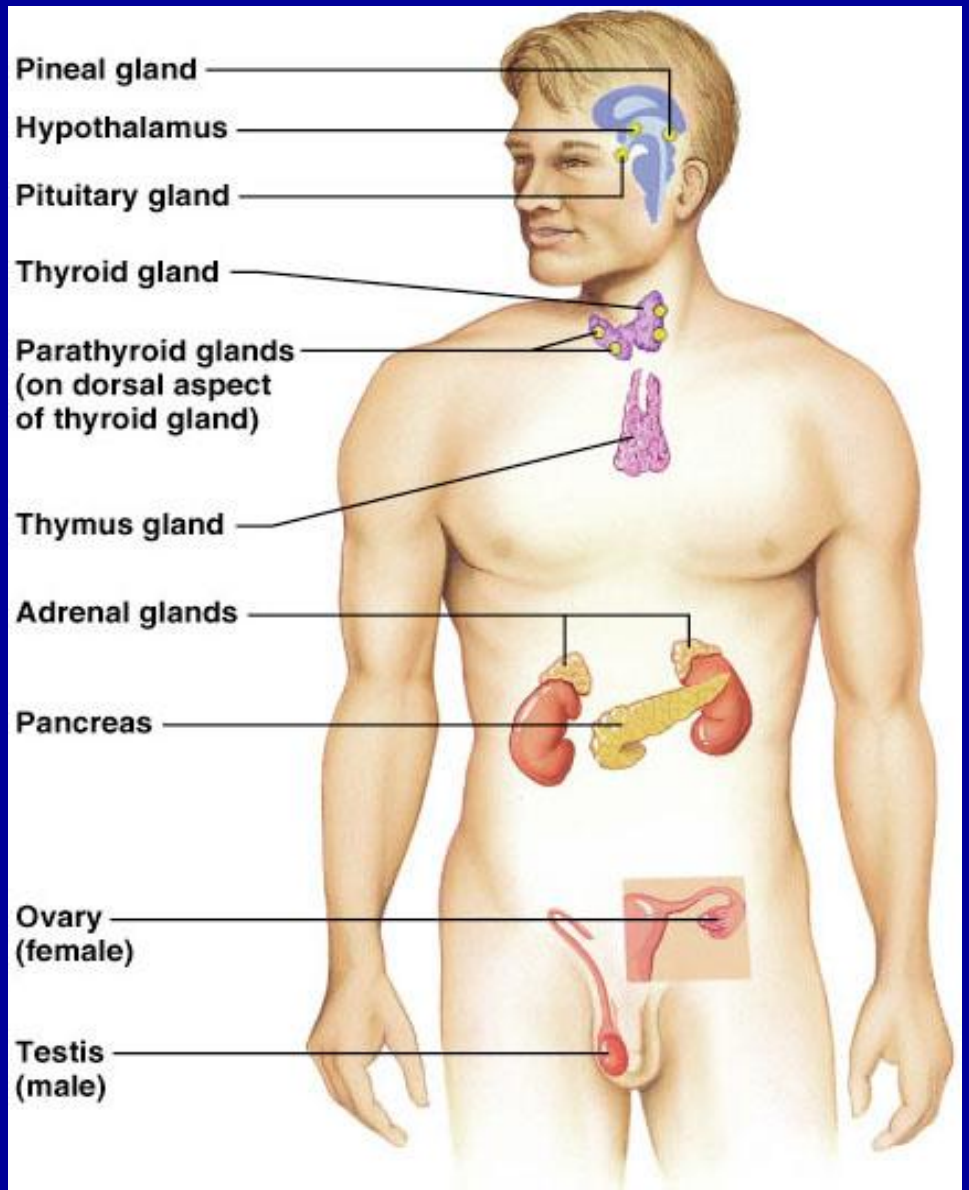
GLANDS

- **Exocrine gland.**
- **Endocrine gland.**

What is hormone?.

- **Chemical substance secreted in a small amount from endocrine gland directly to the blood stream in response to stimulus to cause physiological responses at the target tissues.**

ENDOCRINE GLANDS



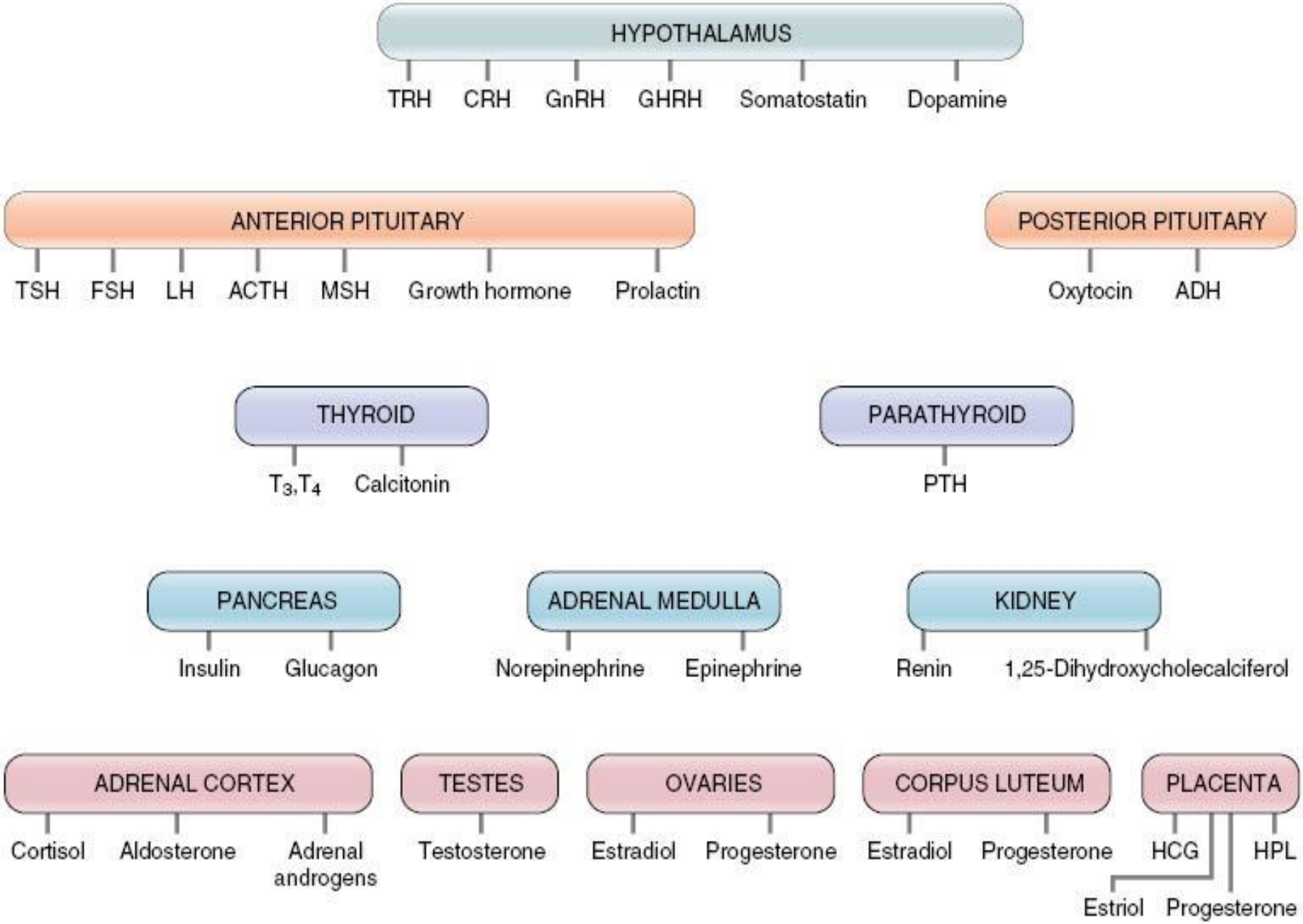


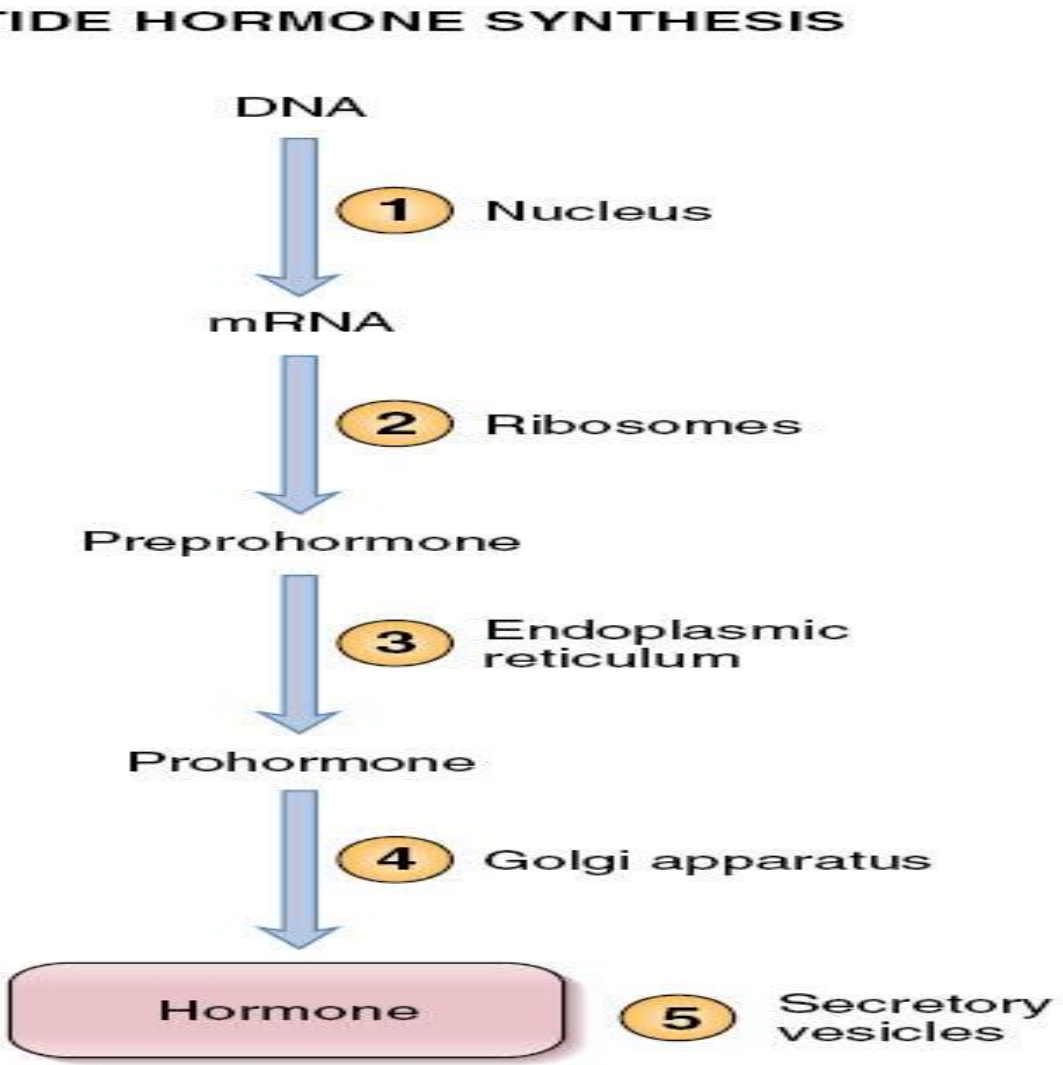
Table 9-1 Commonly Used Abbreviations in Endocrine Physiology

Abbreviation	Hormone	Abbreviation	Hormone
ACTH	Adrenocorticotropic hormone	LH	Luteinizing hormone
ADH	Antidiuretic hormone	MIT	Monoiodotyrosine
CRH	Corticotropin-releasing hormone	MSH	Melanocyte-stimulating hormone
DHEA	Dehydroepiandrosterone	PIF	Prolactin-inhibiting factor
DIT	Diiodotyrosine	POMC	Pro-opiomelanocortin
DOC	11-Deoxycorticosterone	PTH	Parathyroid hormone
FSH	Follicle-stimulating hormone	PTU	Propylthiouracil
GHRH	Growth hormone–releasing hormone	SRIF	Somatotropin release–inhibiting factor
GnRH	Gonadotropin-releasing hormone	T_3	Triiodothyronine
HCG	Human chorionic gonadotropin	T_4	Thyroxine
HGH	Human growth hormone	TBG	Thyroxine-binding globulin
HPL	Human placental lactogen	TRH	Thyrotropin-releasing hormone
IGF	Insulin-like growth factor	TSH	Thyroid-stimulating hormone

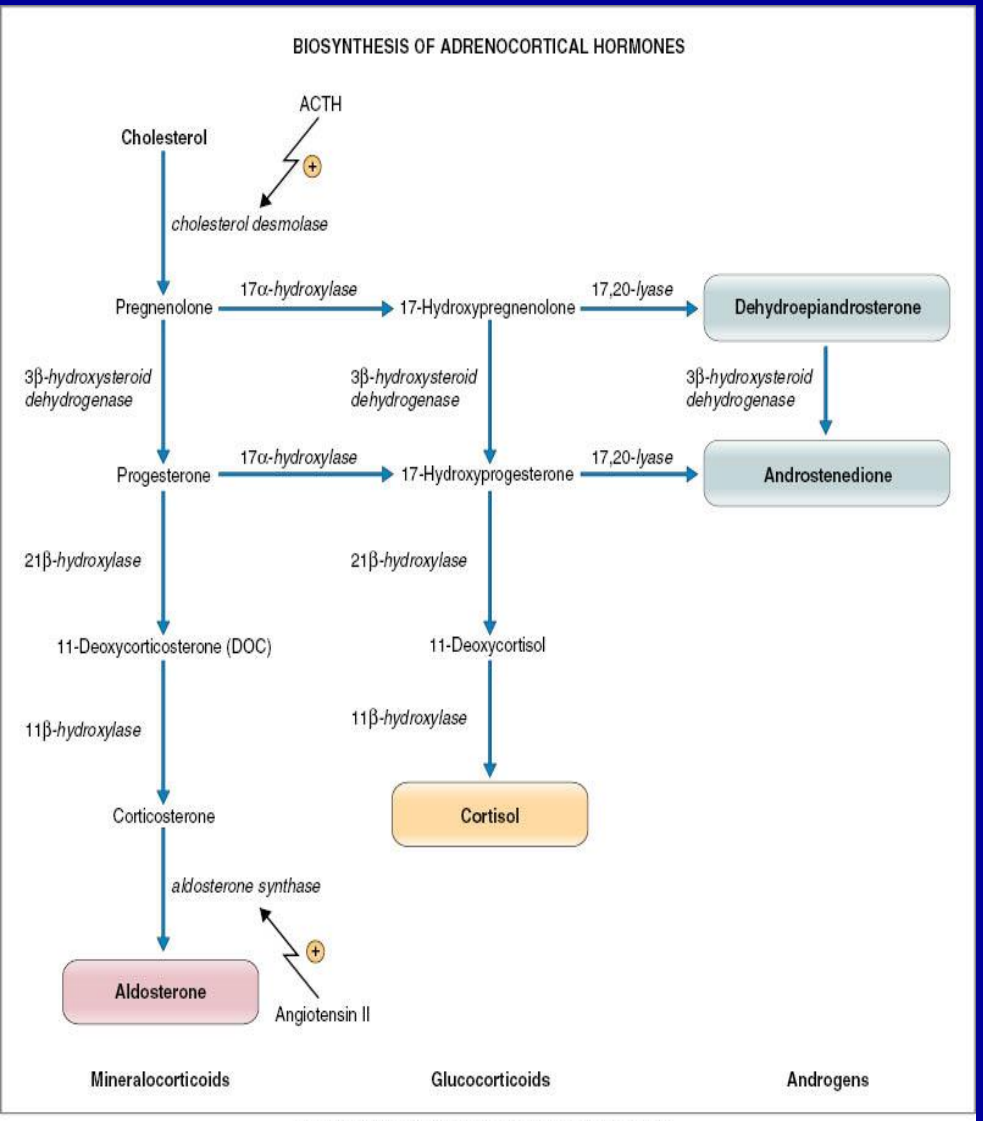
CHEMICAL CLASSIFICATION OF HORMONES

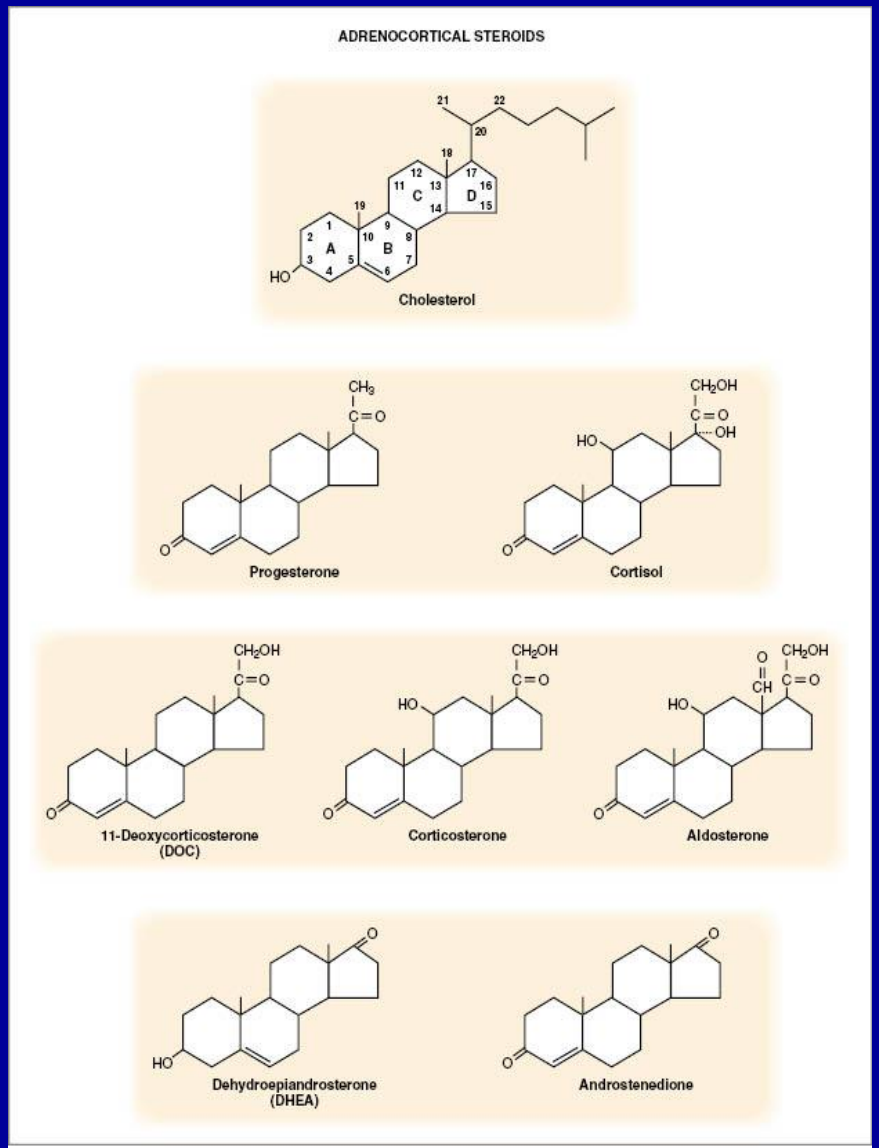
- **Peptides or proteins hormones.**
- **Steroid hormones.**
- **Amine hormones.**

HORMONES SYNTHESIS

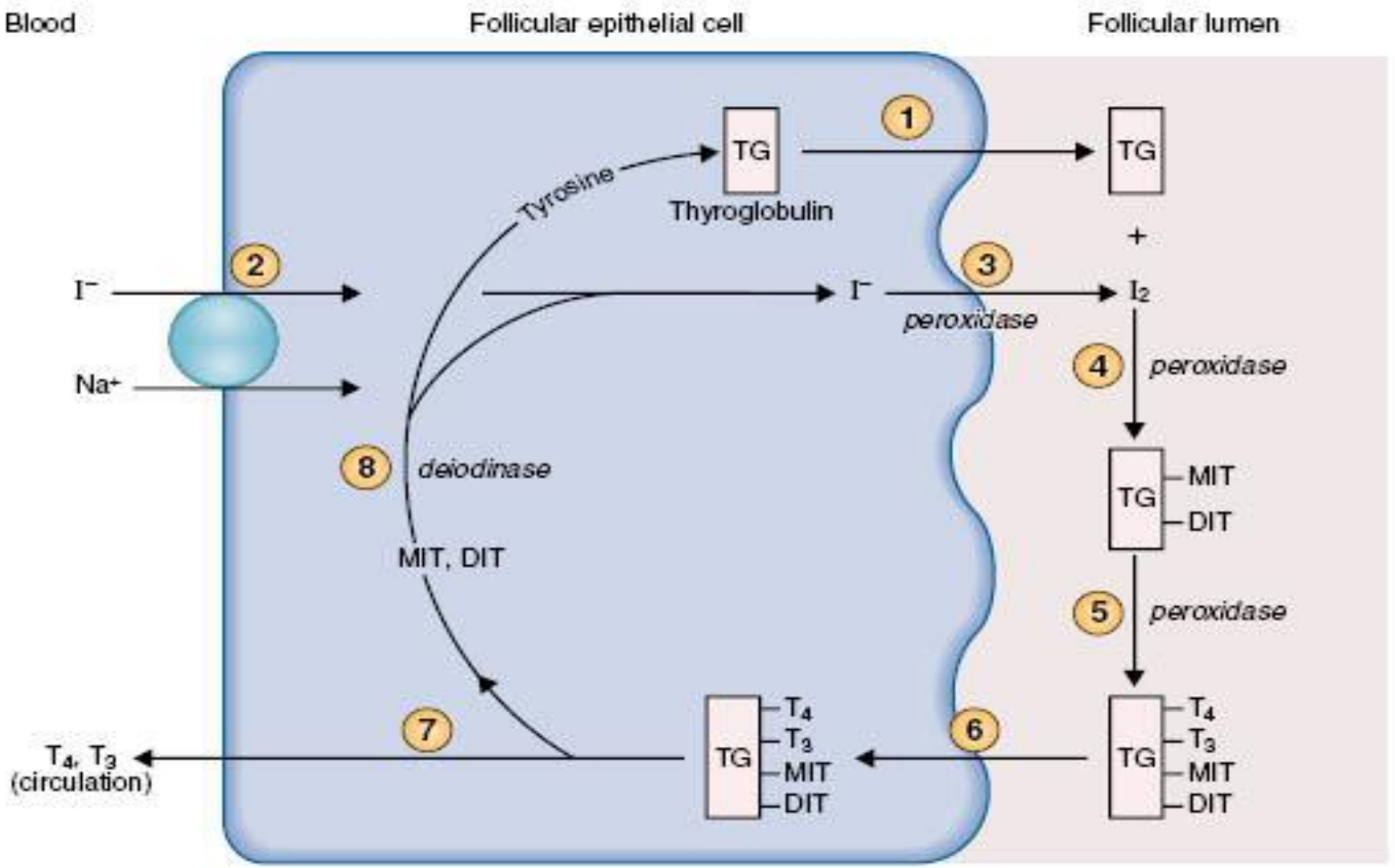


STEROIDS HORMONES





AMINE HORMONE

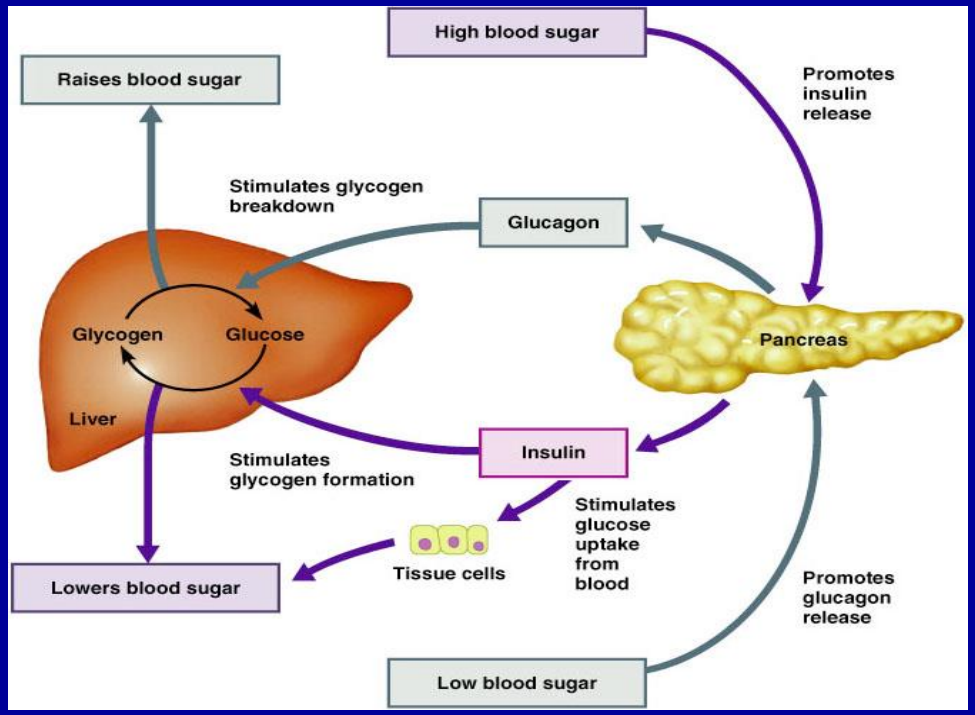


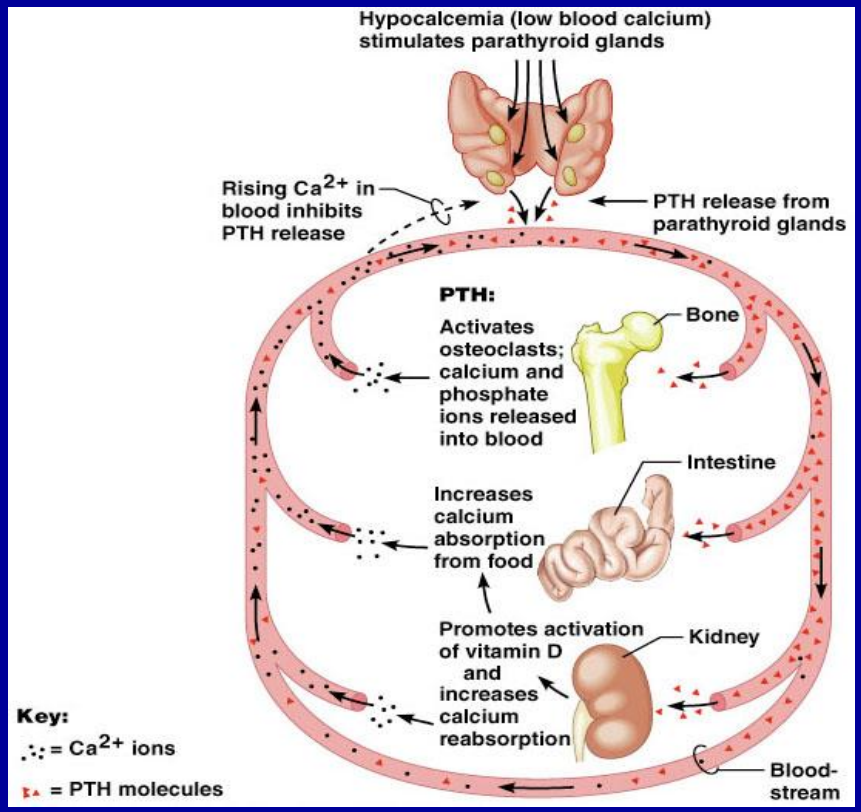
CLASSIFICATION OF STIMULI

- **Humoral Stimuli.**
- **Neural Stimuli.**
- **Hormonal Stimuli.**

Humoral Stimuli

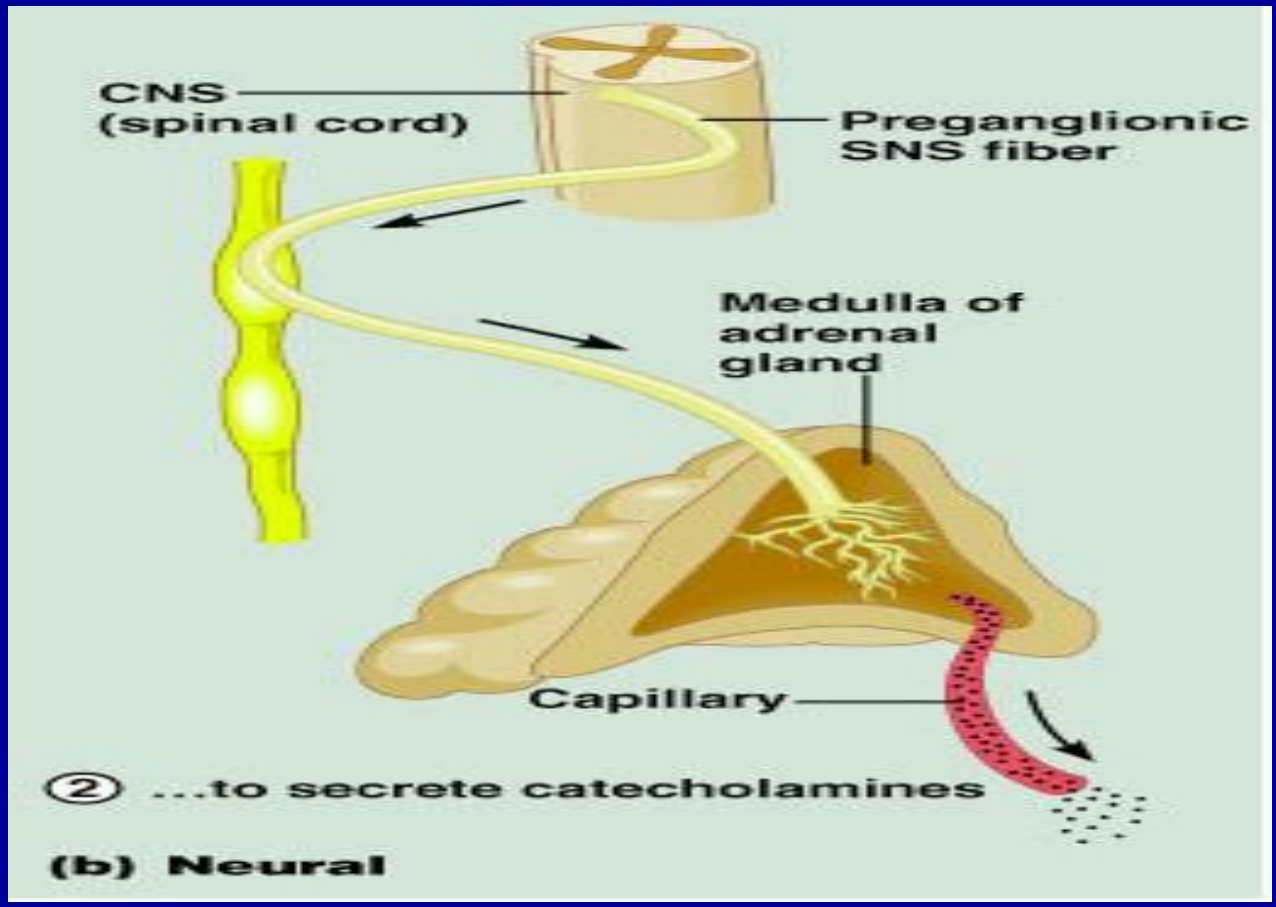
- Secretion of hormones in direct response to changing in blood levels of ions and nutrients





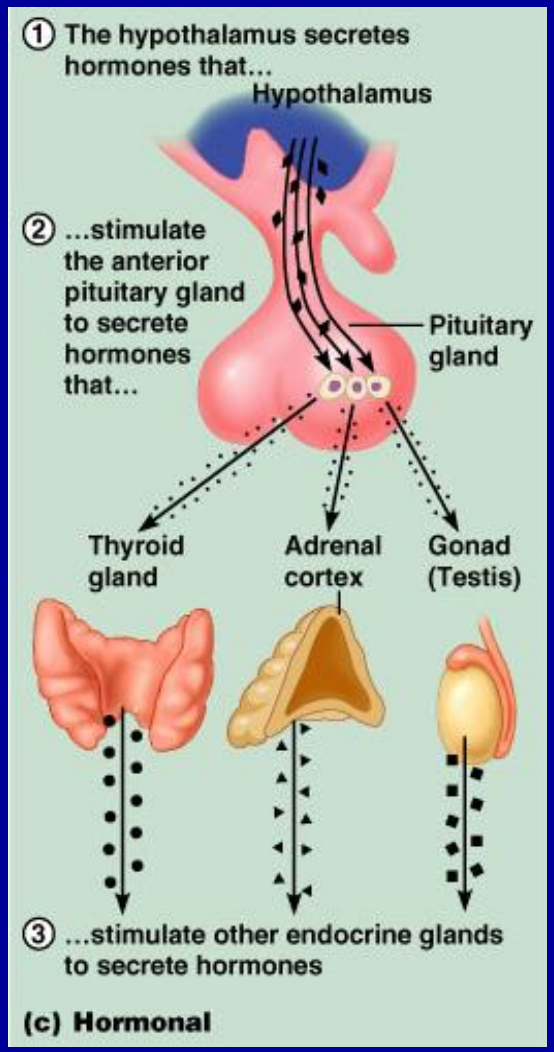
Neural Stimuli

- **Nerve fibers stimulate hormone release.**



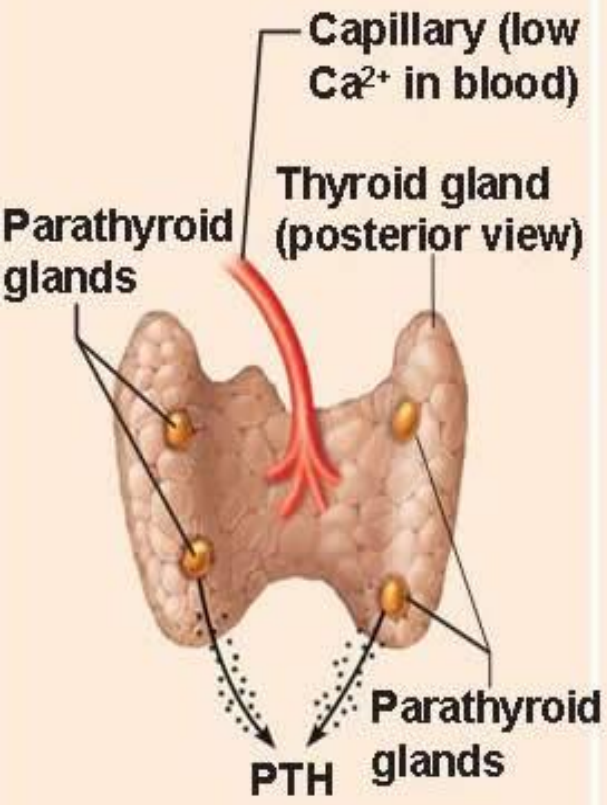
Hormonal Stimuli

- Release of hormones in response to hormones produced by other endocrine gland.



(a) Humoral Stimulus

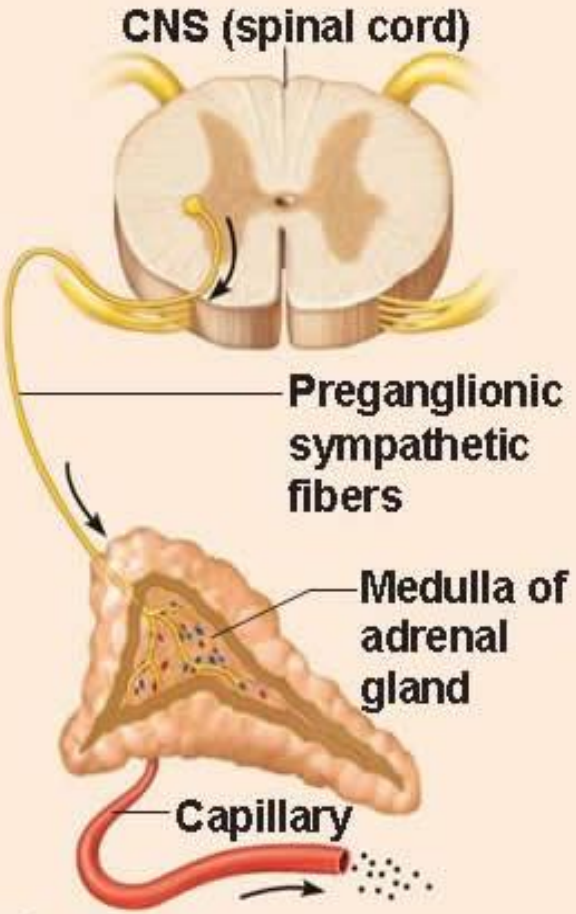
1 Capillary blood contains low concentration of Ca^{2+}, which stimulates...



2 ...secretion of parathyroid hormone (PTH) by parathyroid glands*

(b) Neural Stimulus

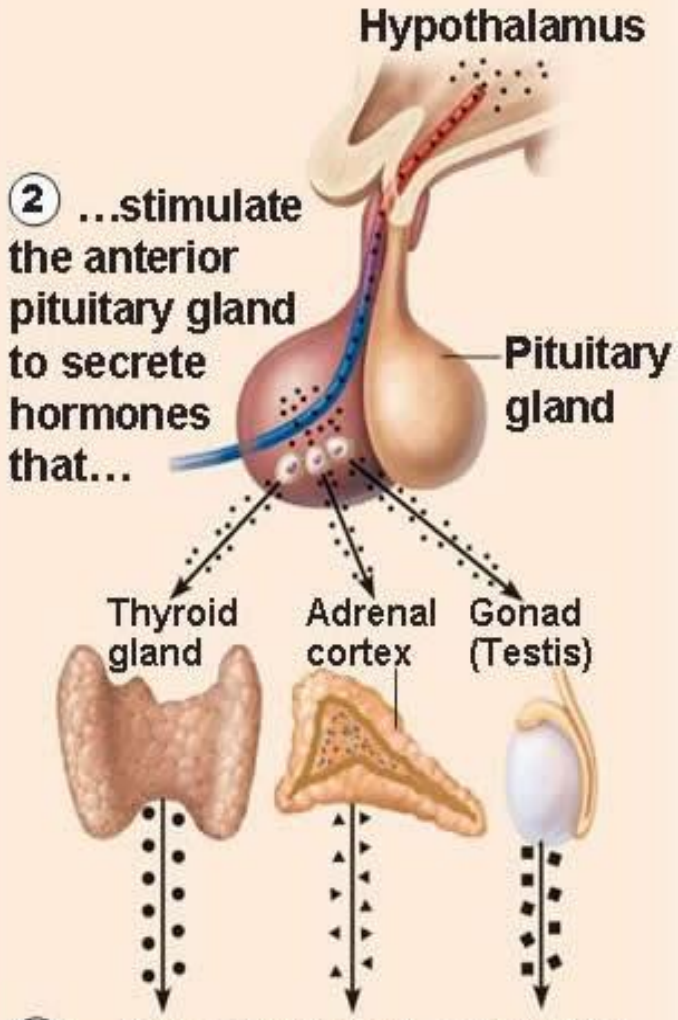
1 Preganglionic sympathetic fibers stimulate adrenal medulla cells...



2 ...to secrete catecholamines (epinephrine and norepinephrine)

(c) Hormonal Stimulus

1 The hypothalamus secretes hormones that...



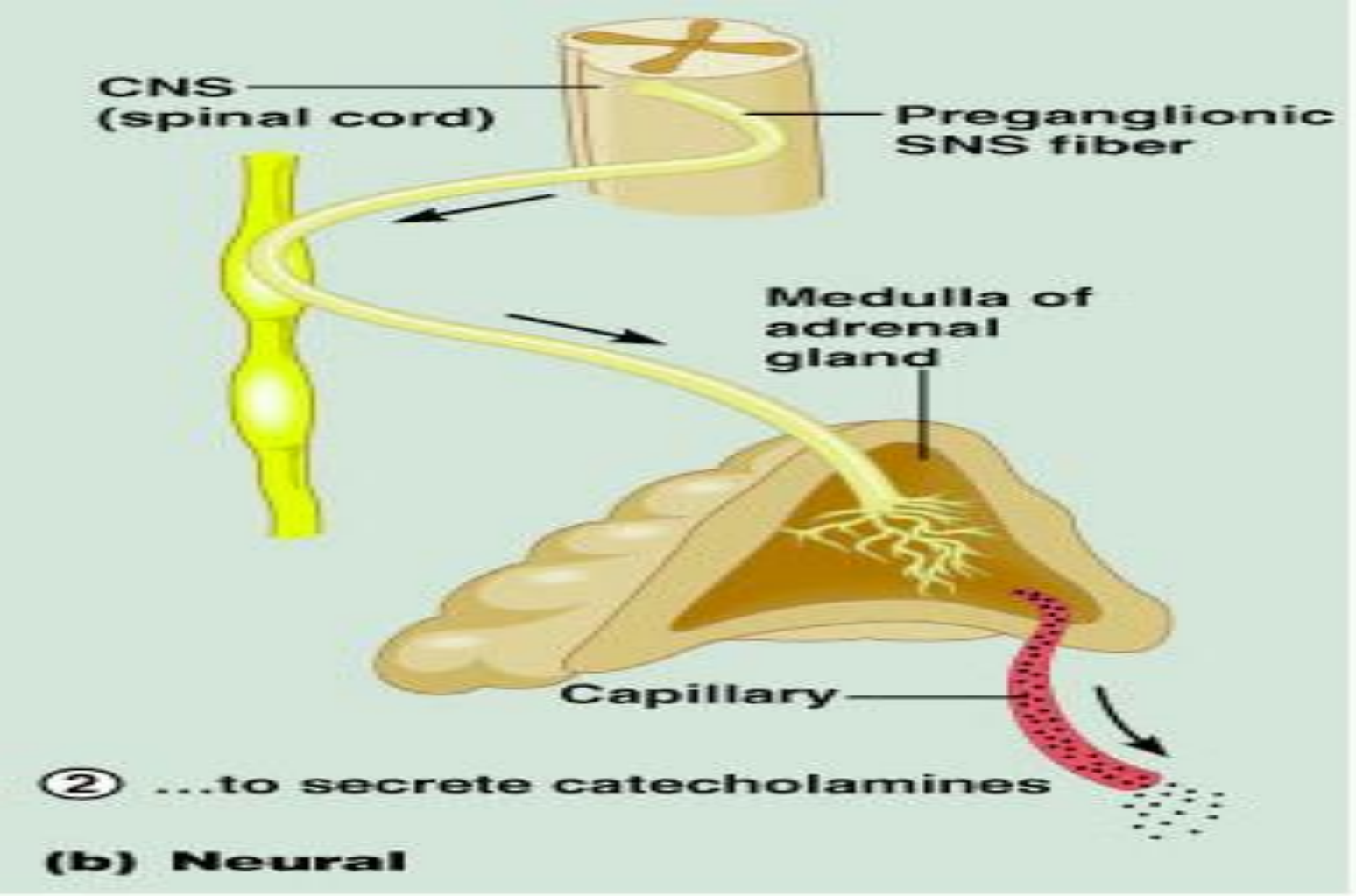
3 ...stimulate other endocrine glands to secrete hormones

REGULATION OF HORMONE SECRETION

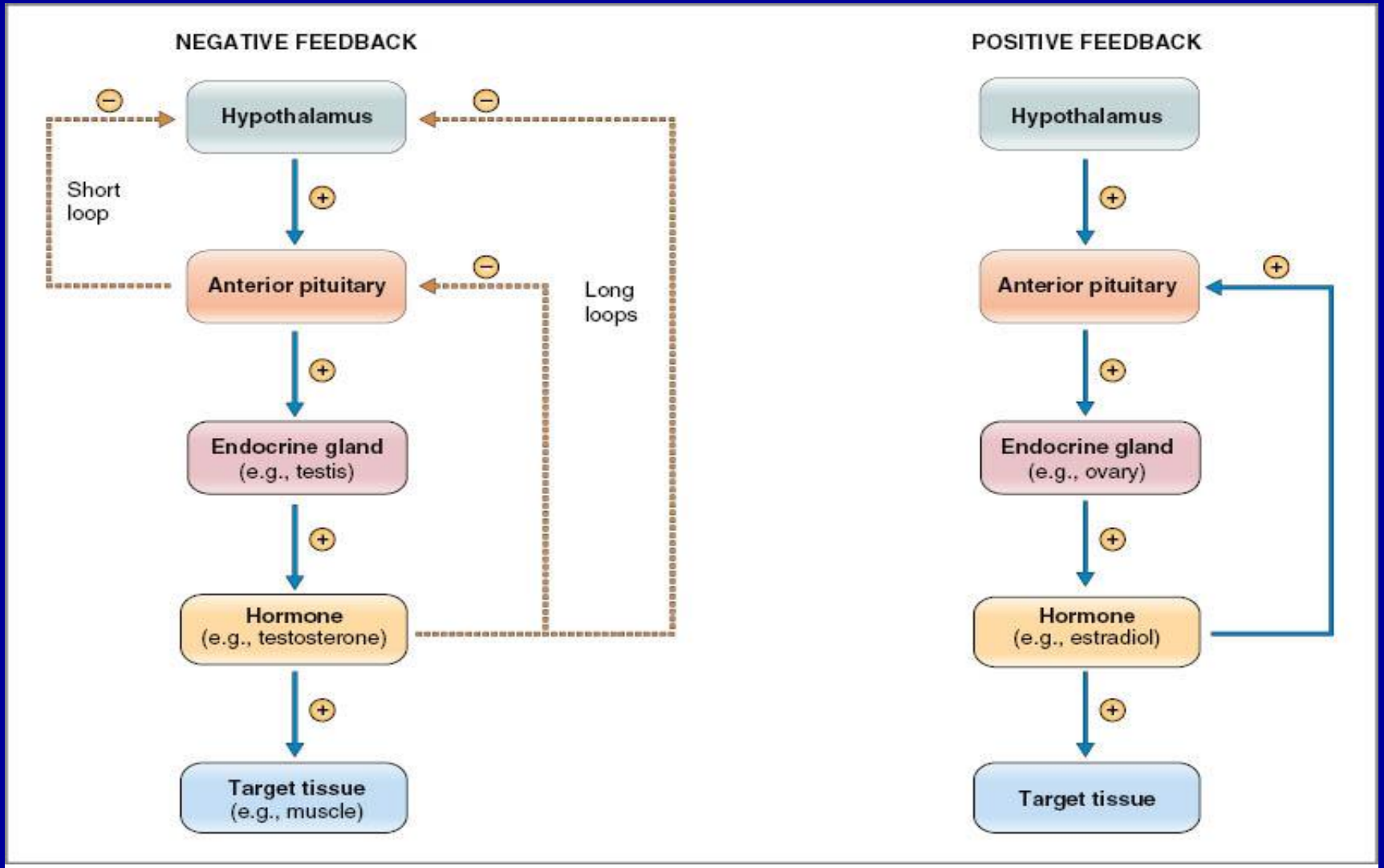
- **Neural mechanism.**

- **Feedback mechanism.**

NEURAL MECHANISM

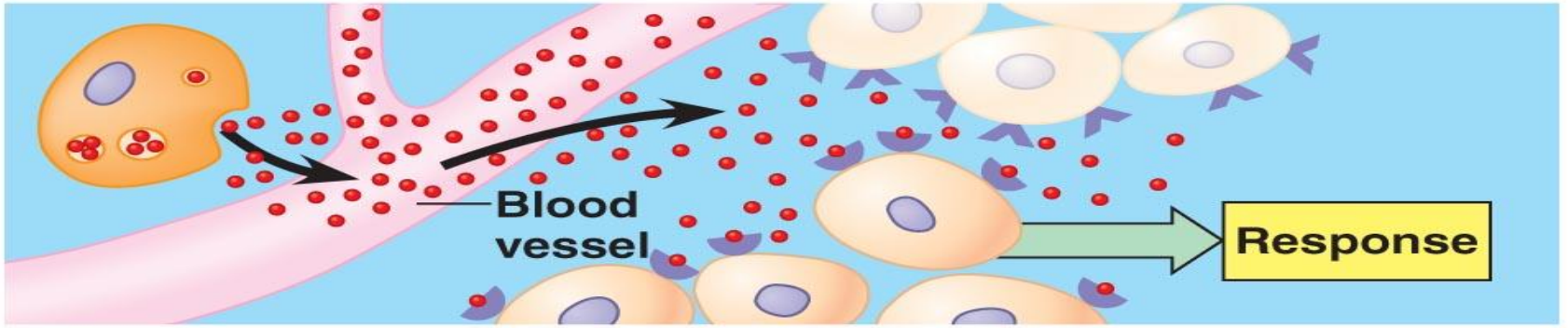


FEEDBACK MECHANISM

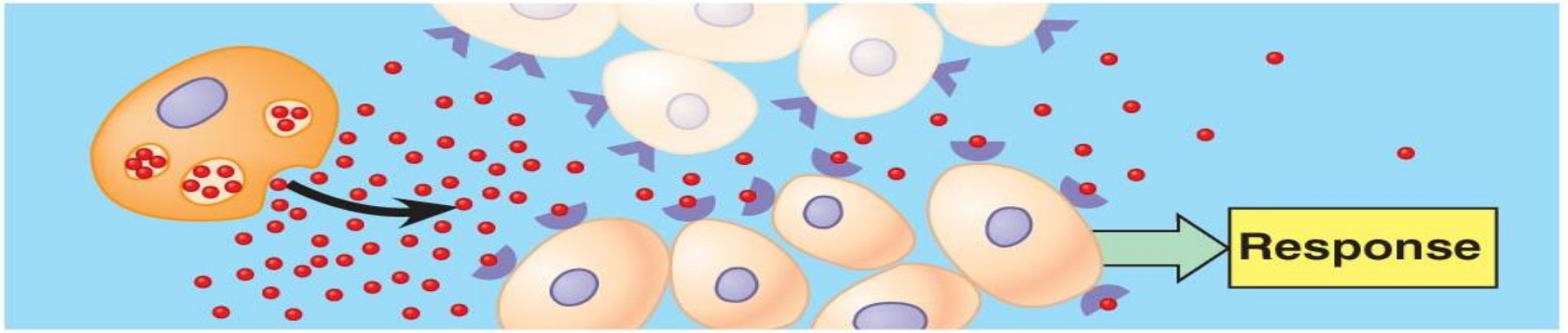


- **Autocrines – chemicals that exert their effects on the same cells that secrete them.**

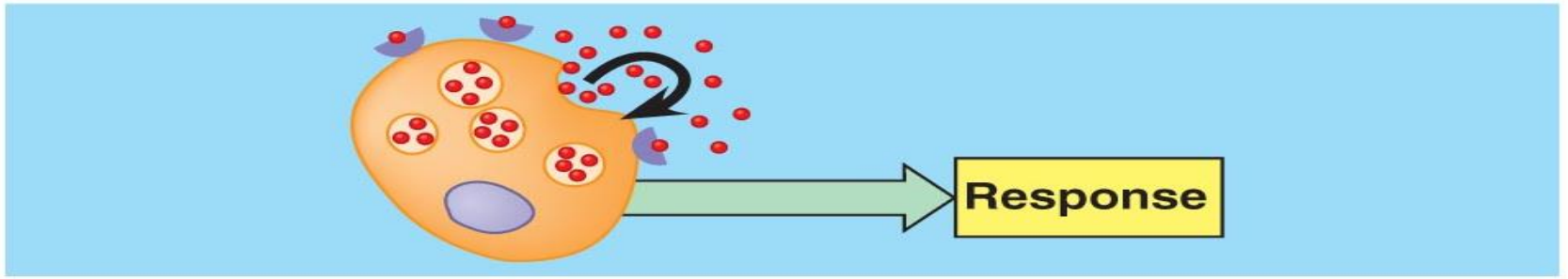
- **Paracrines – locally acting chemicals that affect cells other than those that secrete them.**



(a) Endocrine signaling



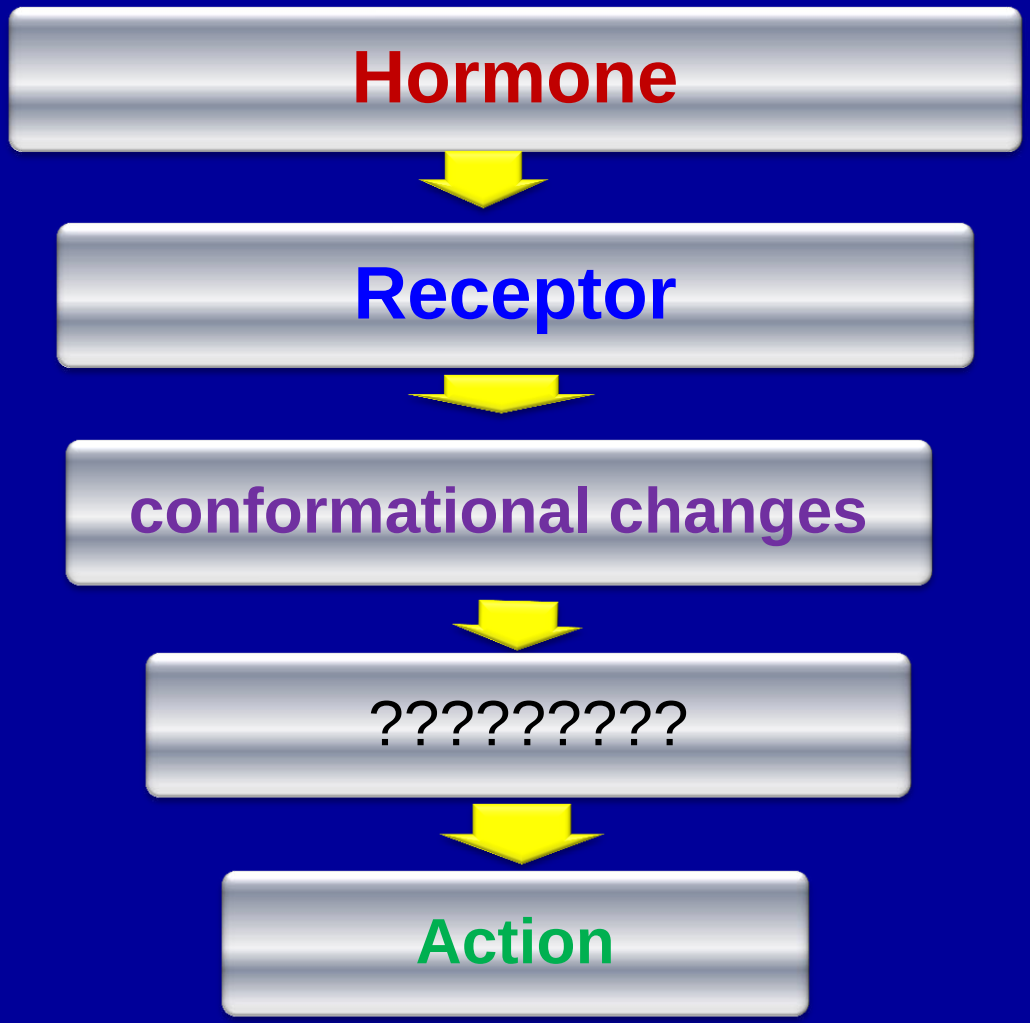
(b) Paracrine signaling



(c) Autocrine signaling

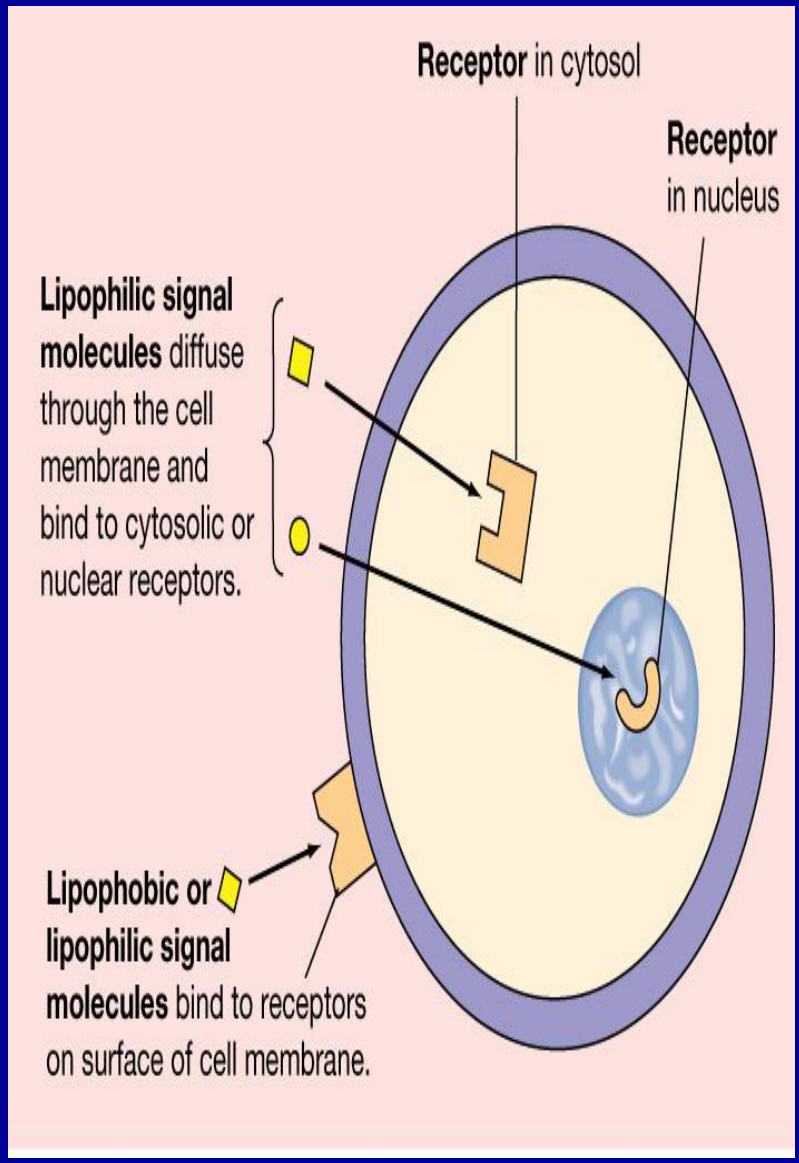
TARGET TISSUE

MECHANISM OF ACTION

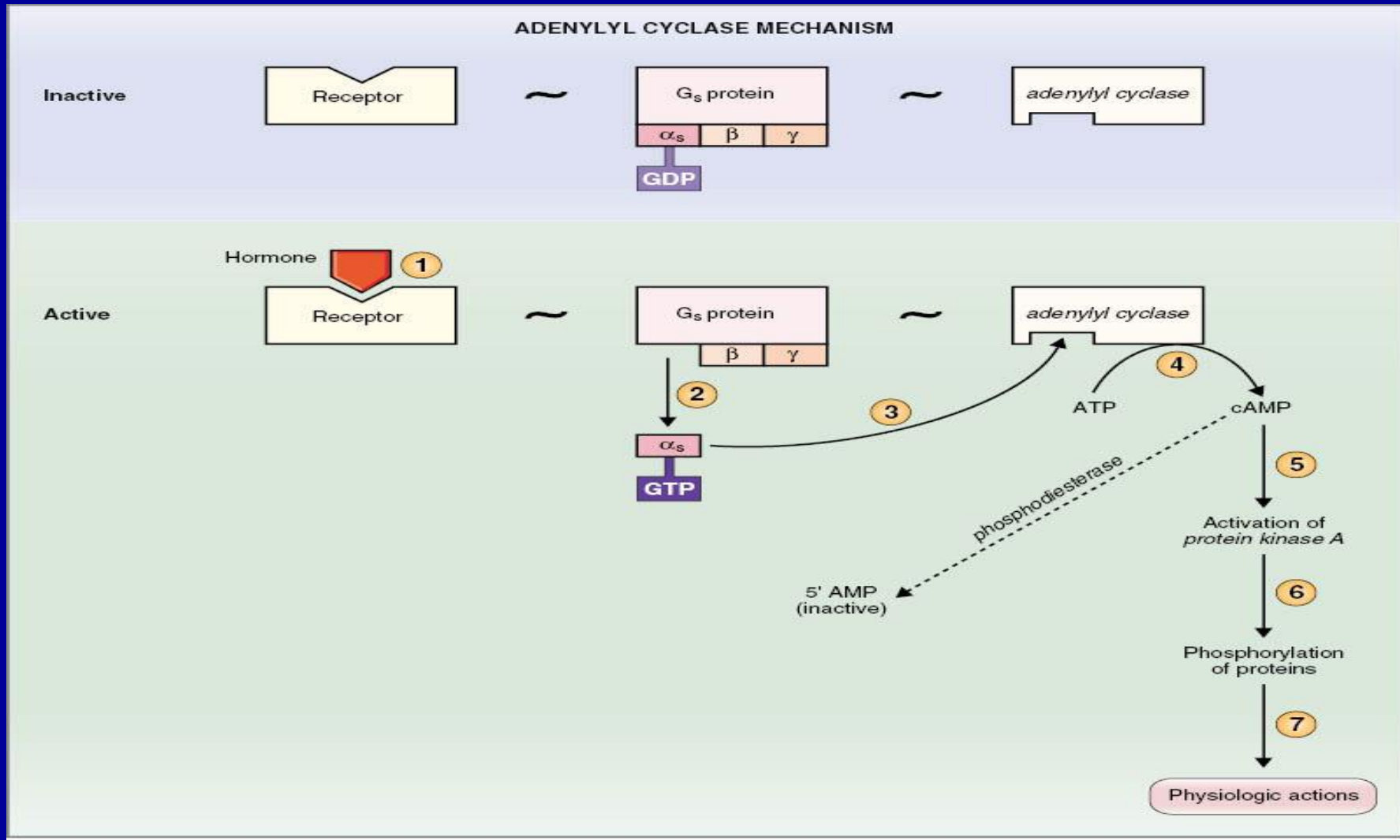


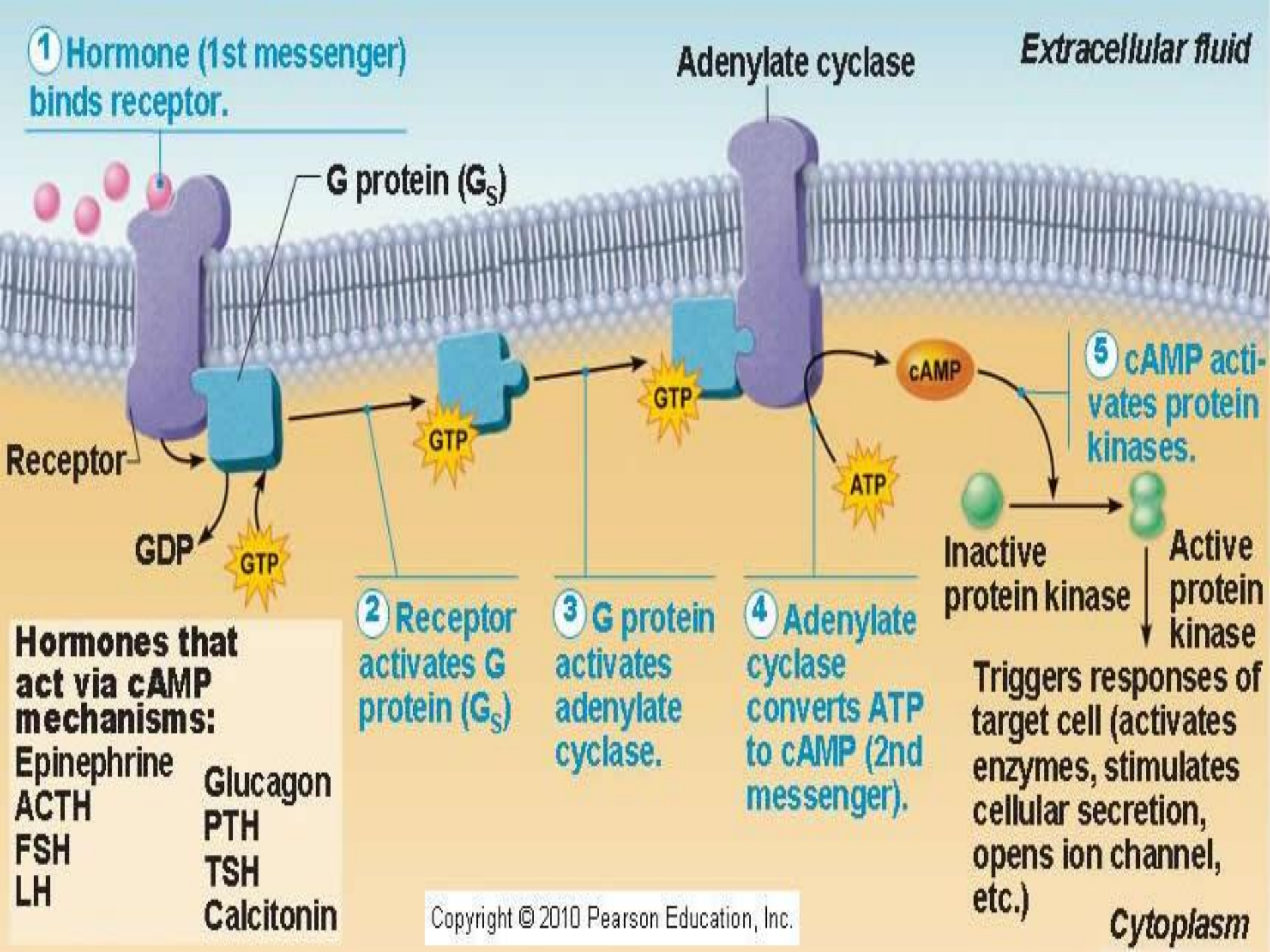
RECEPTOR LOCATIONS

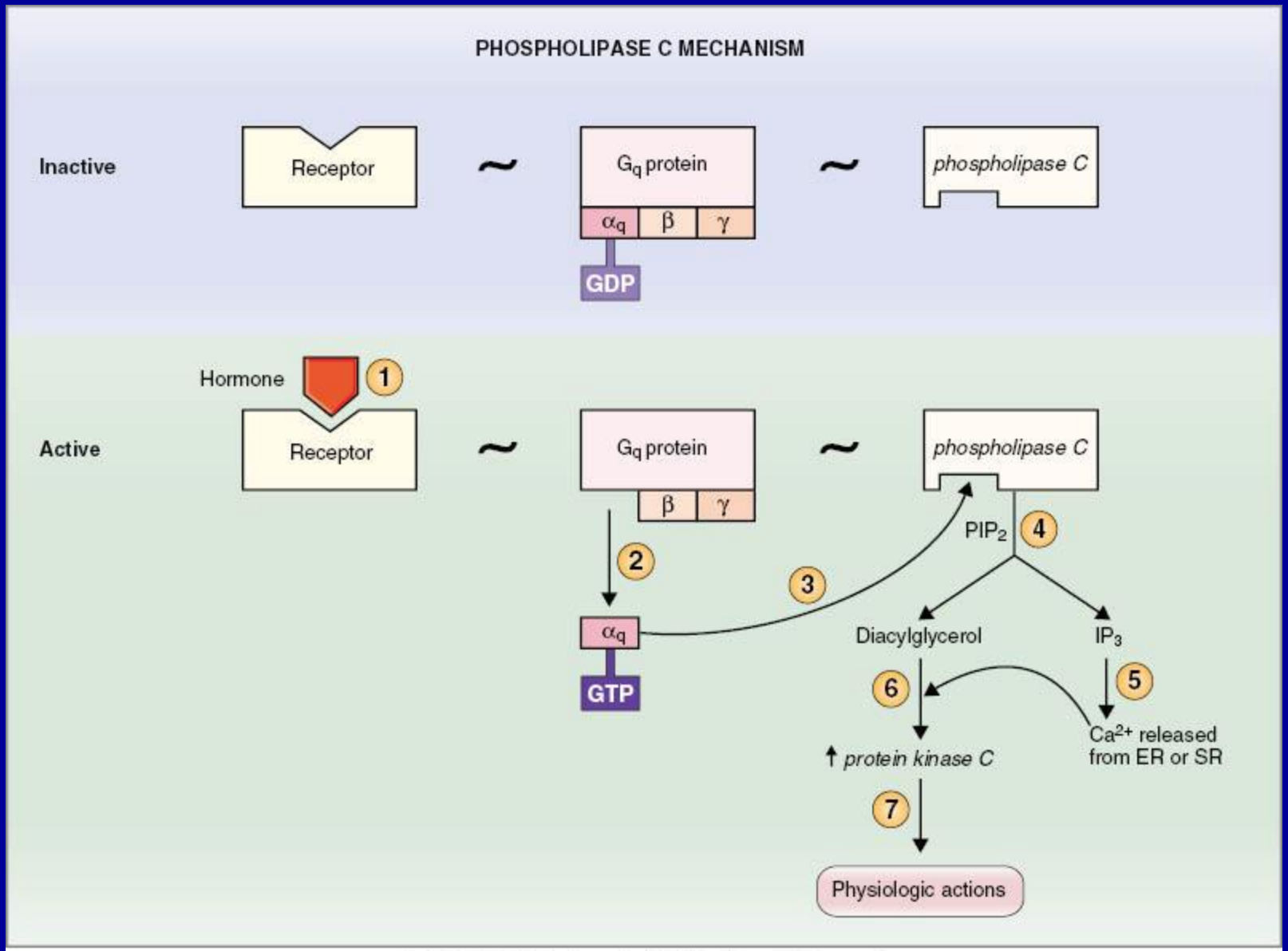
- **Cytosolic or Nuclear**
  - **Lipophilic ligand** enters cell
  - Often activates gene
  - Slower response
- **Cell membrane**
  - **Lipophobic ligand** can't enter cell
  - Outer surface receptor
  - Fast response



SECOND MESSENGER

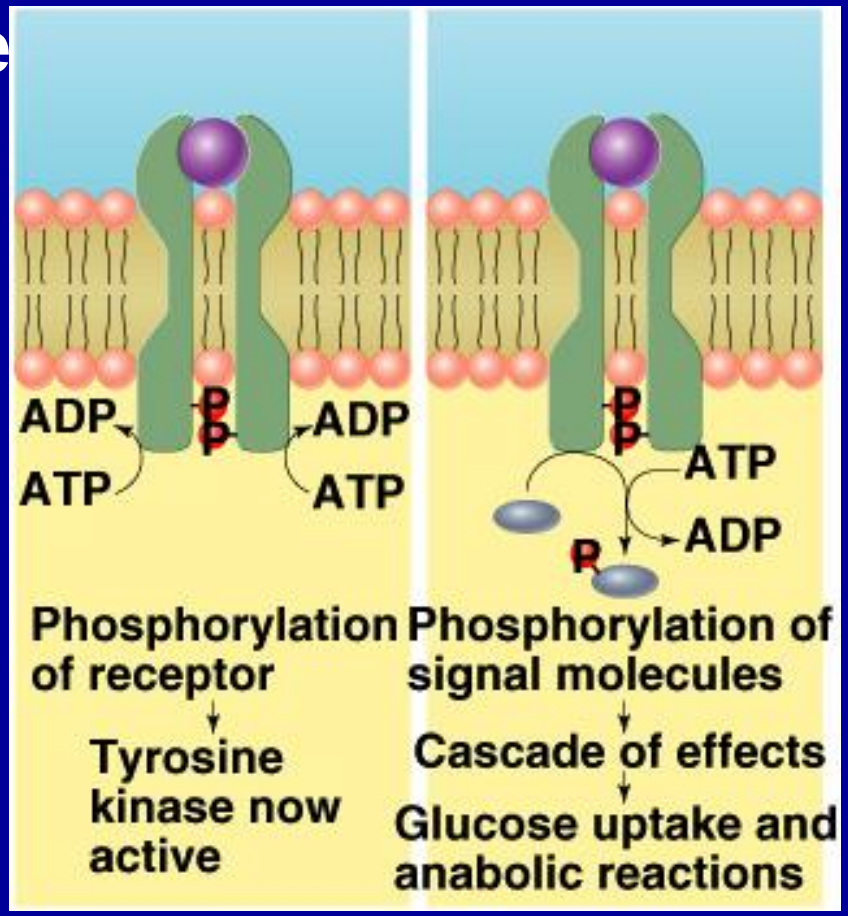




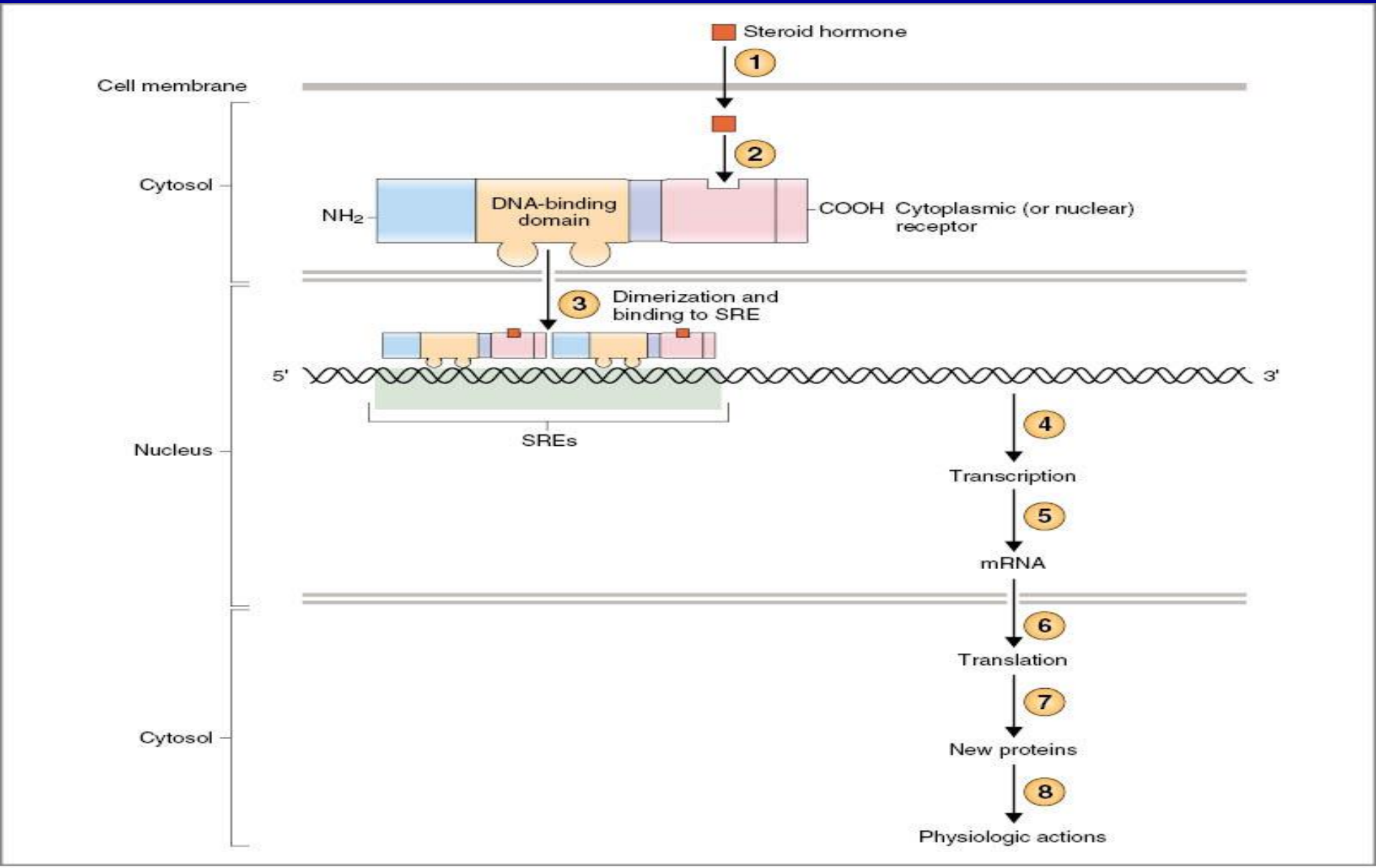


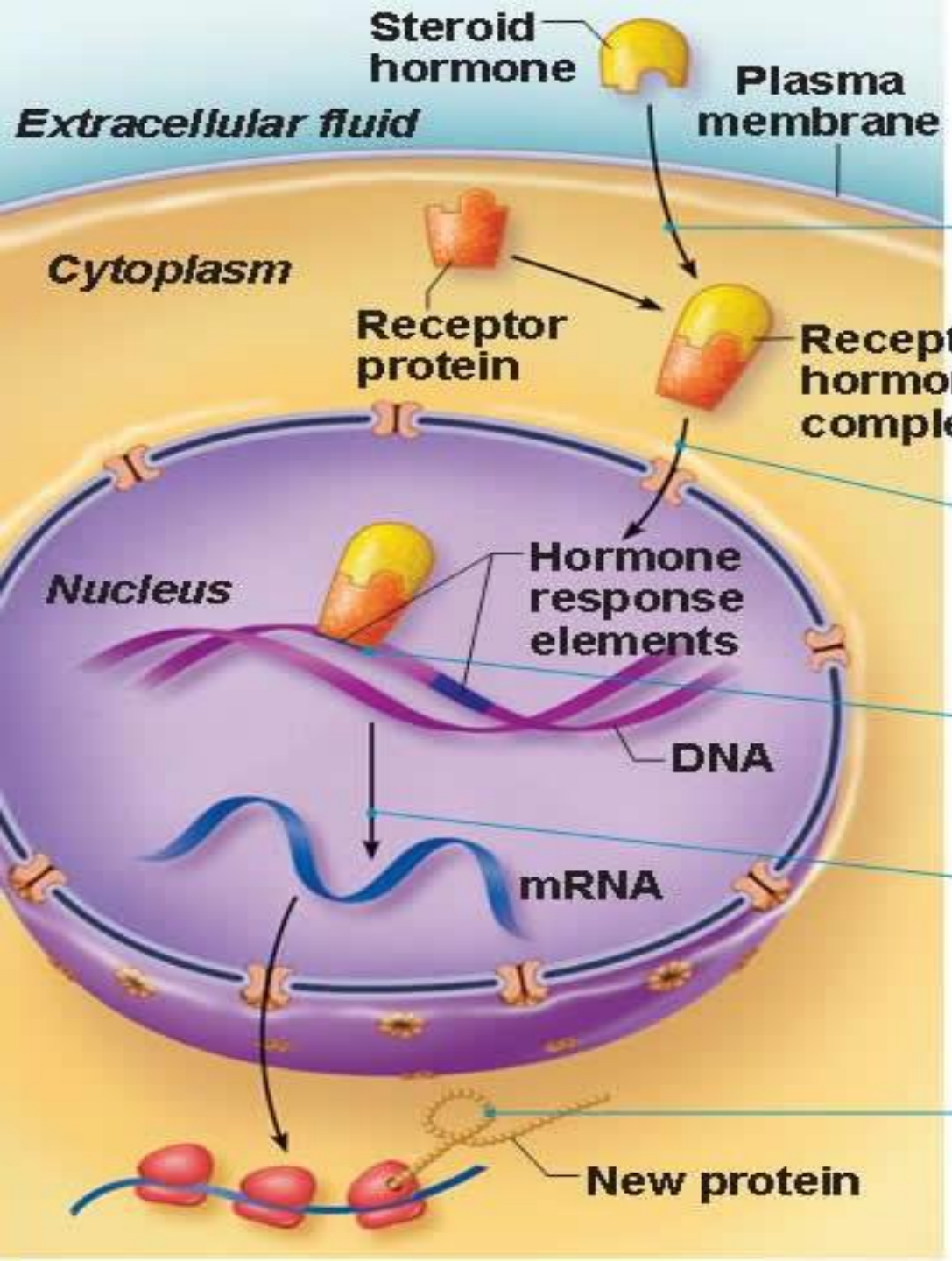
Tyrosine Kinase System

- Activated tyrosine kinase phosphorylates signaling molecules
- Induction of hormone/growth factor effects



STEROID AND THYROID HORMONE MECHANISM





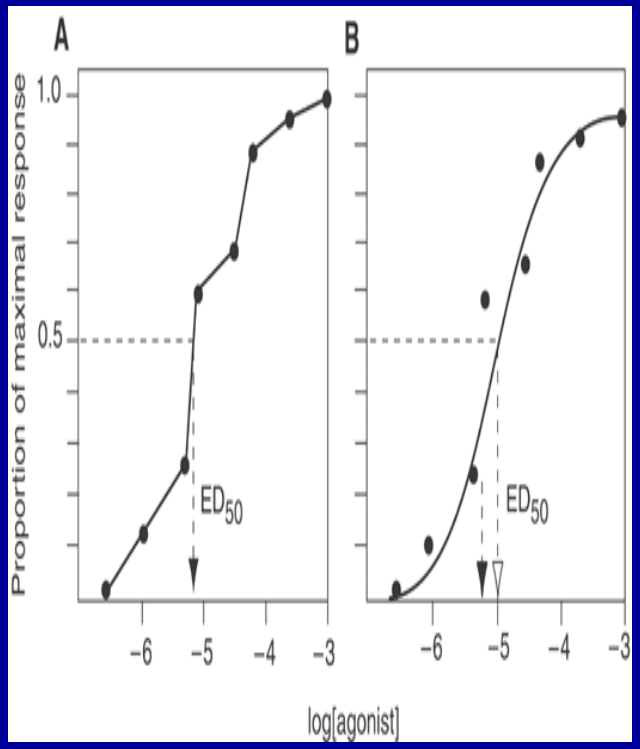
1. The steroid hormone diffuses through the plasma membrane and binds an intracellular receptor.
2. The receptor-hormone complex enters the nucleus.
3. The receptor- hormone complex binds a hormone response element (a specific DNA sequence).
4. Binding initiates transcription of the gene to mRNA.
5. The mRNA directs protein synthesis.

Table 9-3 Mechanisms of Hormone Action

Adenylyl Cyclase Mechanism (cAMP)	Phospholipase C Mechanism (IP_3/Ca^{2+})	Steroid Hormone Mechanism	Tyrosine Kinase Mechanism	Guanylate Cyclase Mechanism (cGMP)
ACTH	GnRH	Glucocorticoids	Insulin	Atrial natriuretic peptide (ANP)
LH	TRH	Estrogen	IGF-1	Endothelial-derived relaxing factor (EDRF)
FSH	GHRH	Progesterone		Nitric oxide (NO)
TSH	Angiotensin II	Testosterone		
ADH (V_2 receptor)	ADH (V_1 receptor)	Aldosterone		
HCG	Oxytocin	1,25-Dihydroxycholecalciferol		
MSH	α_1 Receptors	Thyroid hormones		
CRH				
Calcitonin				
PTH				
Glucagon				
β_1 and β_2 receptors				

REGULATION OF HORMONE RECEPTORS

- **Dose-response relationship.**
- **Sensitivity.**
- **Number.**
- **Affinity.**



DOWN-REGULATION

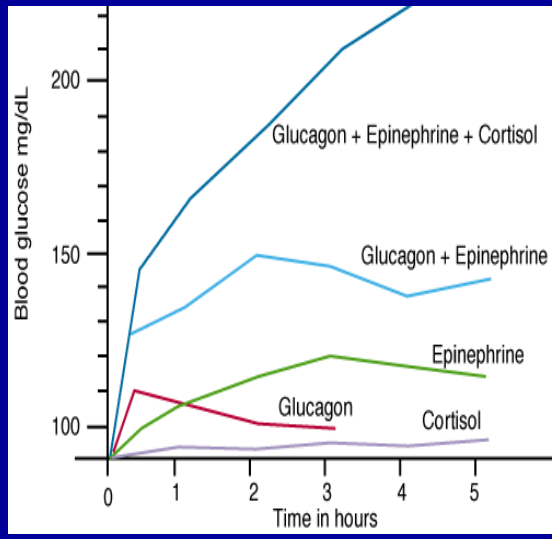
- **Decrease synthesis.**
- **Increase degradation.**
- **Inactivation .**
- **T3.**

UP-REGULATION

- **Increase synthesis.**
- **Decrease degradation.**
- **Activation .**
- **GH, prolactin.**

INTERACTION OF HORMONES AT TARGET CELLS

- **Permissiveness** (Thyroid hormone have permissive effect on growth hormone action)
- **Synergism** (glucagon, cortisol and epinephrine)
- **Antagonism** (Glucagon /insulin)



HORMONE CONCENTRATIONS IN THE BLOOD

- **Concentrations of circulating hormone reflect:**
  - **Rate of release**
  - **Speed of inactivation and removal from the body**
- **Hormones are removed from the blood by:**
  - **Degrading enzymes**
  - **The kidneys**
  - **Liver enzyme systems**

HYPOTHALAMIC-PITUITARY AXIS

OBJECTIVES

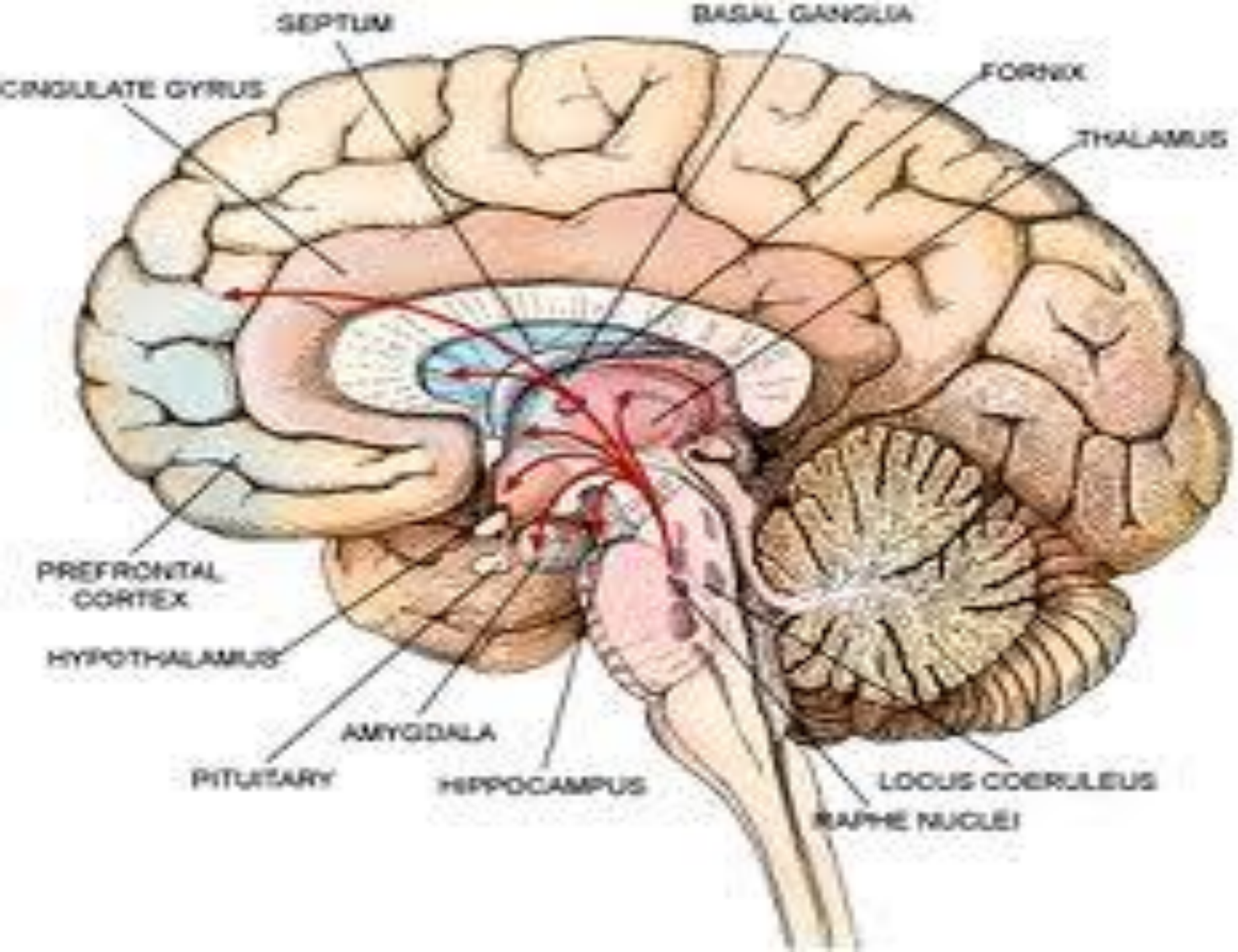
- **By the end of this lecture, students should be able to describe:**
- **Structure of pituitary gland (hypophysis)**
  - **Anterior pituitary (adenohypophysis) cell types and hormones**
  - **Posterior pituitary (neurohypophysis) cell types and hormones**
- **Control of pituitary gland by hypothalamus**
  - **Hypothalamo-hypophysial portal blood vessels (Hypothalamic releasing and inhibiting hormones and median eminence)**
  - **Hypothalamo-hypophysial tract**
- **Feedback mechanisms: positive and negative feedback**

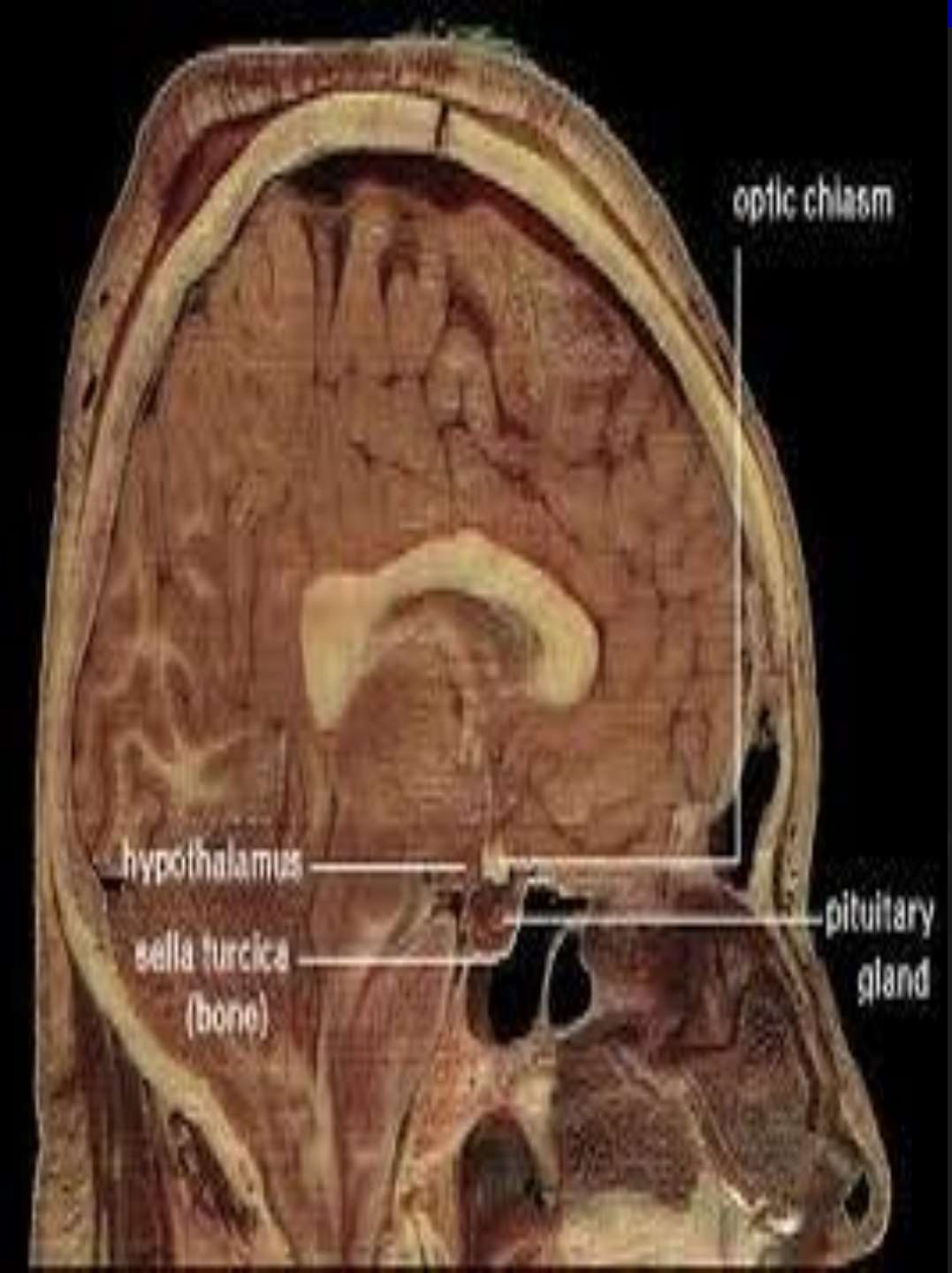
HYPOTHALAMIC-PITUITARY AXIS

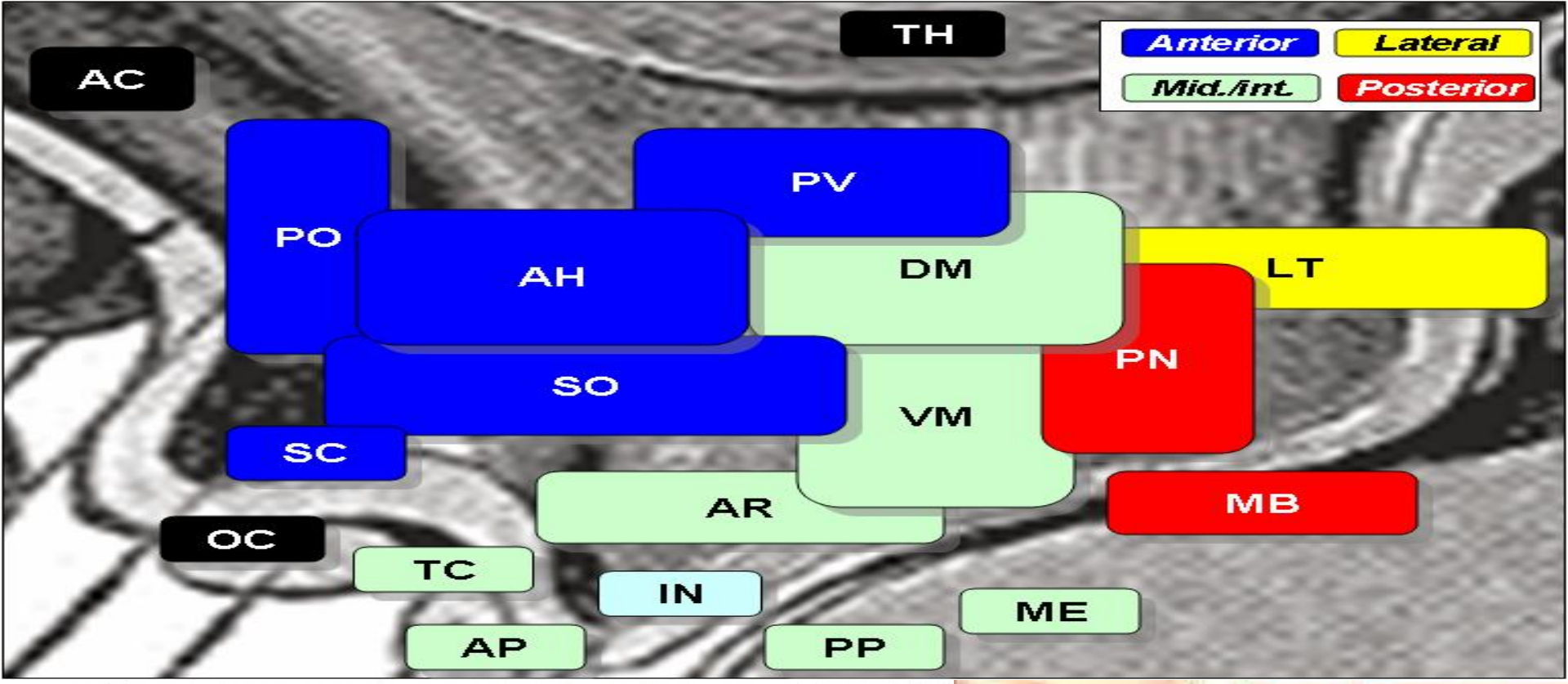
- **Coordinate.**
- **Thyroid gland, adrenal gland, reproductive gland, control growth, milk production, osmoregulation.**

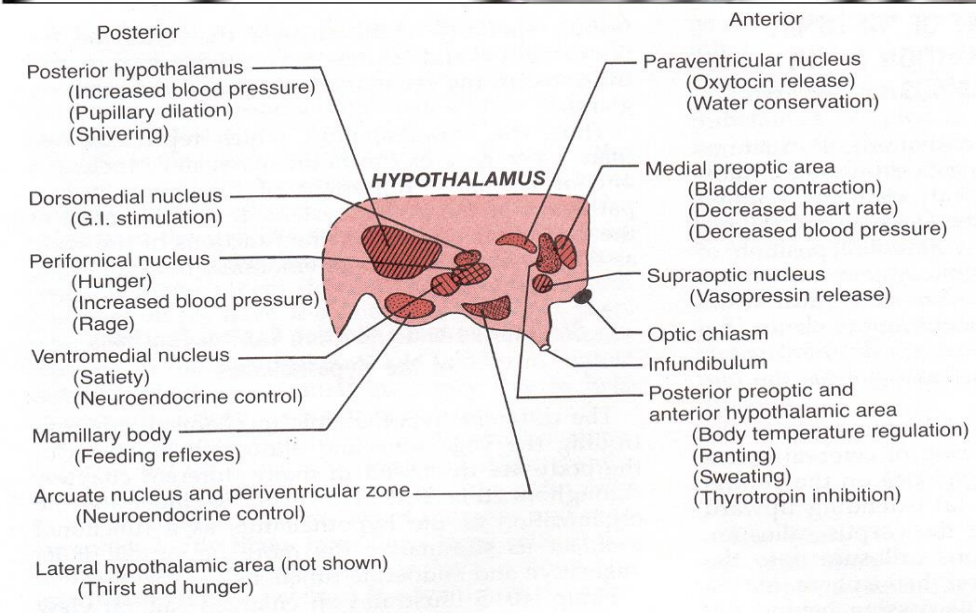
HYPOTHALAMUS

- **Control pituitary gland secretion.**
- **Composed of number of nerve cells.**


SEPTUM
BASAL GANGLIA
CINGULATE GYRUS
FORNIX
THALAMUS
PREFRONTAL CORTEX
HYPOTHALAMUS
AMYGDALA
PITUITARY
HIPPOCAMPUS
LOCUS COERULEUS
RAPHE NUCLEI


optic chiasm
hypothalamus
sella turcica
(bone)
pituitary
gland





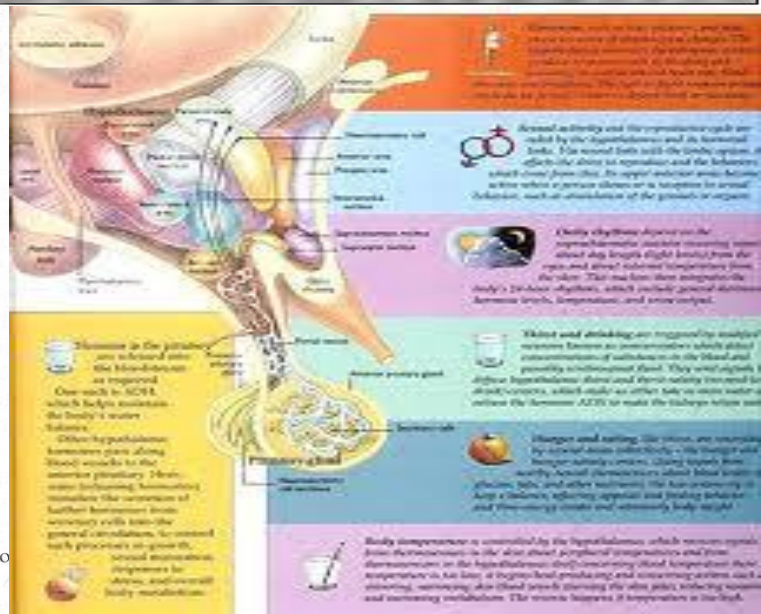


Figure 40–5 Contro
hypothalamus.

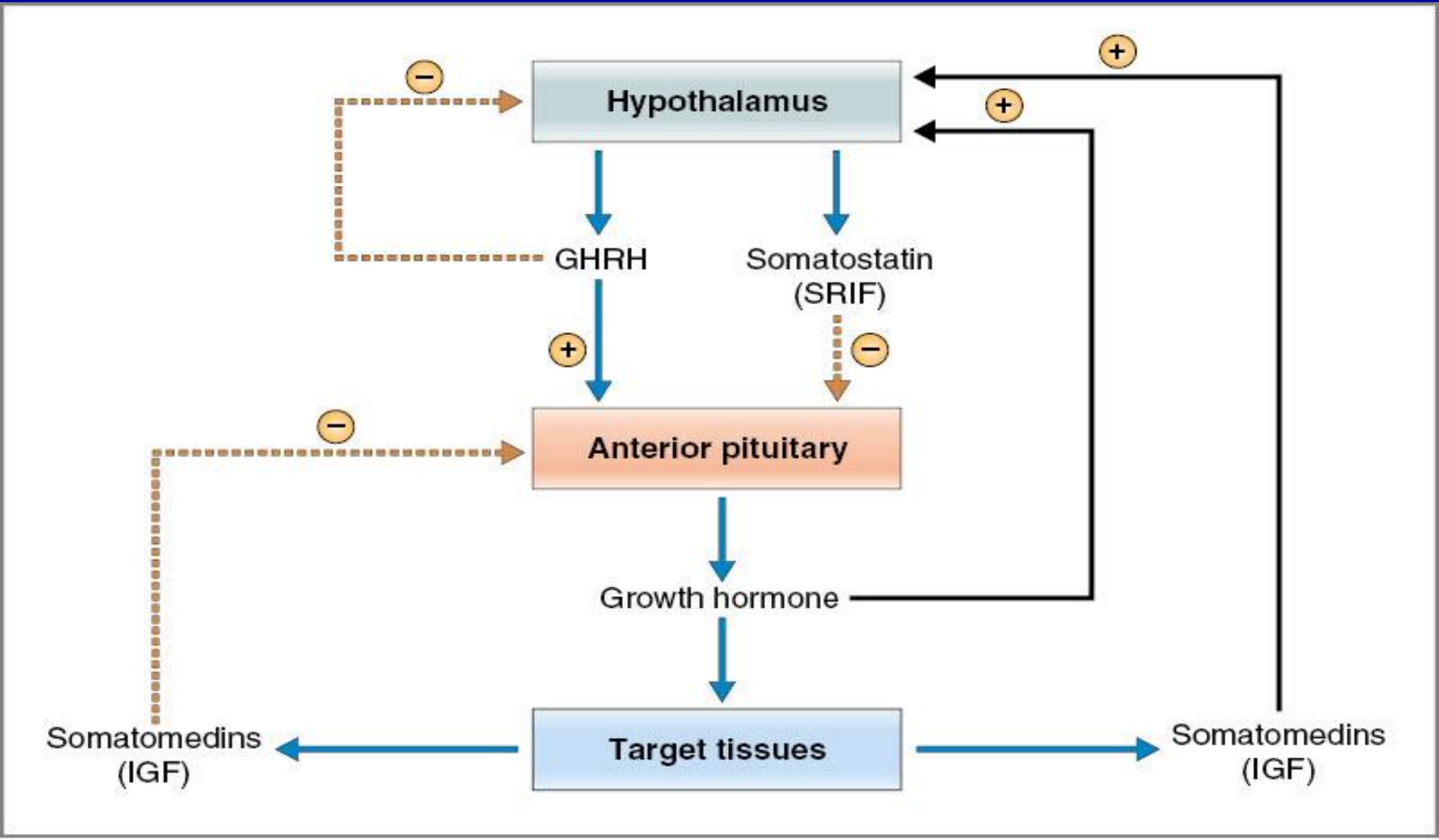
HORMONES

- TRH.
- CRH.
- GnRH.
- PIF.
- GHRH.
- SRIF.

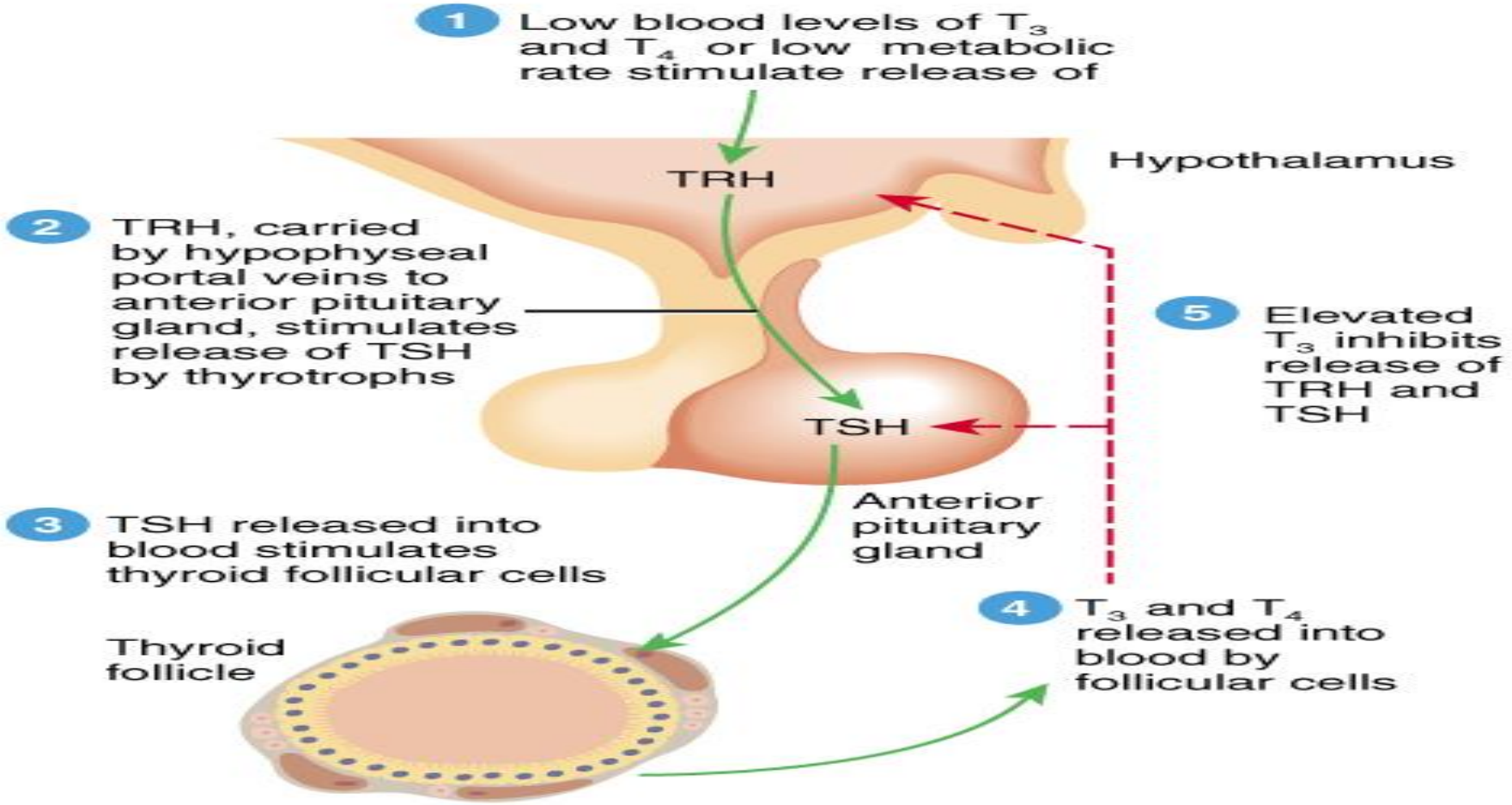
Table 9-2 Summary of Endocrine Glands and Actions of Hormones

Gland of Origin	Hormones*	Chemical Classification†	Major Actions
Hypothalamus	Thyrotropin-releasing hormone (TRH)	Peptide	Stimulates secretion of TSH and prolactin
	Corticotropin-releasing hormone (CRH)	Peptide	Stimulates secretion of ACTH
	Gonadotropin-releasing hormone (GnRH)	Peptide	Stimulates secretion of LH and FSH
	Somatostatin or somatotropin release-inhibiting hormone (SRIF)	Peptide	Inhibits secretion of growth hormone
	Dopamine or prolactin-inhibiting factor (PIF)	Amine	Inhibits secretion of prolactin
	Growth hormone–releasing hormone (GHRH)	Peptide	Stimulates secretion of growth hormone

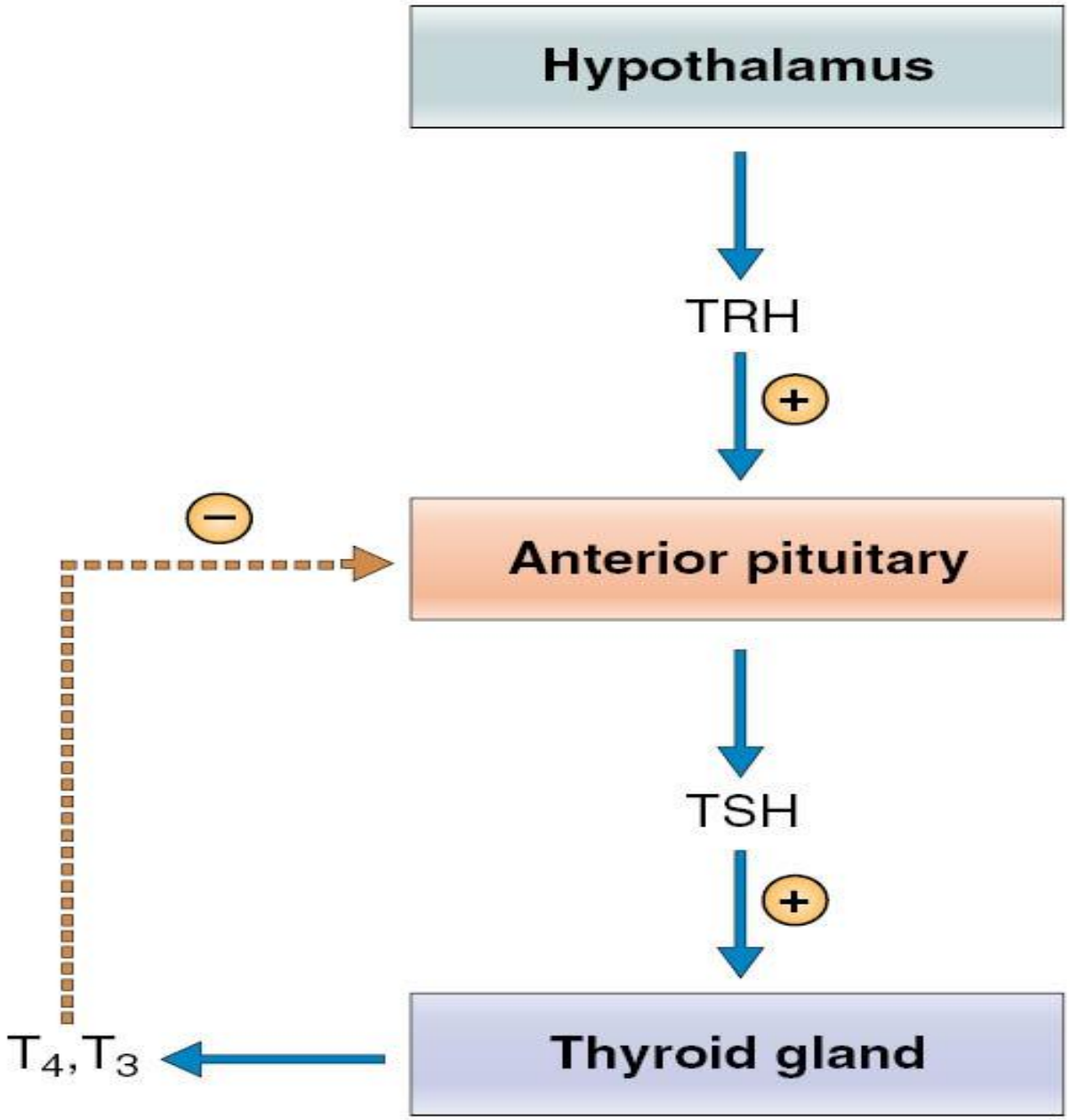
GHRH/GHIH(SRIF)



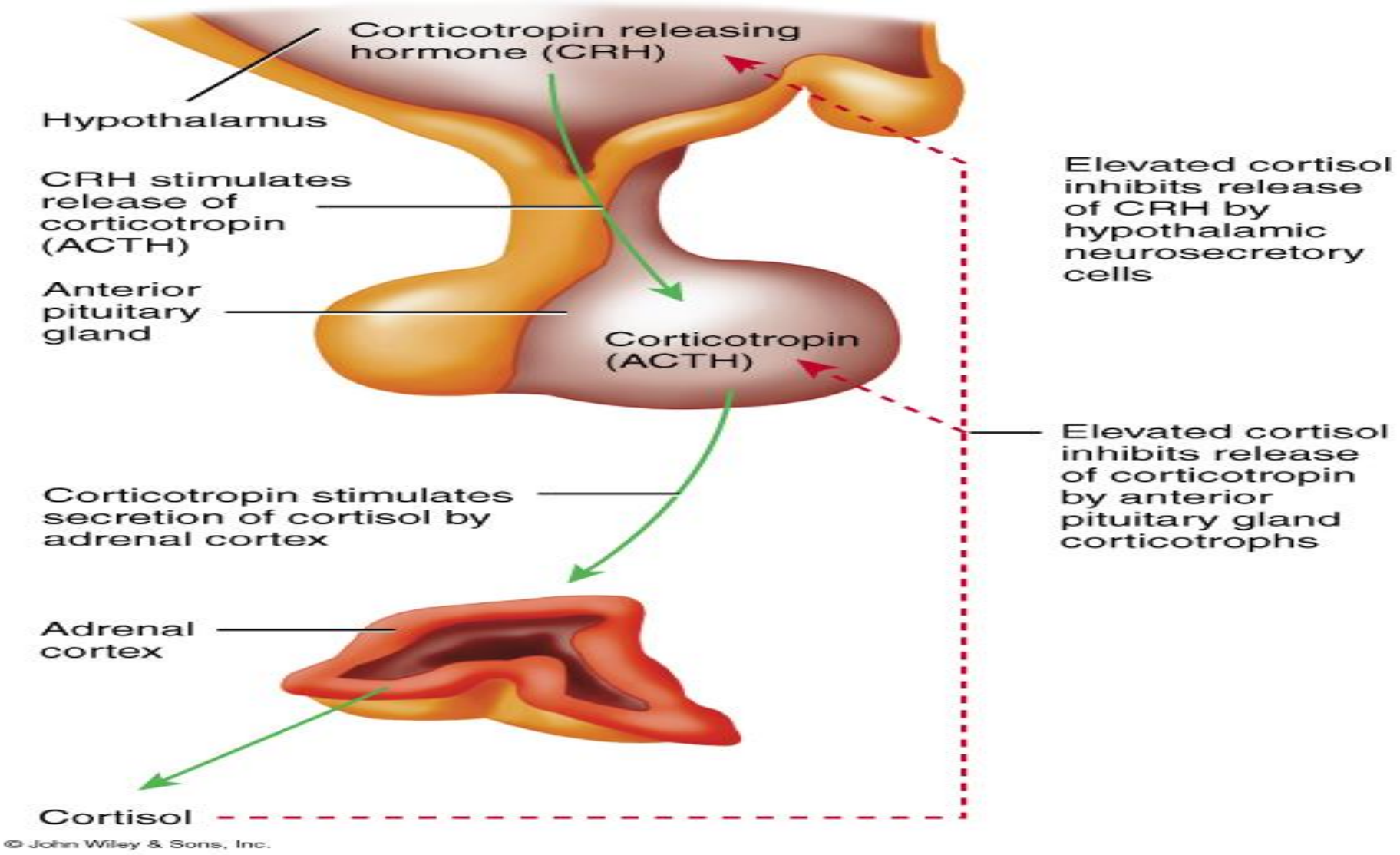
TRH

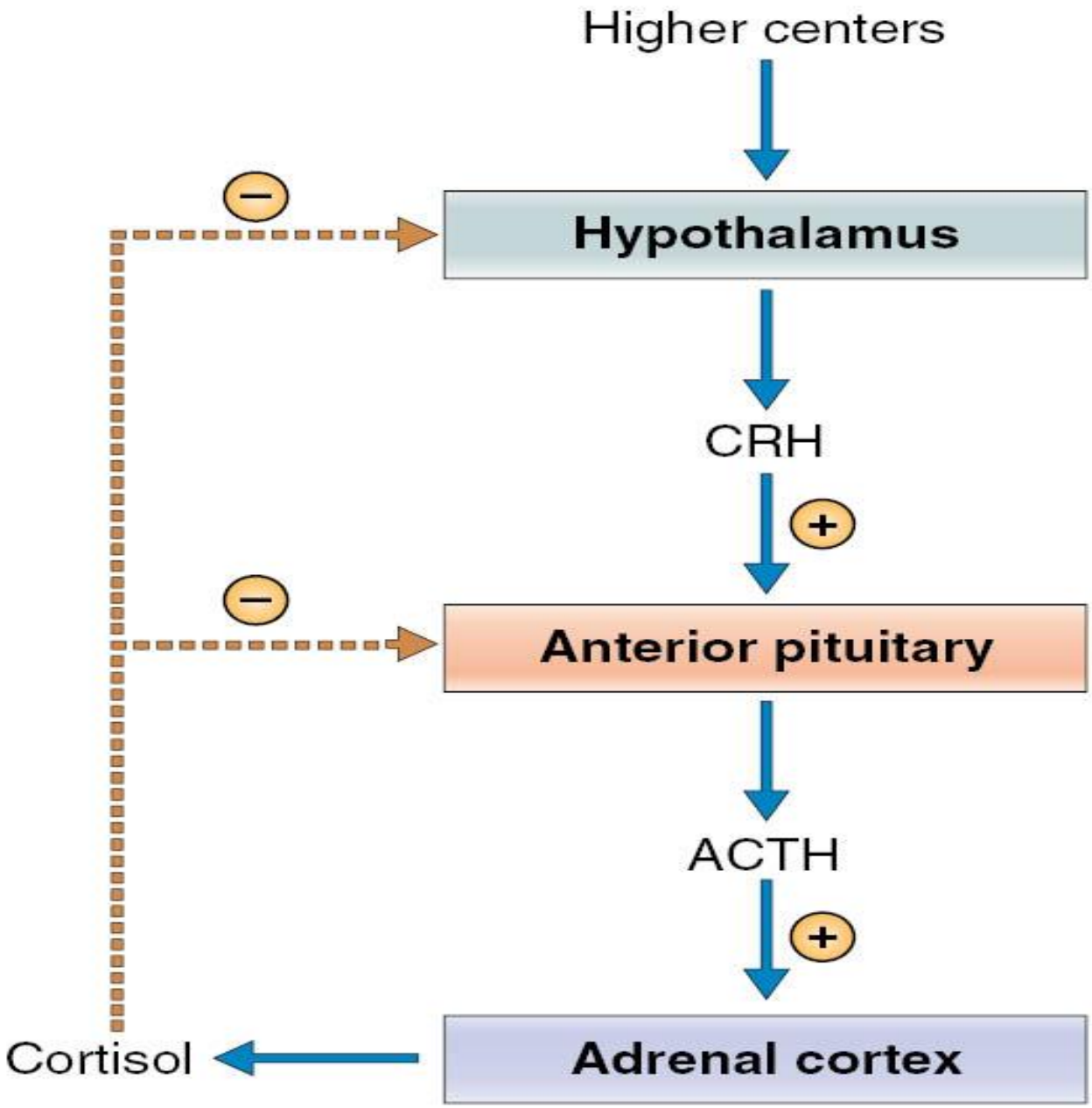


Key:
TRH = Thyrotropin releasing hormone
TSH = Thyroid-stimulating hormone
T_3 = Triiodothyronine
T_4 = Thyroxine (Tetraiodothyronine)


Hypothalamus
TRH
+
−
Anterior pituitary
TSH
+
T_4, T_3
Thyroid gland

CRH





GnRH

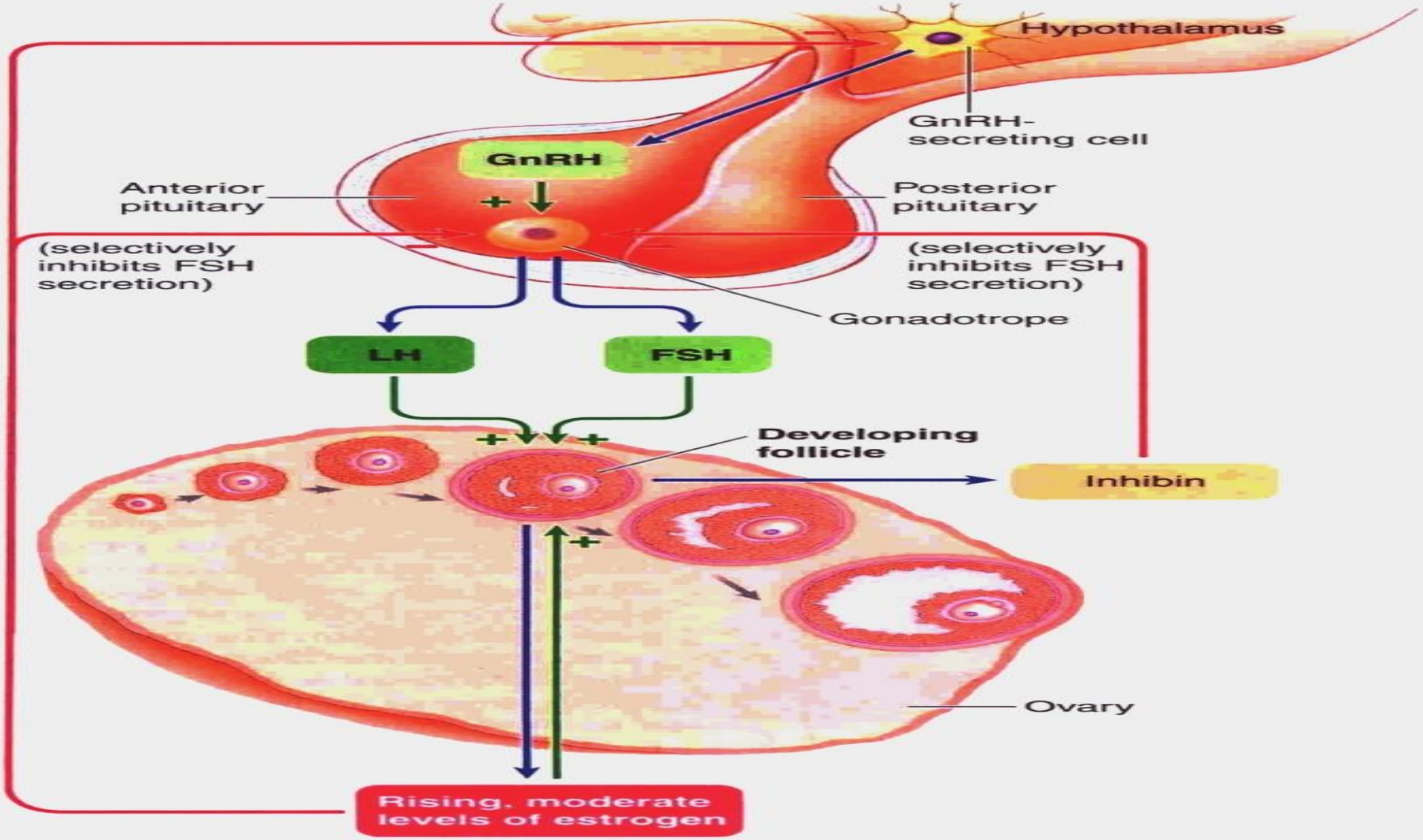
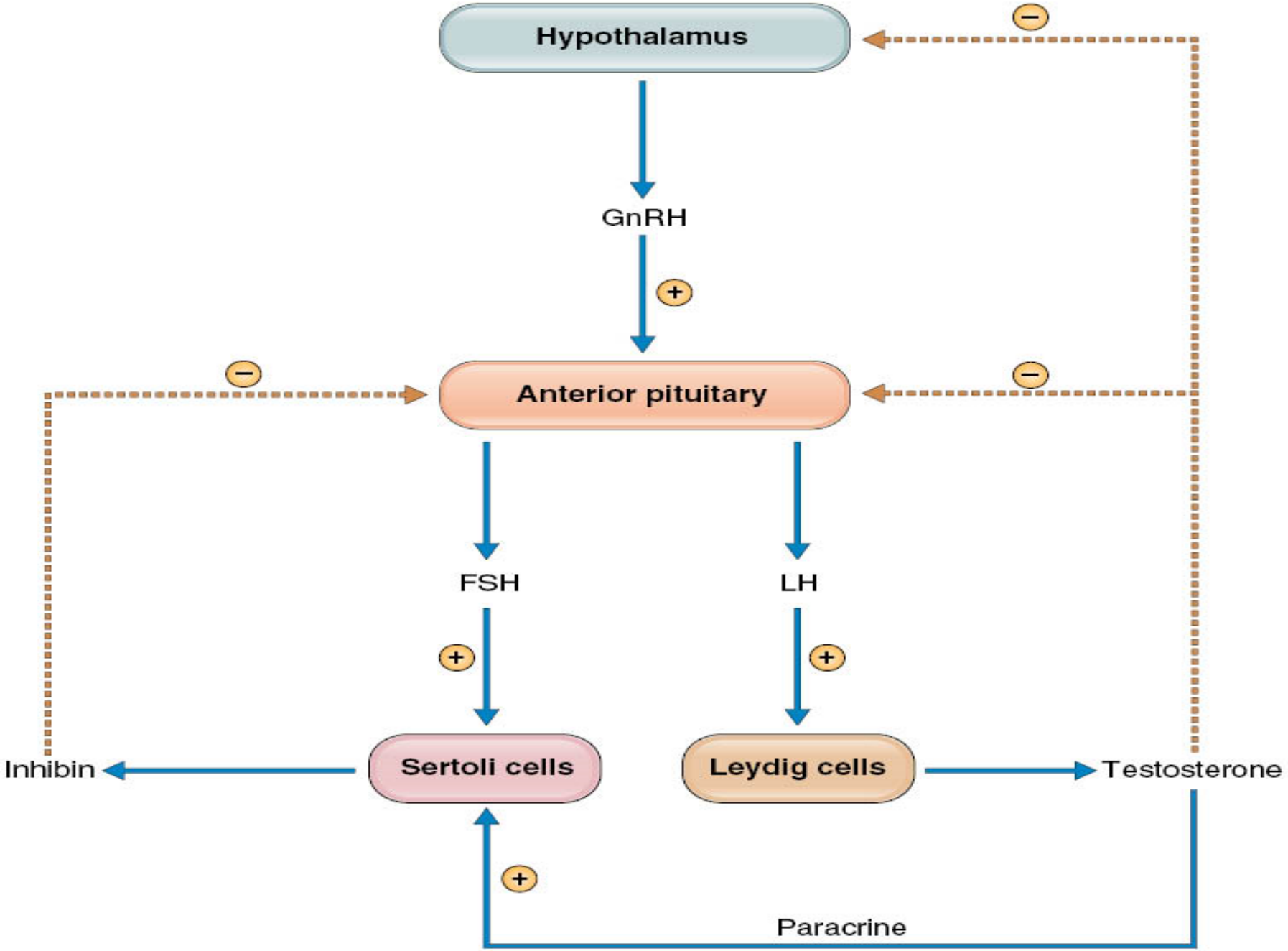
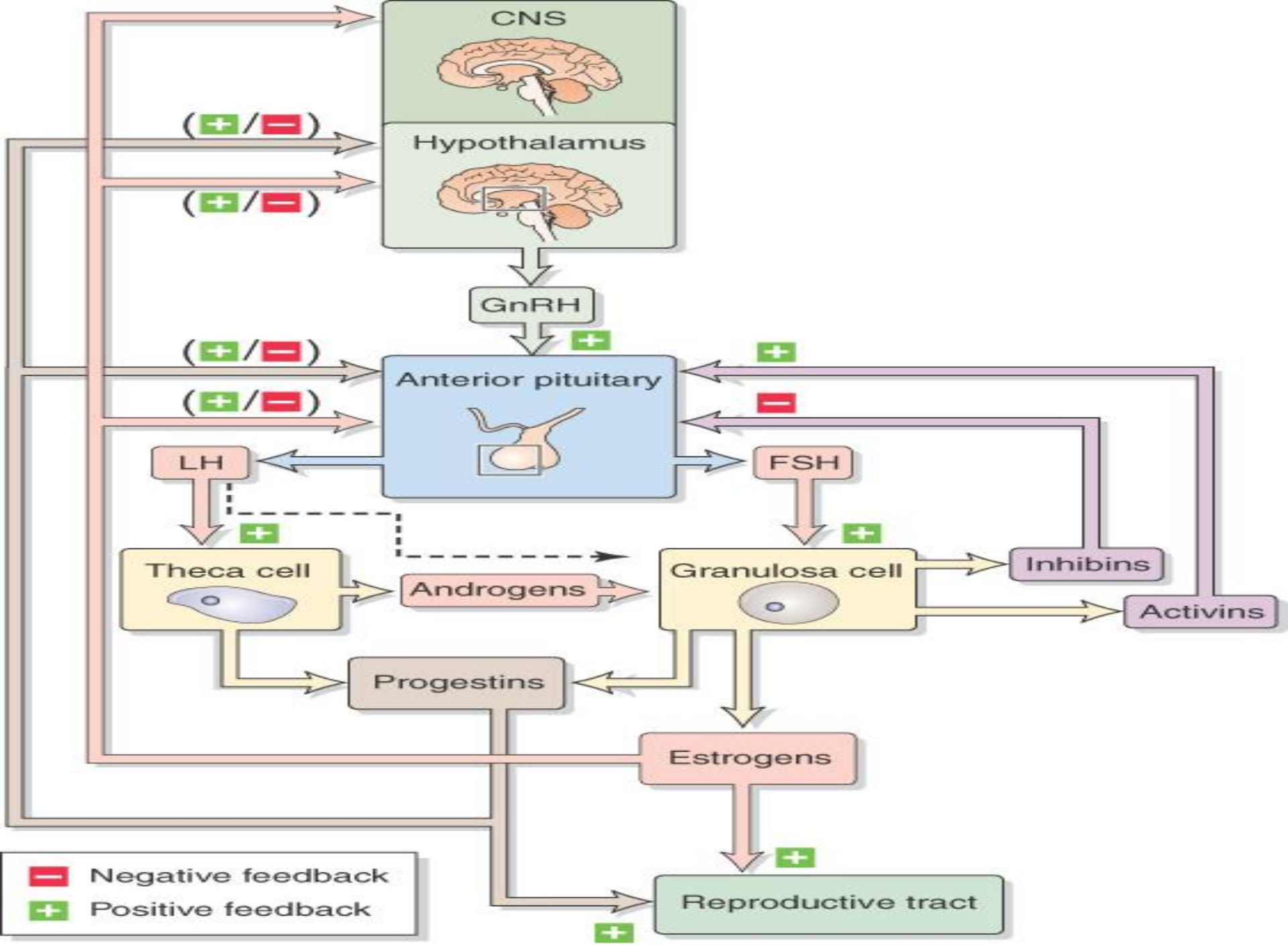


FIGURE 20-20 Feedback control of FSH and tonic LH secretion during the follicular phase.





PIH

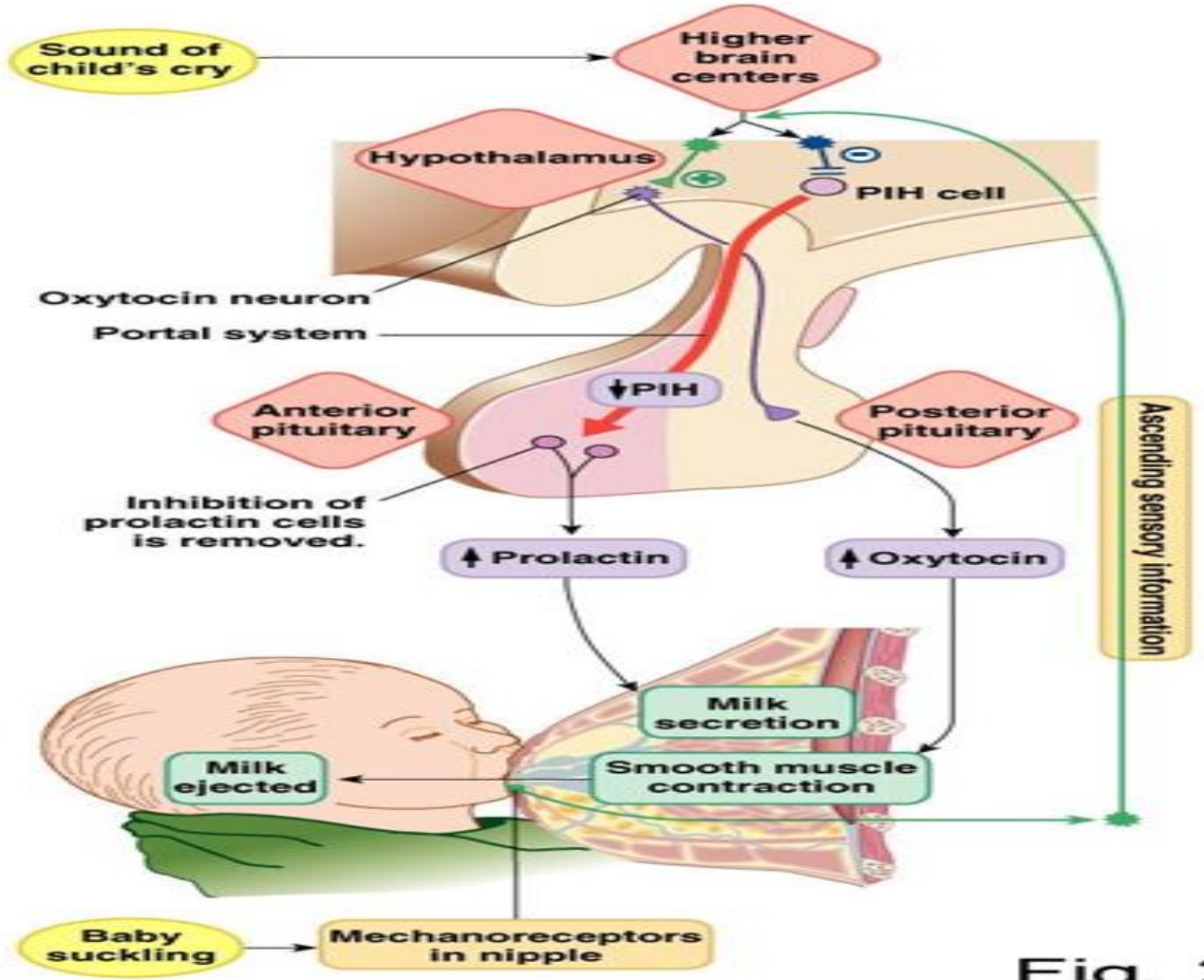
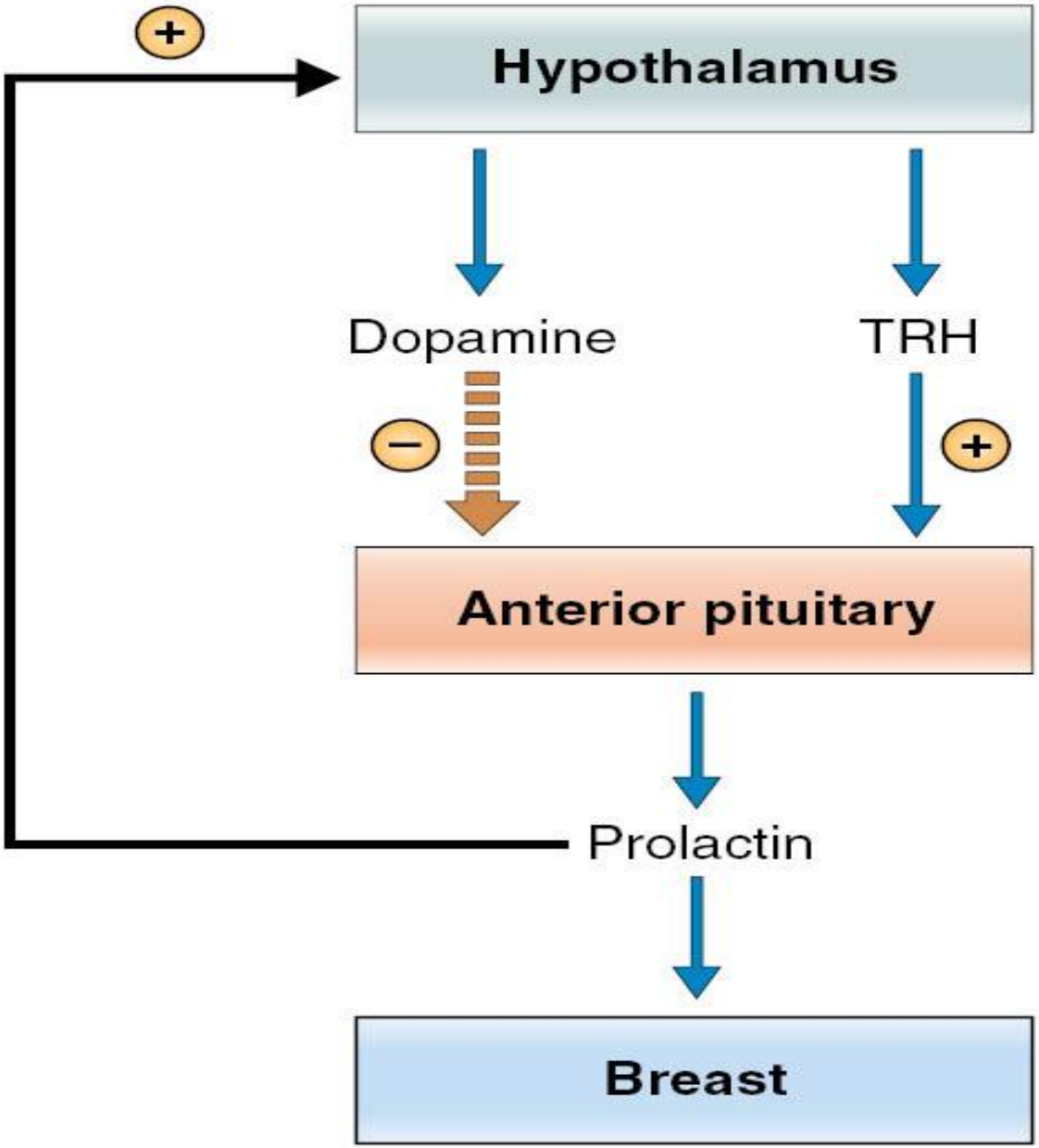
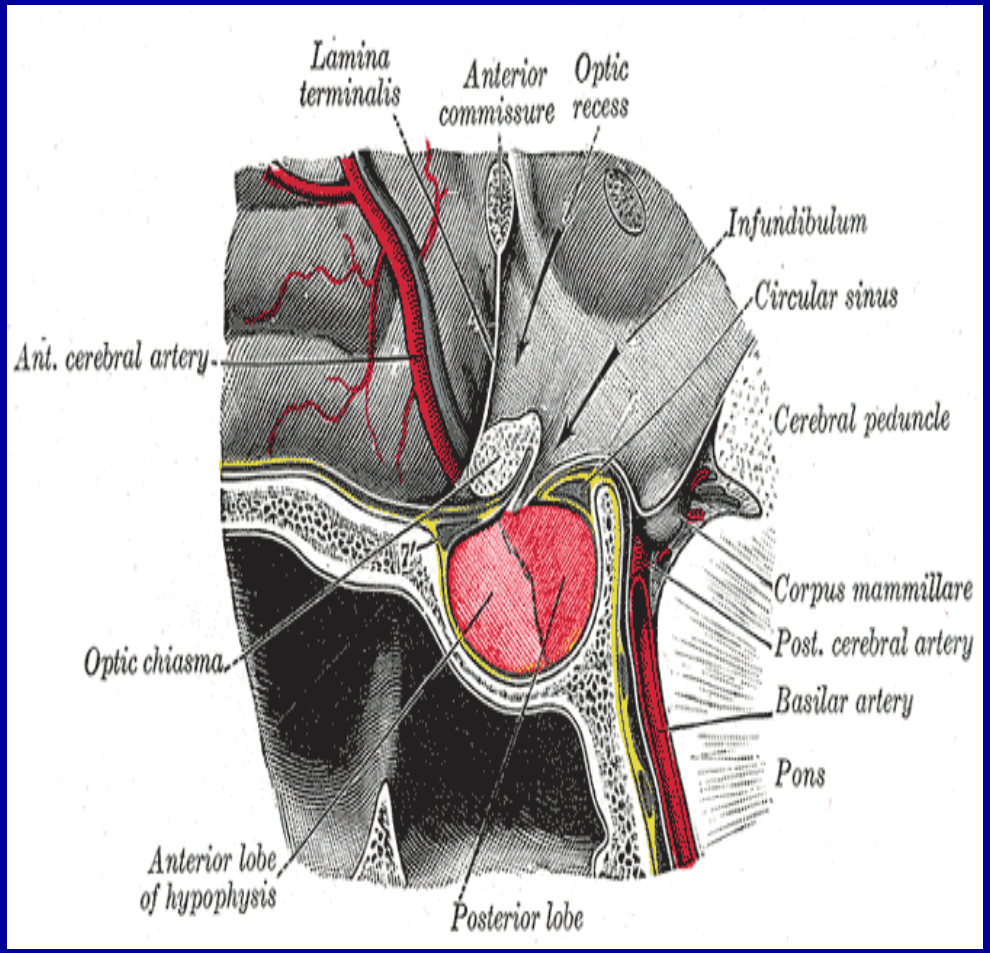


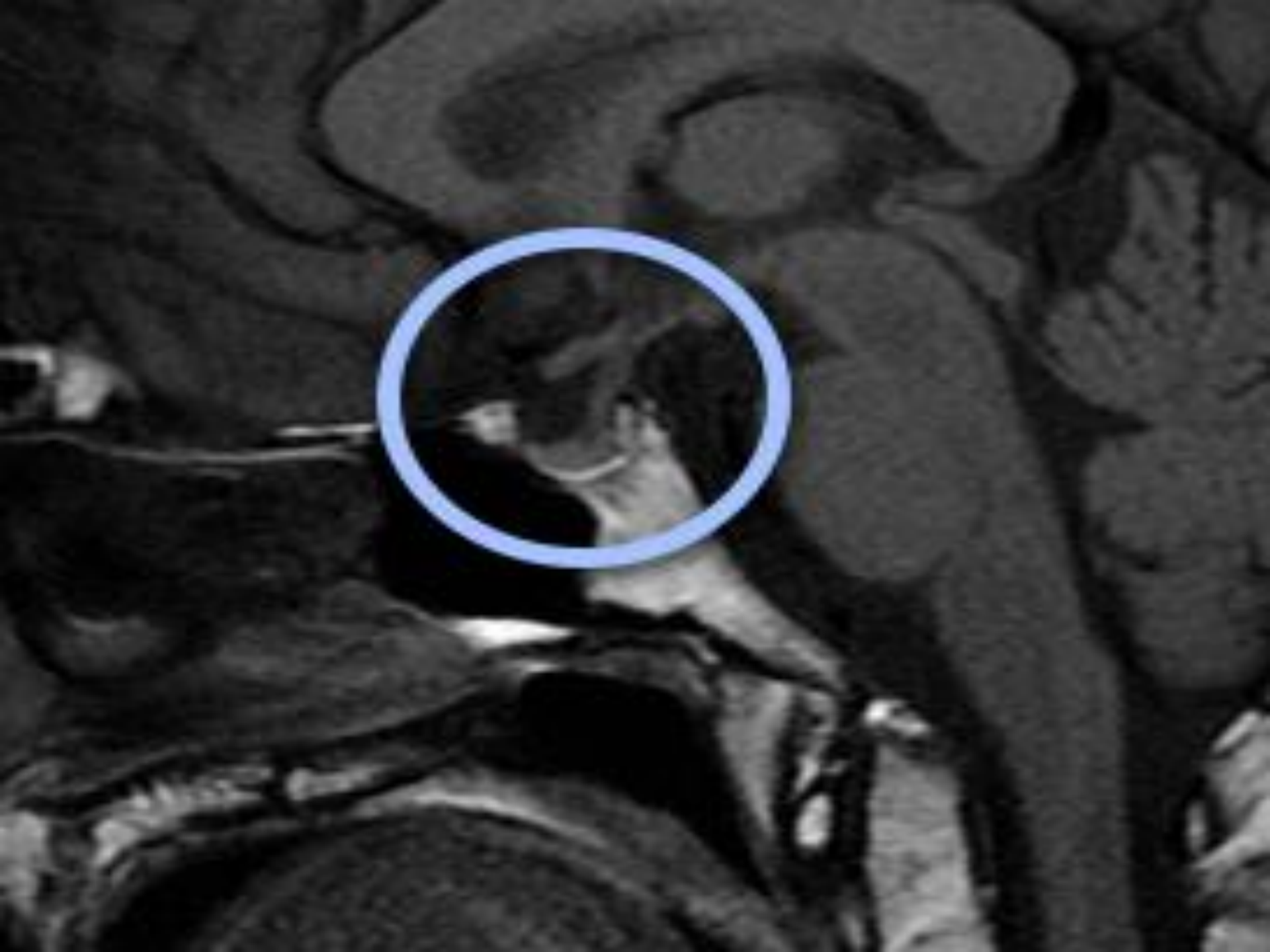
Fig. 26-23

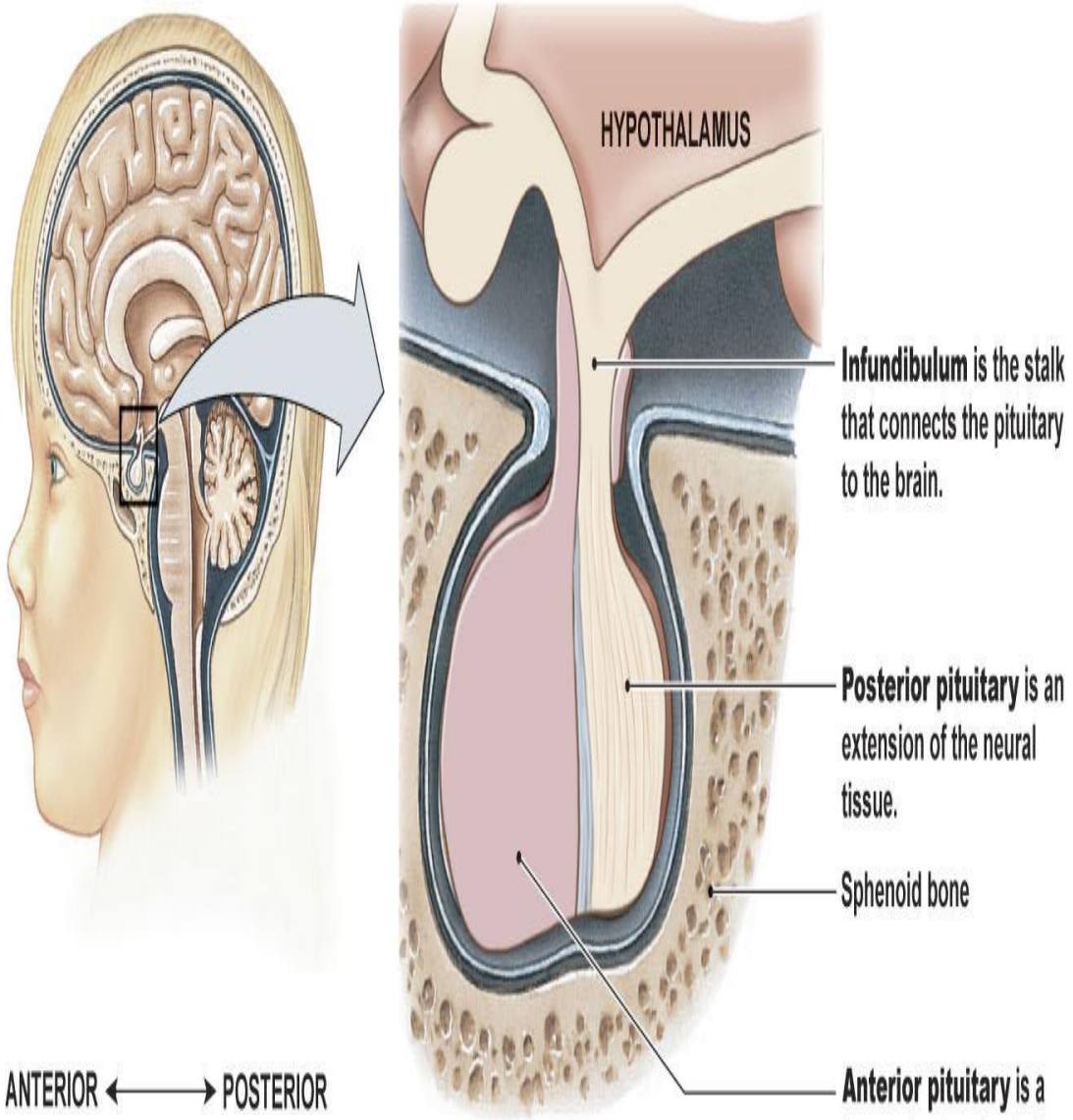


PITUITARY GLAND

- **Hypophysis.**
- **1cm .**
- **0.5-1 gram.**







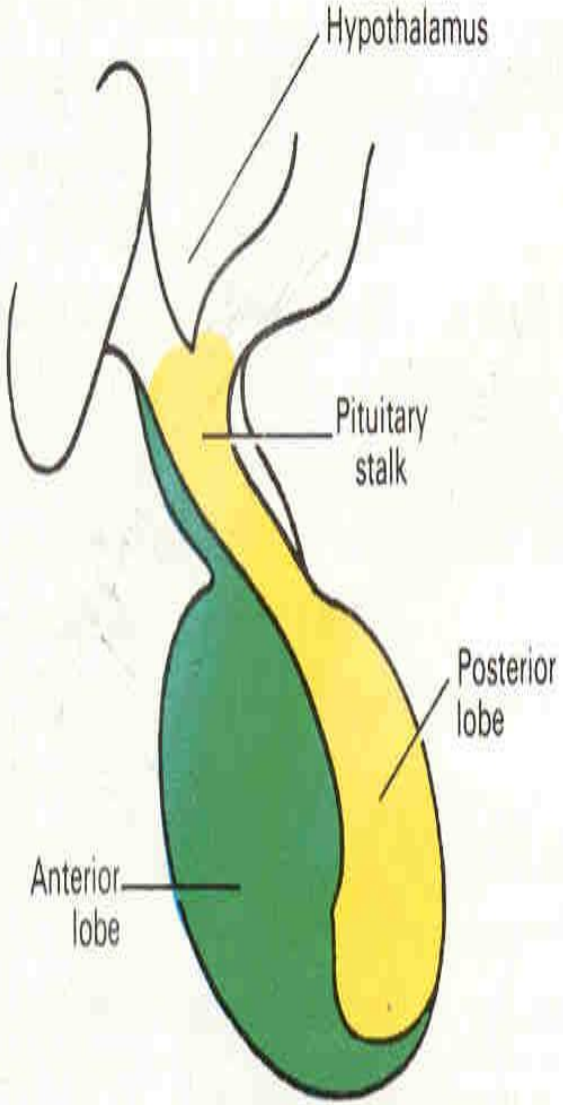


Figure 14:3 The parts of the pituitary gland and its relation to the hypothalamus.

STRUCTURE

Anterior lobe (adenohypophysis).

Posterior lobe (neurohypophysis).

Infundibulum.

Hypothalamus

Mammillary body

Median eminence

Optic chiasm

Infundibulum

Anterior pituitary

- Pars tuberalis
- Pars intermedia
- Pars distalis

Posterior pituitary

- Infundibular stalk
- Pars nervosa

Hypophyseal fossa in sella turcica of sphenoid bone

RELATIONSHIP OF THE HYPOTHALAMUS TO THE POSTERIOR PITUITARY

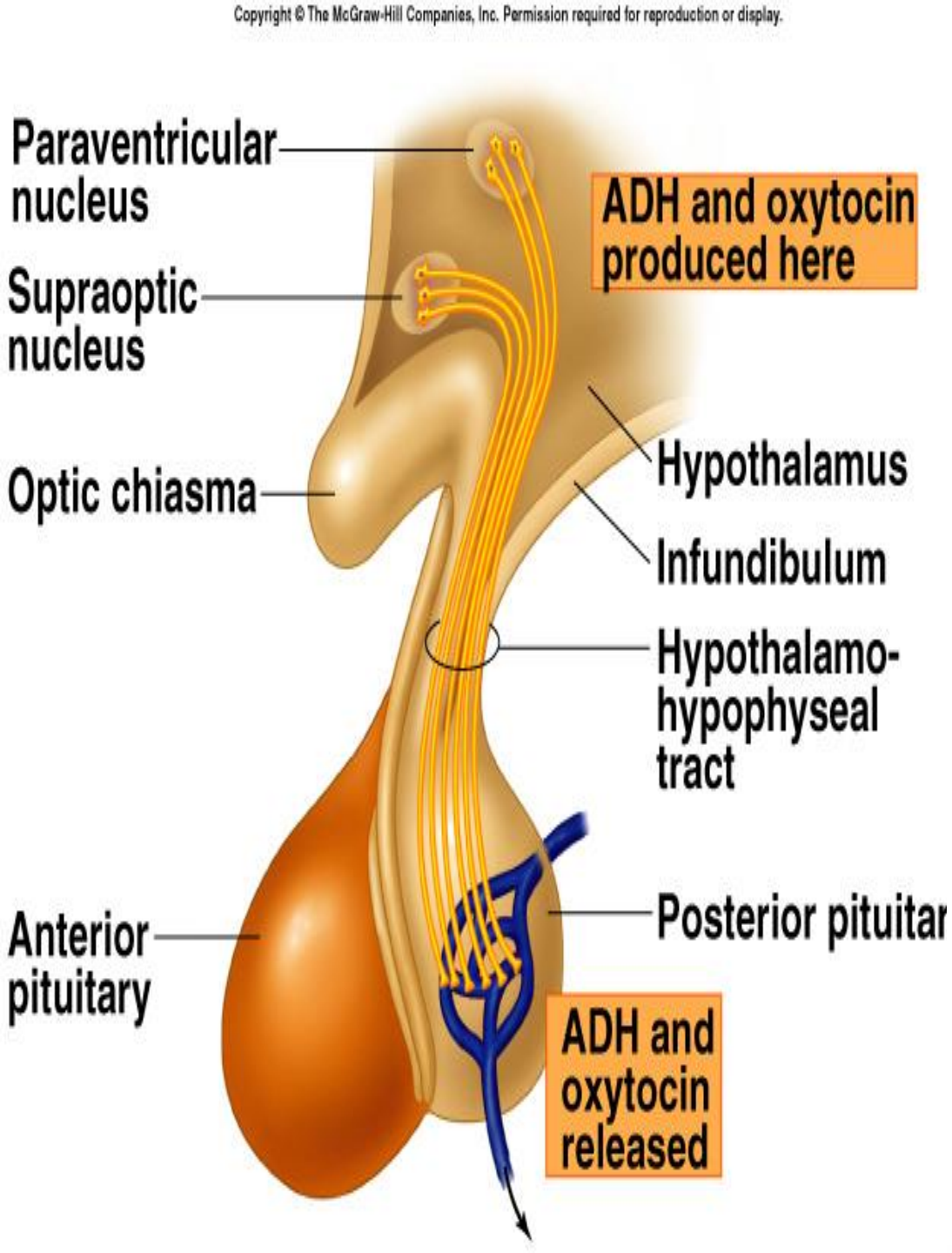
- **Collection of nerve axons +supporting cells.**

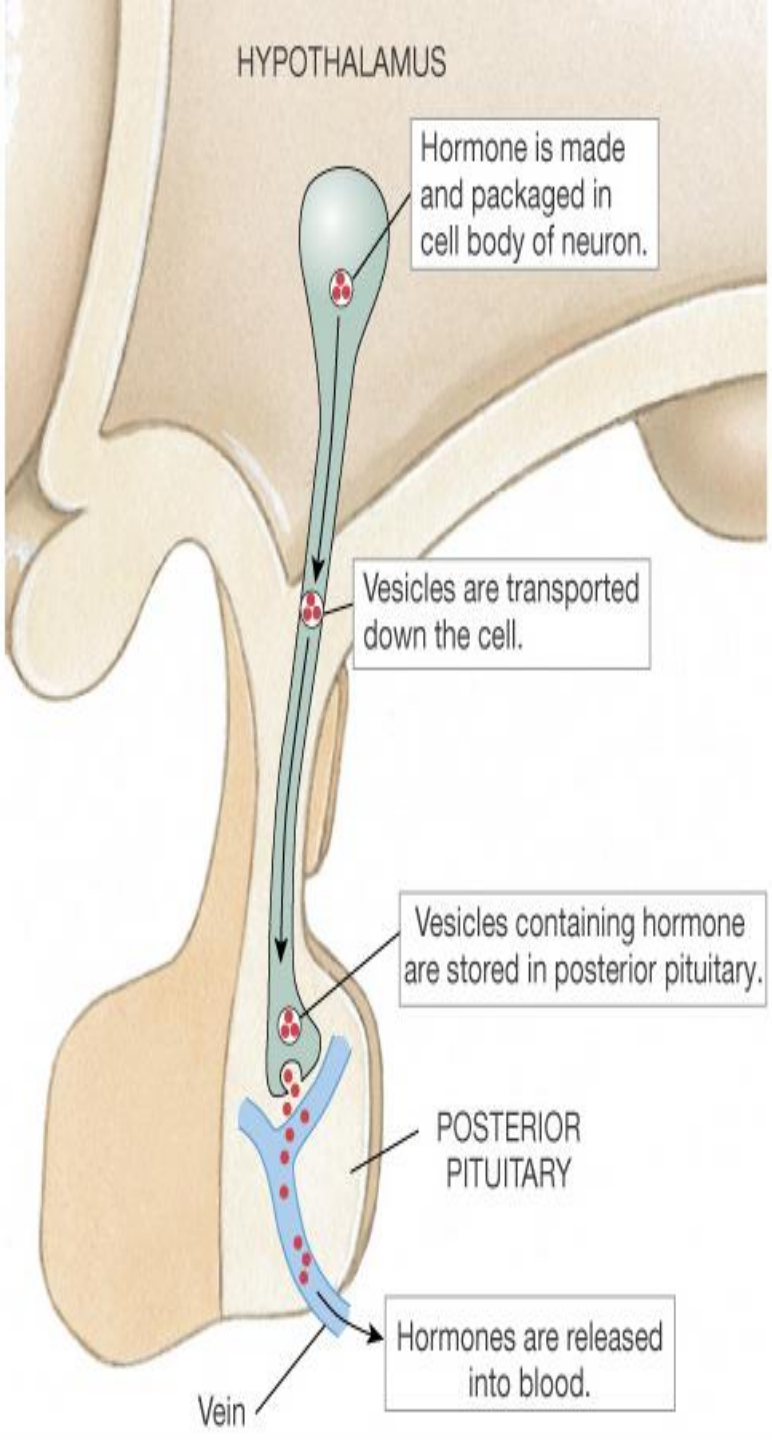
1- Antidiuretic hormone (ADH).

Supraoptic nuclei.

2- Oxytocin.

Paraventricular nuclei.





HYPOTHALAMO-NEURO HYPOPHYSIAL TRACT

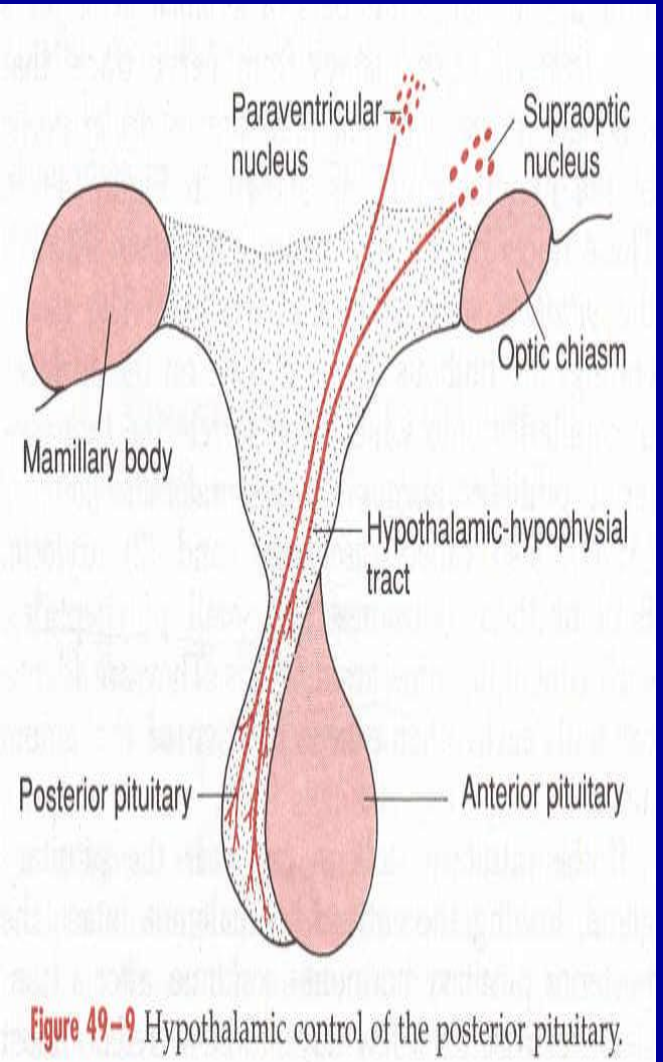
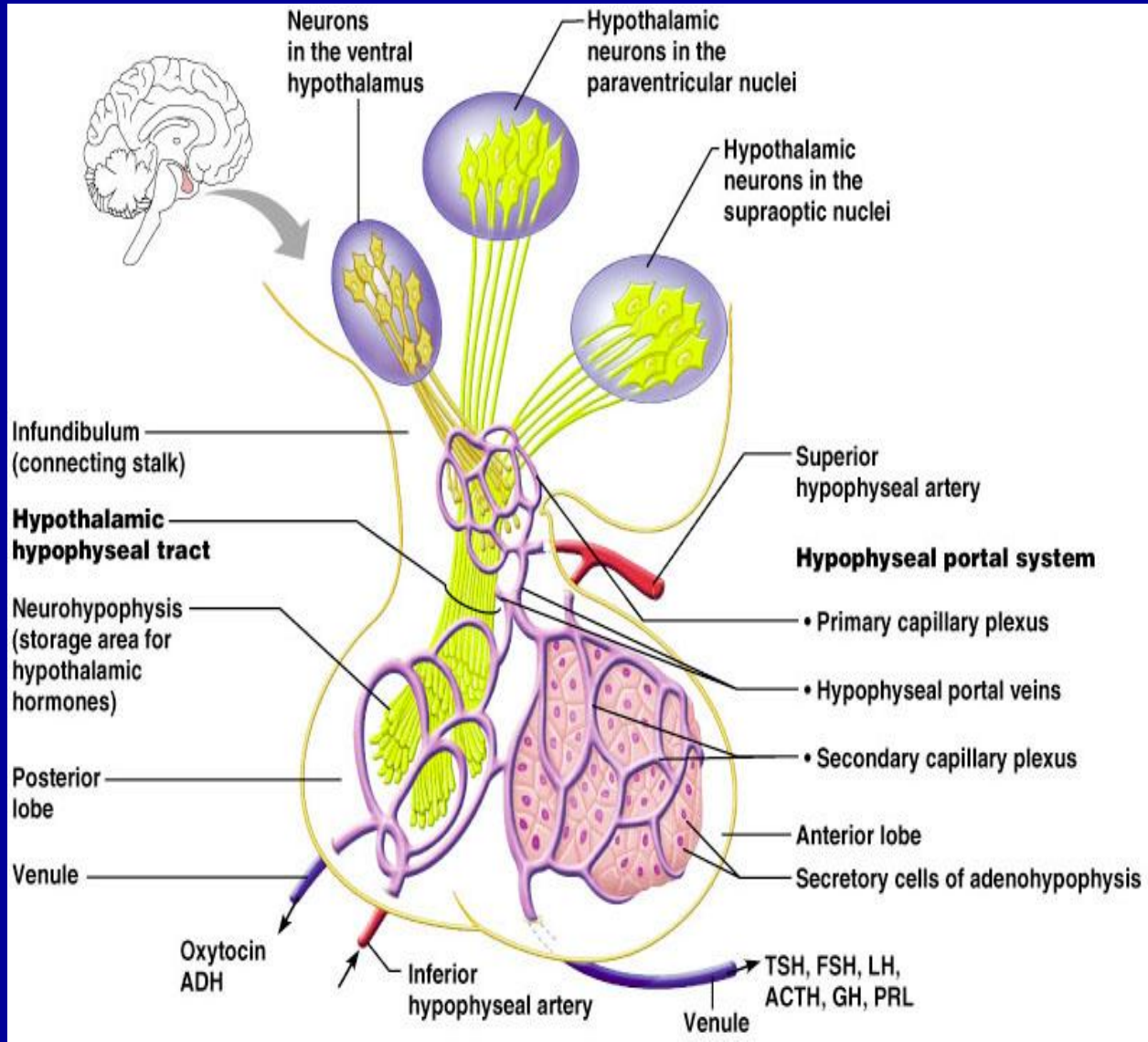


Figure 49–9 Hypothalamic control of the posterior pituitary.



RELATIONSHIP OF THE HYPOTHALAMUS TO THE ANTERIOR PITUITARY

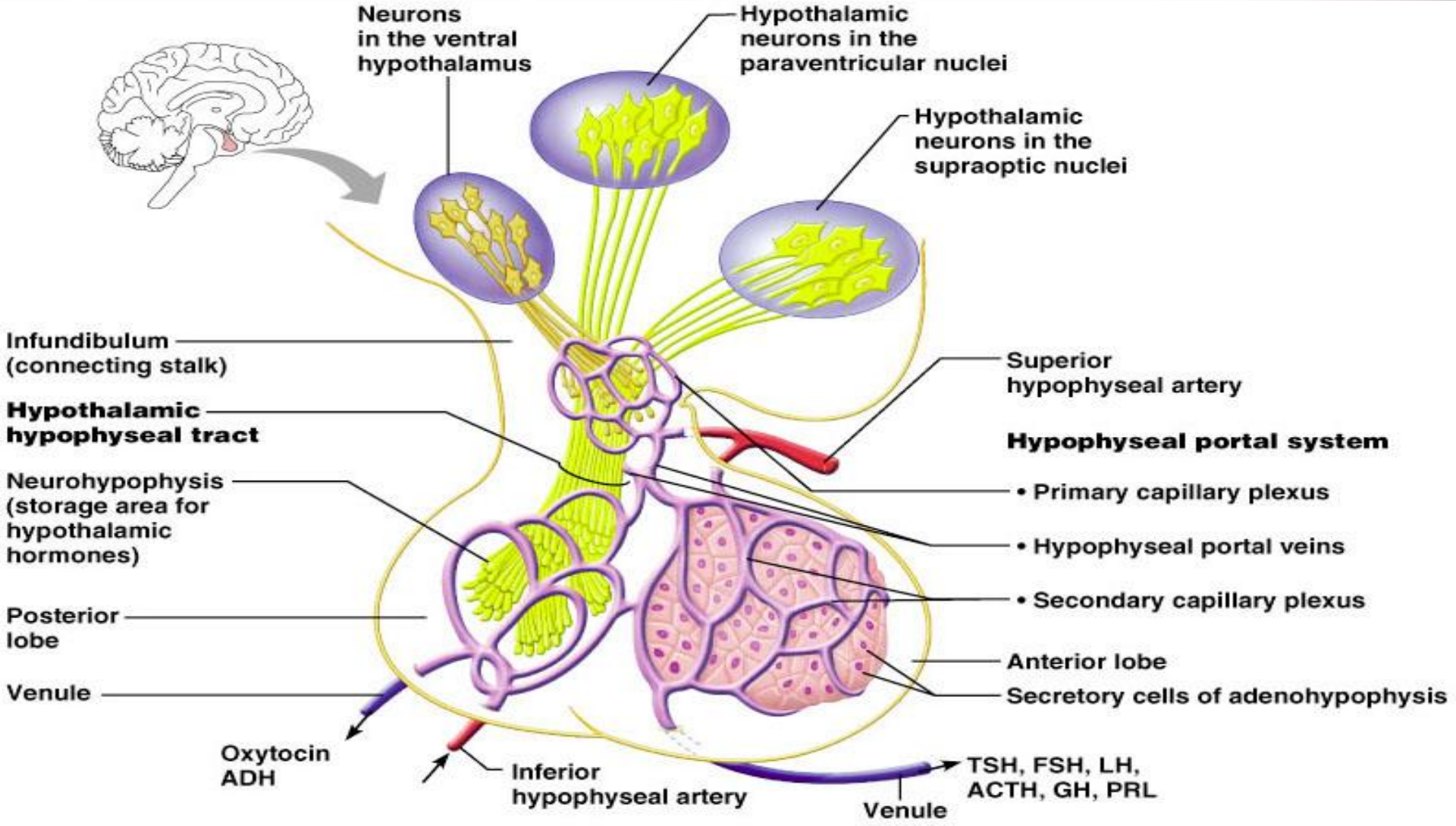
collection of endocrine glands.

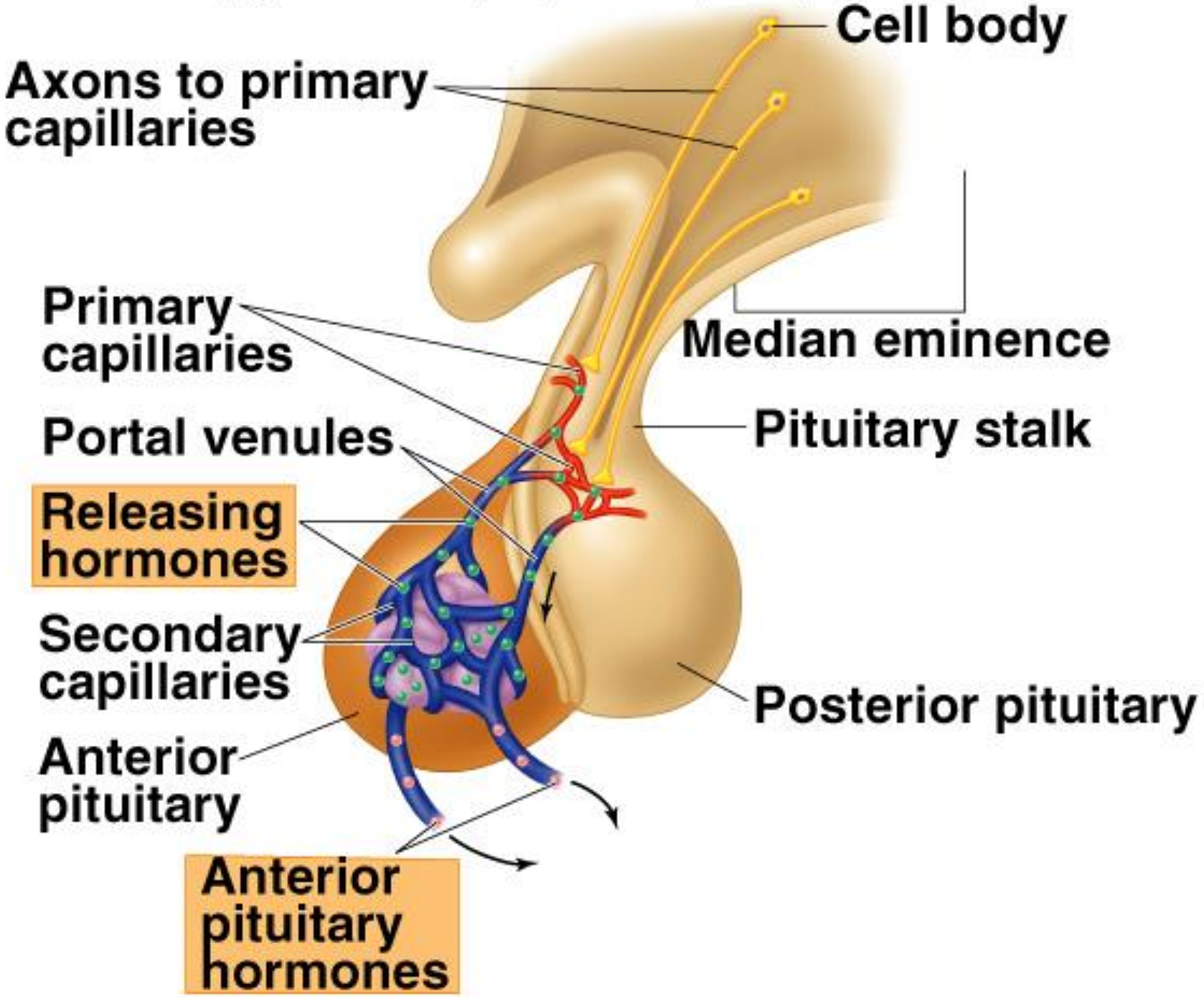
1- TSH
2- FSH
3- LH
4- GH
5- PROLACTIN
6- ACTH.

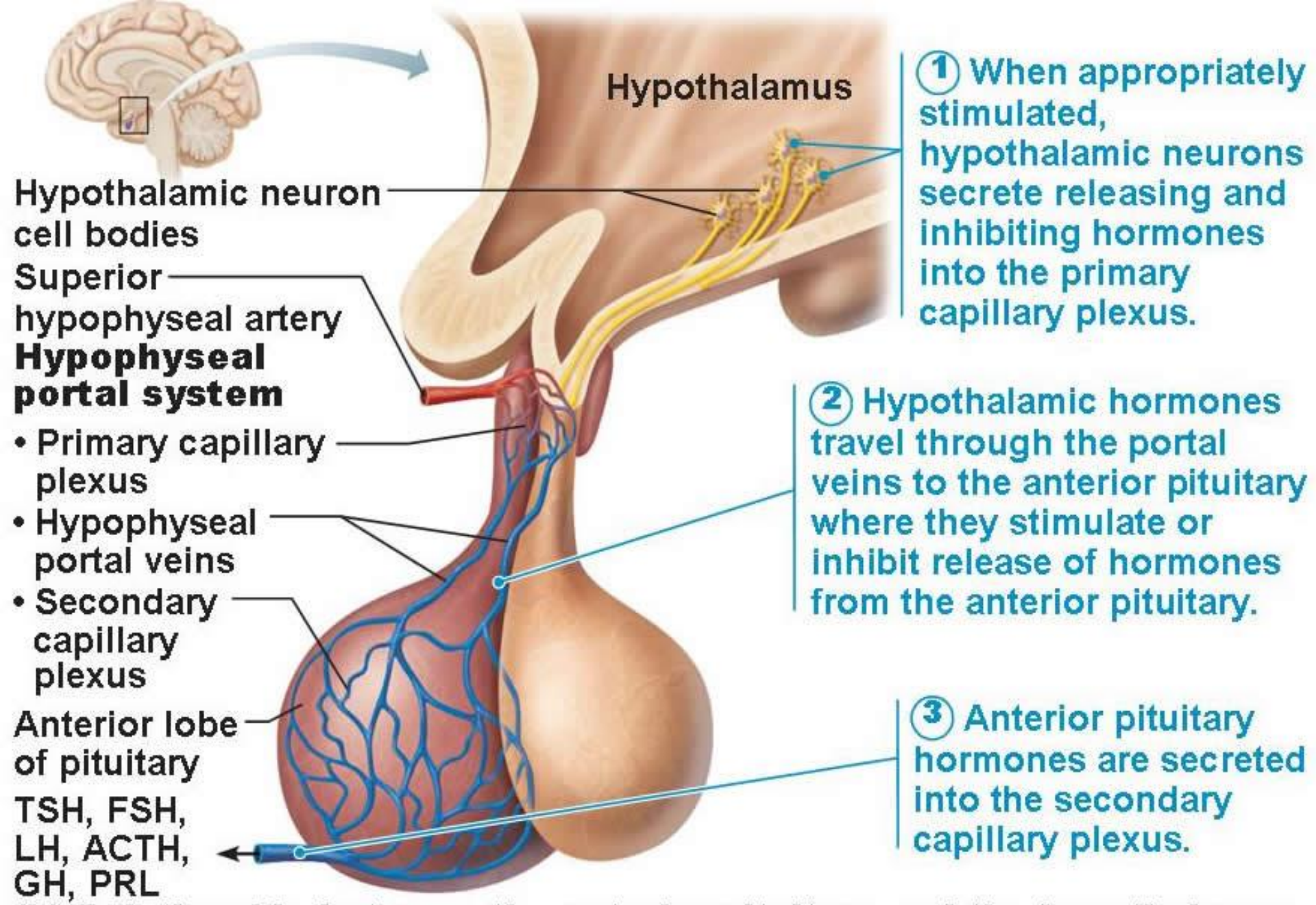
Table 11.6 | Anterior Pituitary Hormones

Hormone	Target Tissue	Principal Actions	Regulation of Secretion
ACTH (adrenocorticotropic hormone)	Adrenal cortex	Stimulates secretion of glucocorticoids	Stimulated by CRH (corticotropin-releasing hormone); inhibited by glucocorticoids
TSH (thyroid-stimulating hormone)	Thyroid gland	Stimulates secretion of thyroid hormones	Stimulated by TRH (thyrotropin-releasing hormone); inhibited by thyroid hormones
GH (growth hormone)	Most tissue	Promotes protein synthesis and growth; lipolysis and increased blood glucose	Inhibited by somatostatin; stimulated by growth hormone-releasing hormone
FSH (follicle-stimulating hormone)	Gonads	Promotes gamete production and stimulates estrogen production in females	Stimulated by GnRH (gonadotropin-releasing hormone); inhibited by sex steroids and inhibin
PRL (prolactin)	Mammary glands and other sex accessory organs	Promotes milk production in lactating females; additional actions in other organs	Inhibited by PIH (prolactin-inhibiting hormone)
LH (luteinizing hormone)	Gonads	Stimulates sex hormone secretion; ovulation and corpus luteum formation in females; stimulates testosterone secretion in males	Stimulated by GnRH; inhibited by sex steroids

HYPOTHALAMIC-HYPOPHYSIAL PORTAL SYSTEM

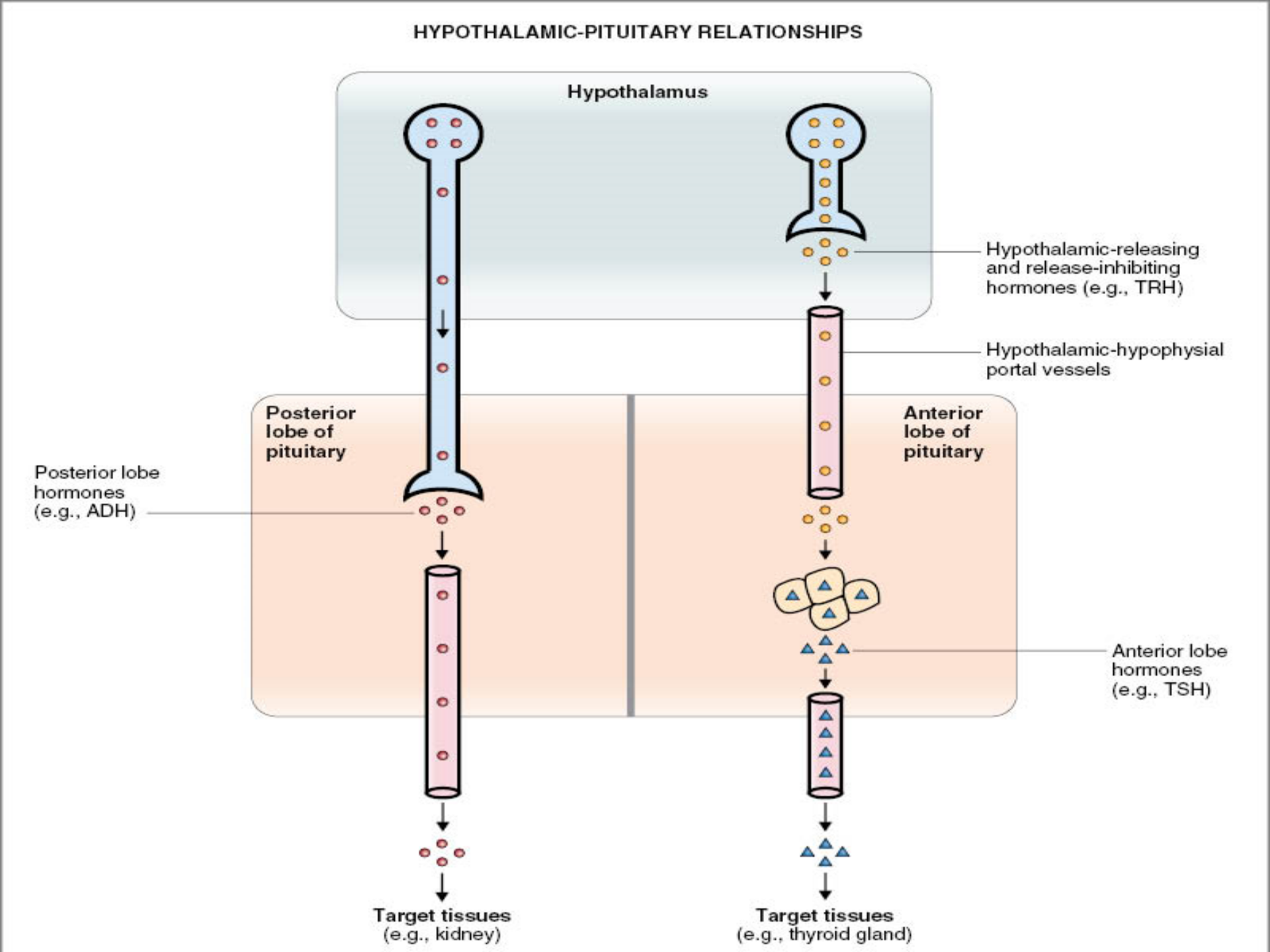






(b) Relationship between the anterior pituitary and the hypothalamus

- **Both neural and endocrine.**


HYPOTHALAMIC-PITUITARY RELATIONSHIPS
Hypothalamus
Hypothalamic-releasing and release-inhibiting hormones (e.g., TRH)
Hypothalamic-hypophysial portal vessels
Posterior lobe of pituitary
Anterior lobe of pituitary
Posterior lobe hormones (e.g., ADH)
Anterior lobe hormones (e.g., TSH)
Target tissues (e.g., kidney)
Target tissues (e.g., thyroid gland)

NEGATIVE FEEDBACK MECHANISM

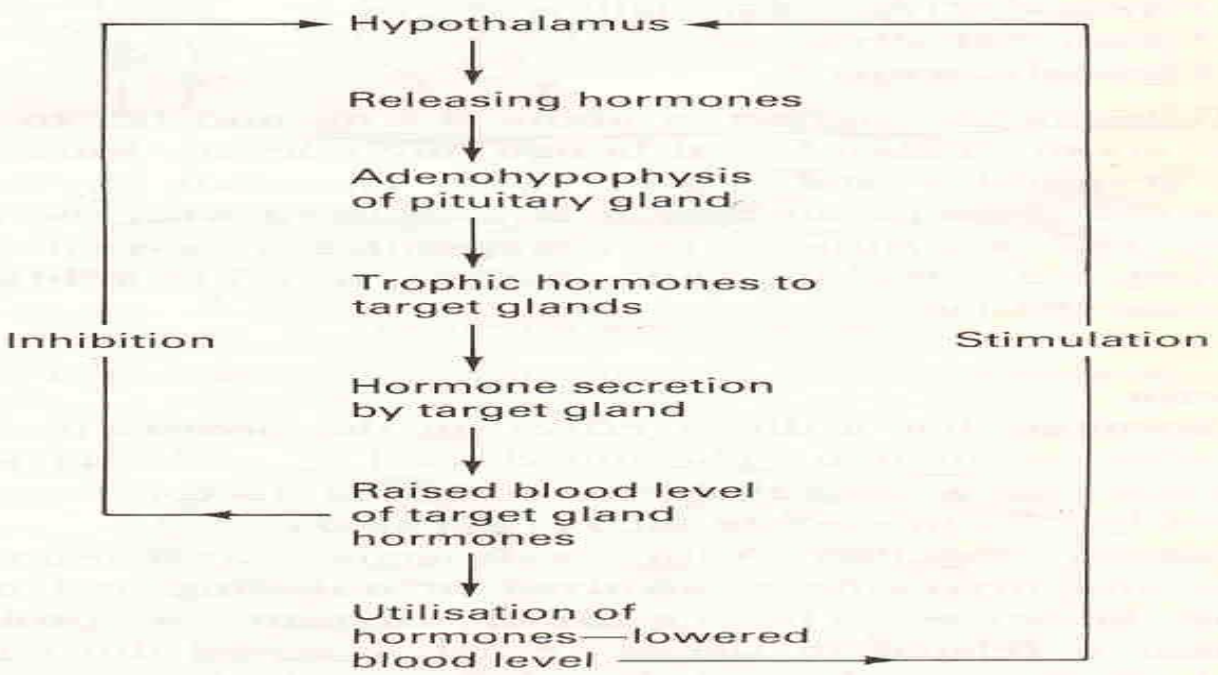
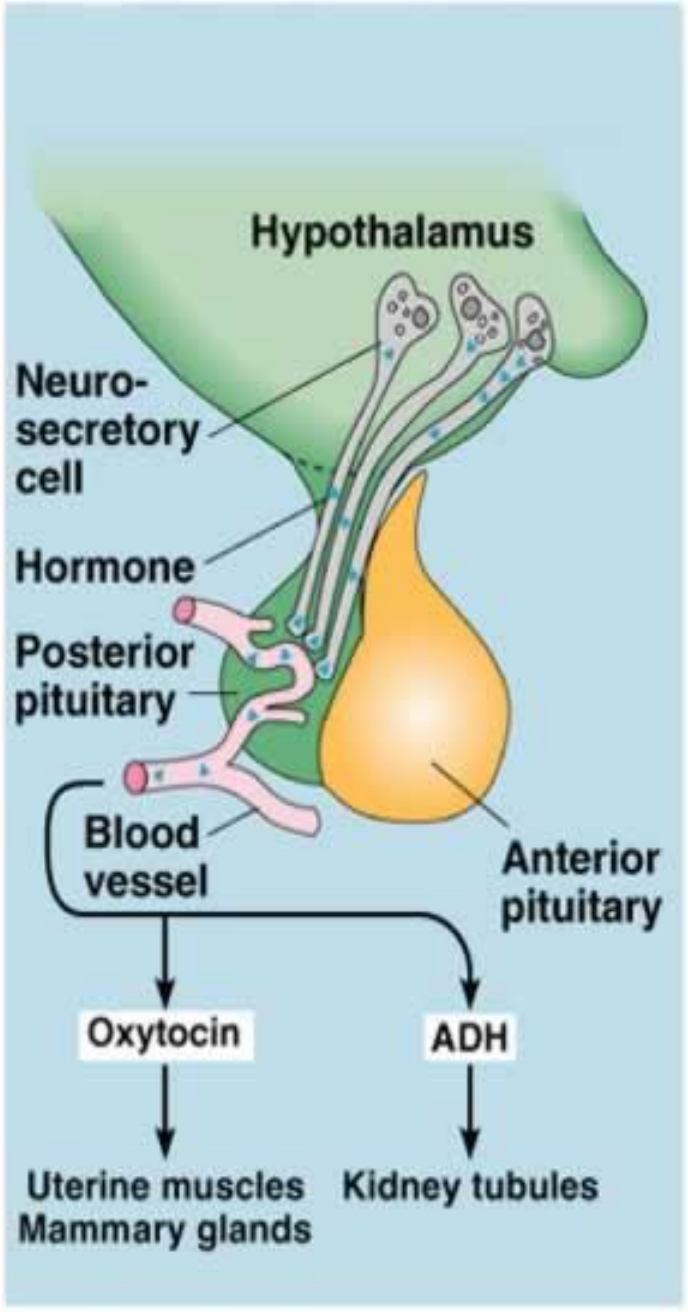
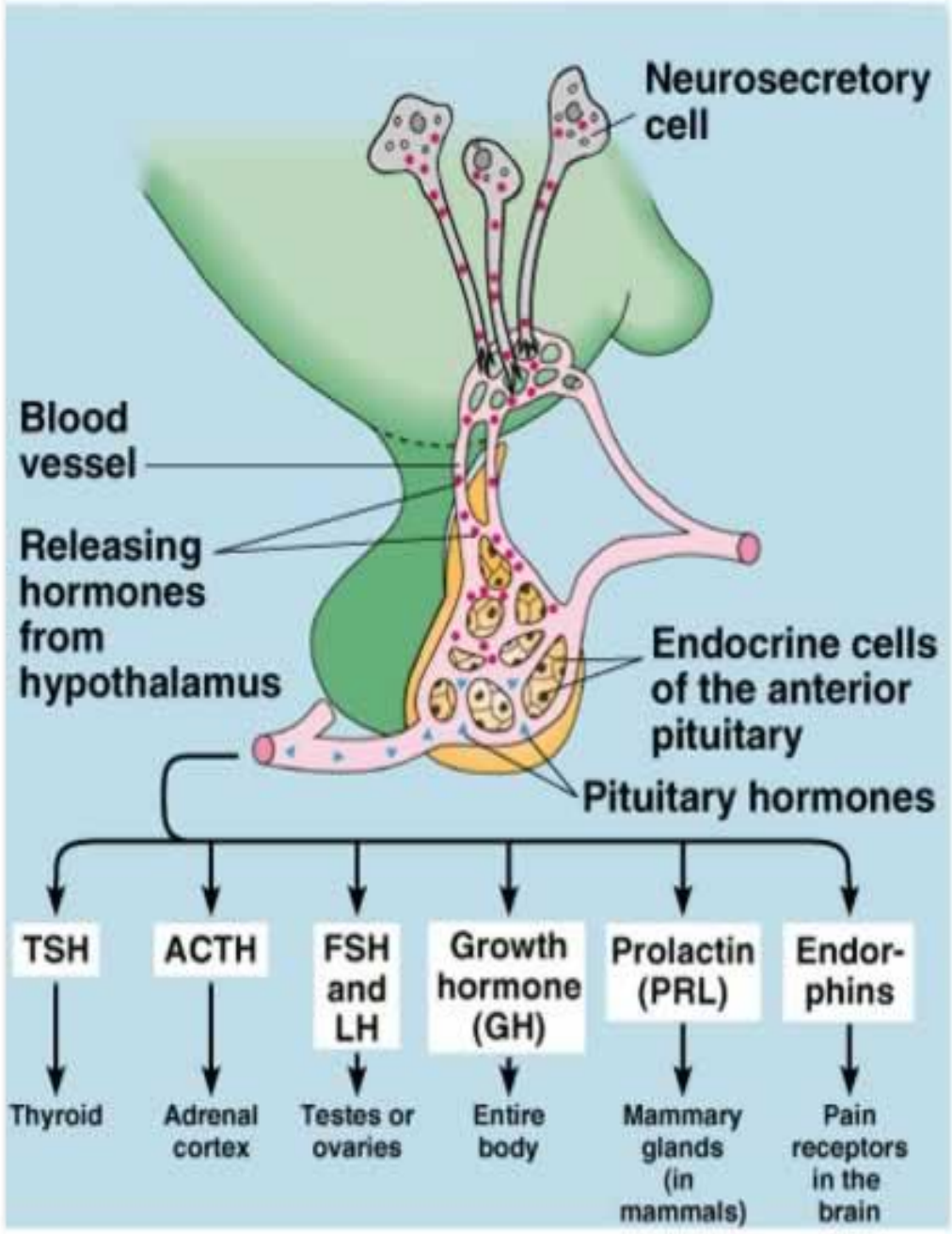


Figure 14:4 Diagram of the negative feedback regulation of the secretions of hormones by the anterior lobe of the pituitary gland.



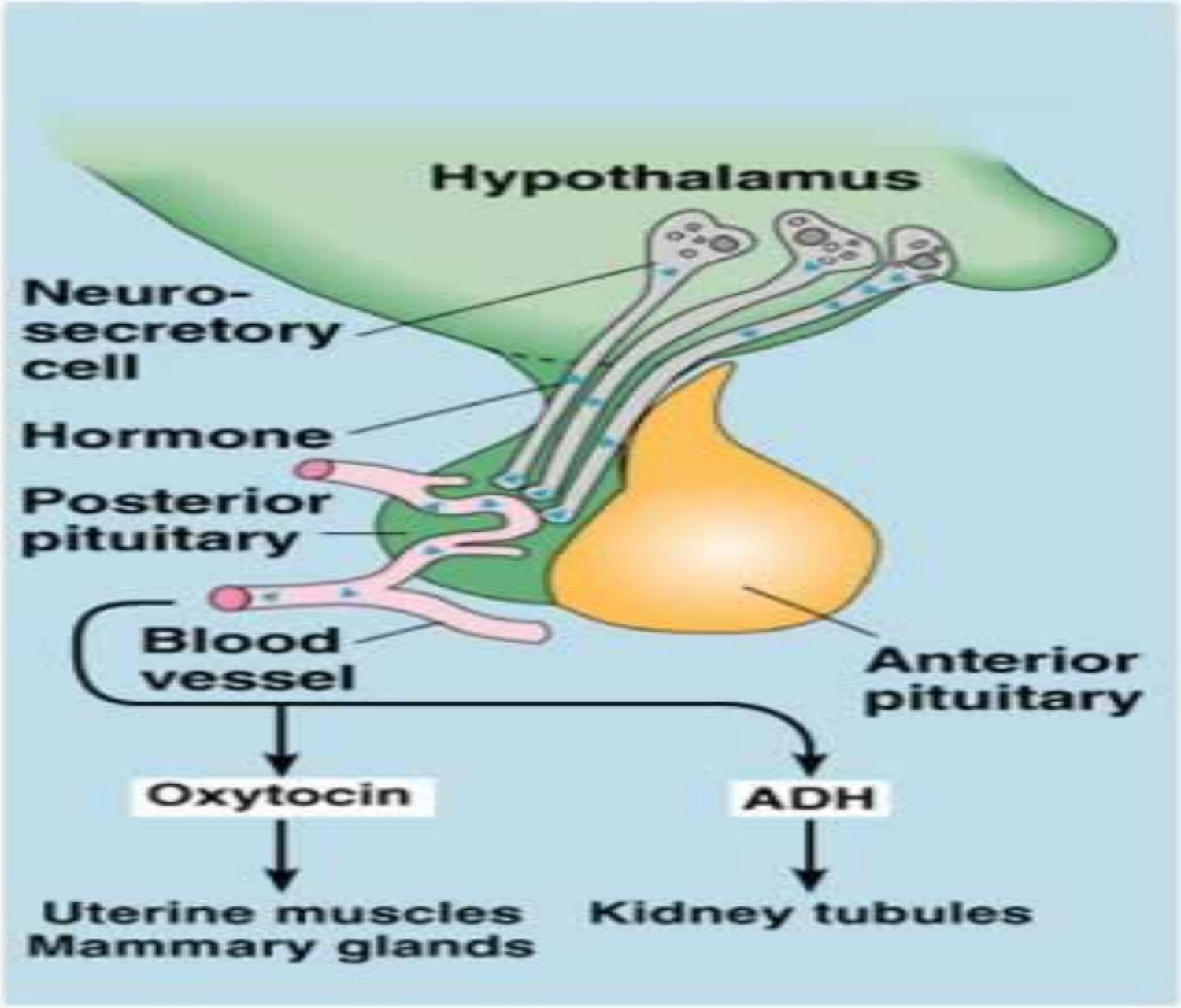


POSTERIOR PITUITARY

HORMONES

1- Antidiuretic hormone (ADH).

2- Oxytocin.



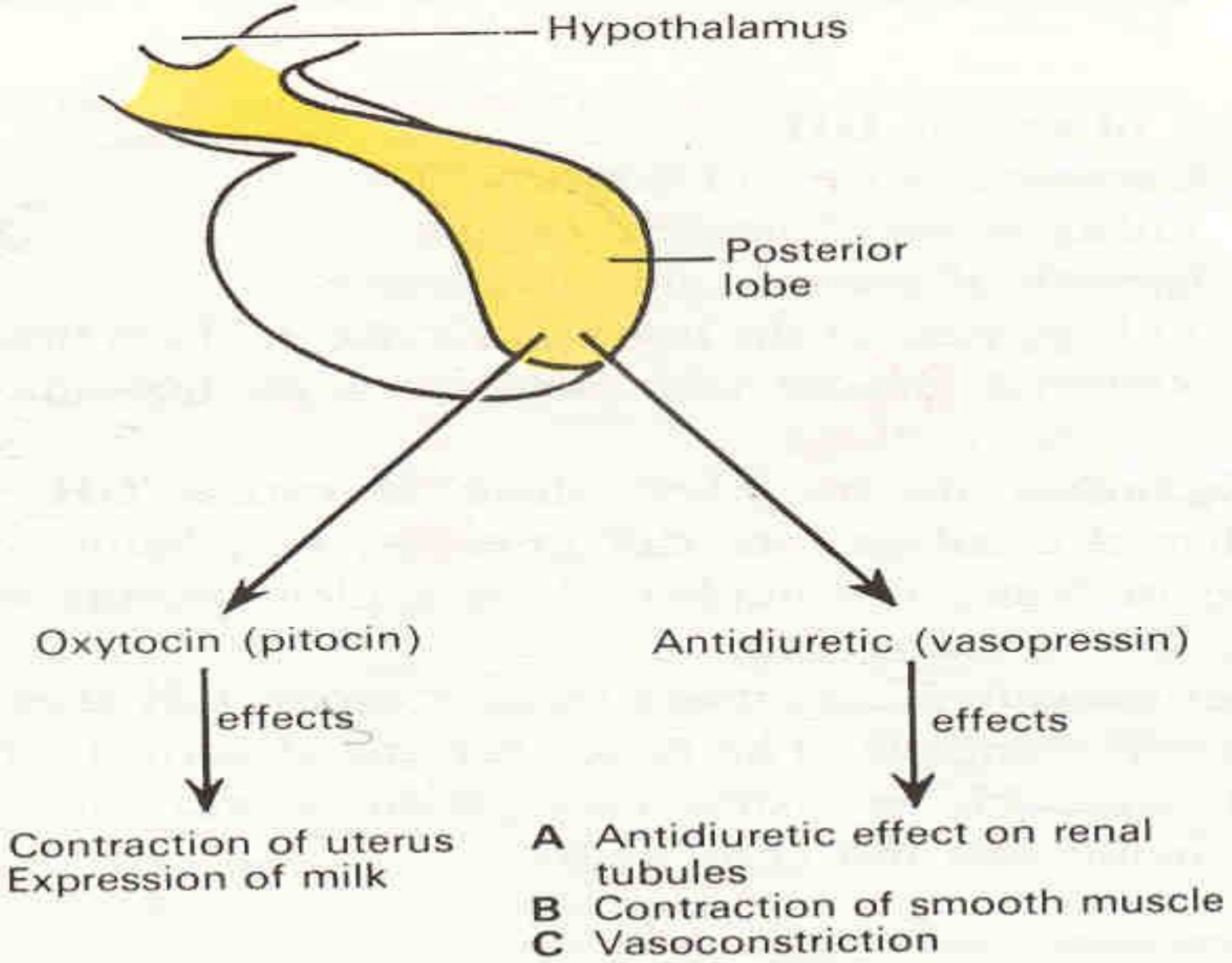
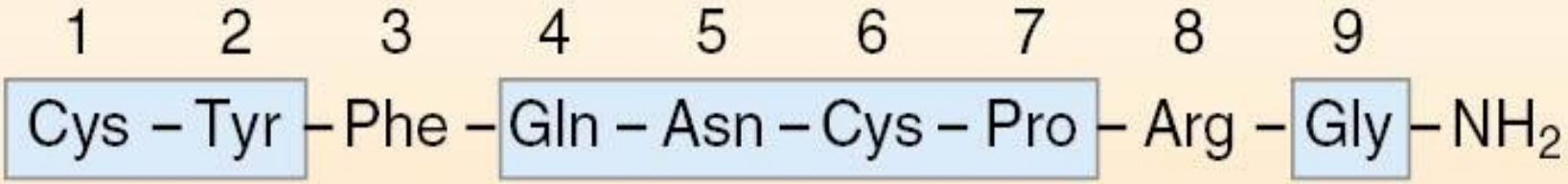
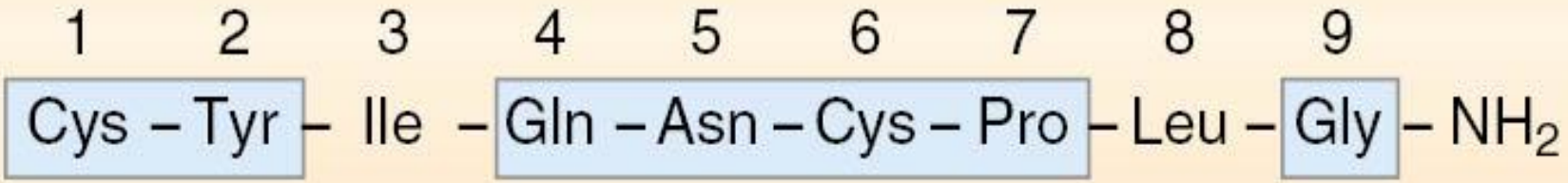


Figure 14:7 The hormones secreted by the posterior lobe of the pituitary gland (neurohypophysis) and their main functions.

ADH



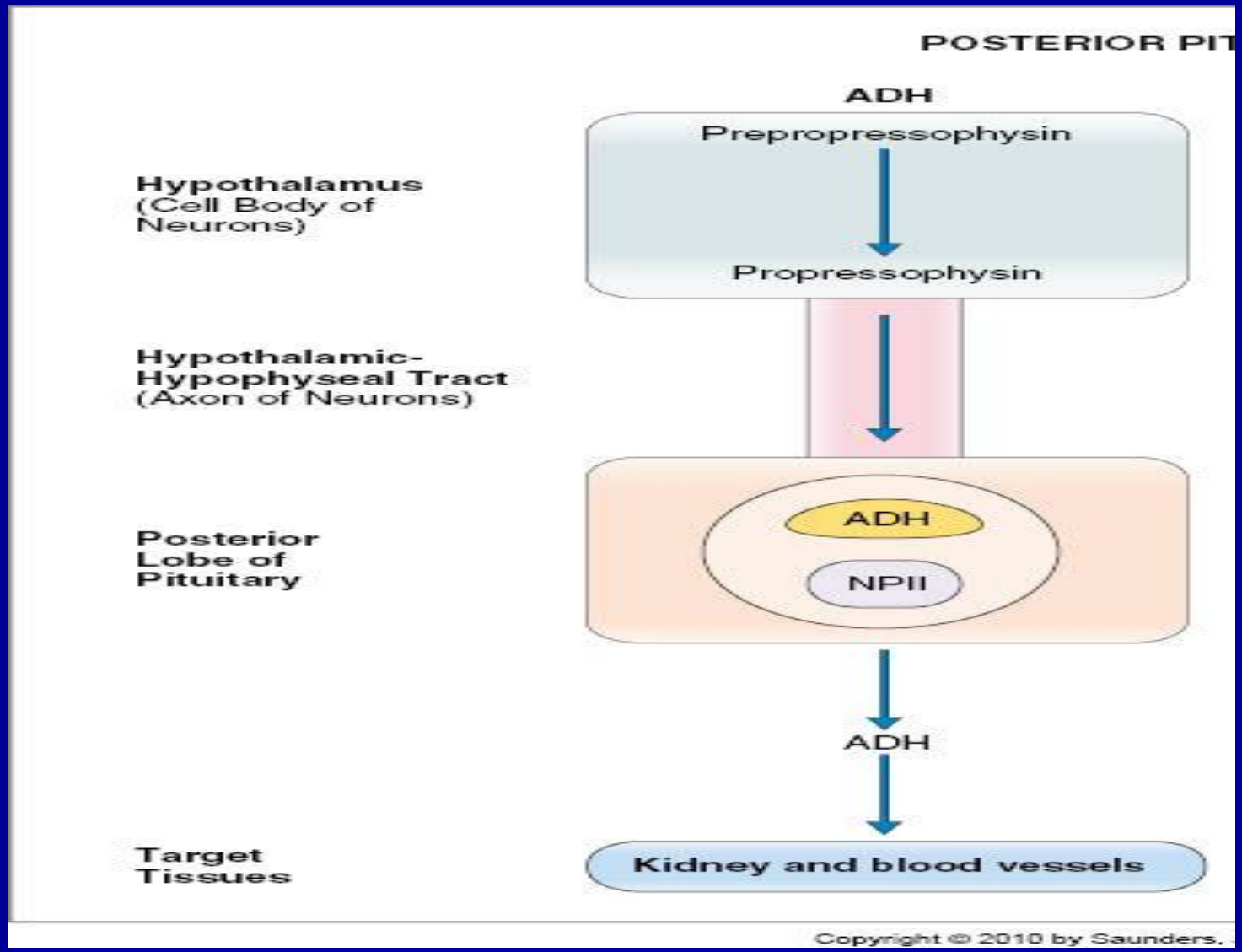
Oxytocin



ADH

- Vasopressin.
- Nonapeptides.
- Supraoptic nuclei.
- Prepropressophysin.
- Neurophysins-2.

REGULATION OF SECRETION



ACTION

1- Osmolarity regulation.

Increase water reabsorption.

V2 receptor

↓

Gs protein.

↓

Adenylyl cyclase.

↓

cAMP

↓

Phosphrylation
Insertion.

2- Contraction of vascular smooth muscle.

V1 receptor.

↓

Gs protein.

↓

Phospholipase C.

IP3, Ca

↓

contraction

Table 9-6 Factors Affecting Antidiuretic Hormone Secretion

Stimulatory Factors	Inhibitory Factors
Increased serum osmolarity	Decreased serum osmolarity
Decreased ECF volume	Ethanol
Angiotensin II	α-Adrenergic agonists
Pain	Atrial natriuretic peptide (ANP)
Nausea	
Hypoglycemia	
Nicotine	
Opiates	
Antineoplastic drugs	

ABNORMALITIES

- **Diabetes insipidus**

1- central.

2- nephrogenic.

- **Syndrom of inappropriate ADH (SIADH).**

OXYTOCYIN

- **Nonapeptides.**
- **Paraventricular nuclei.**
- **Preprooxyphysin.**
- **Neurophysins-1.**

REGULATION OF SECRETION

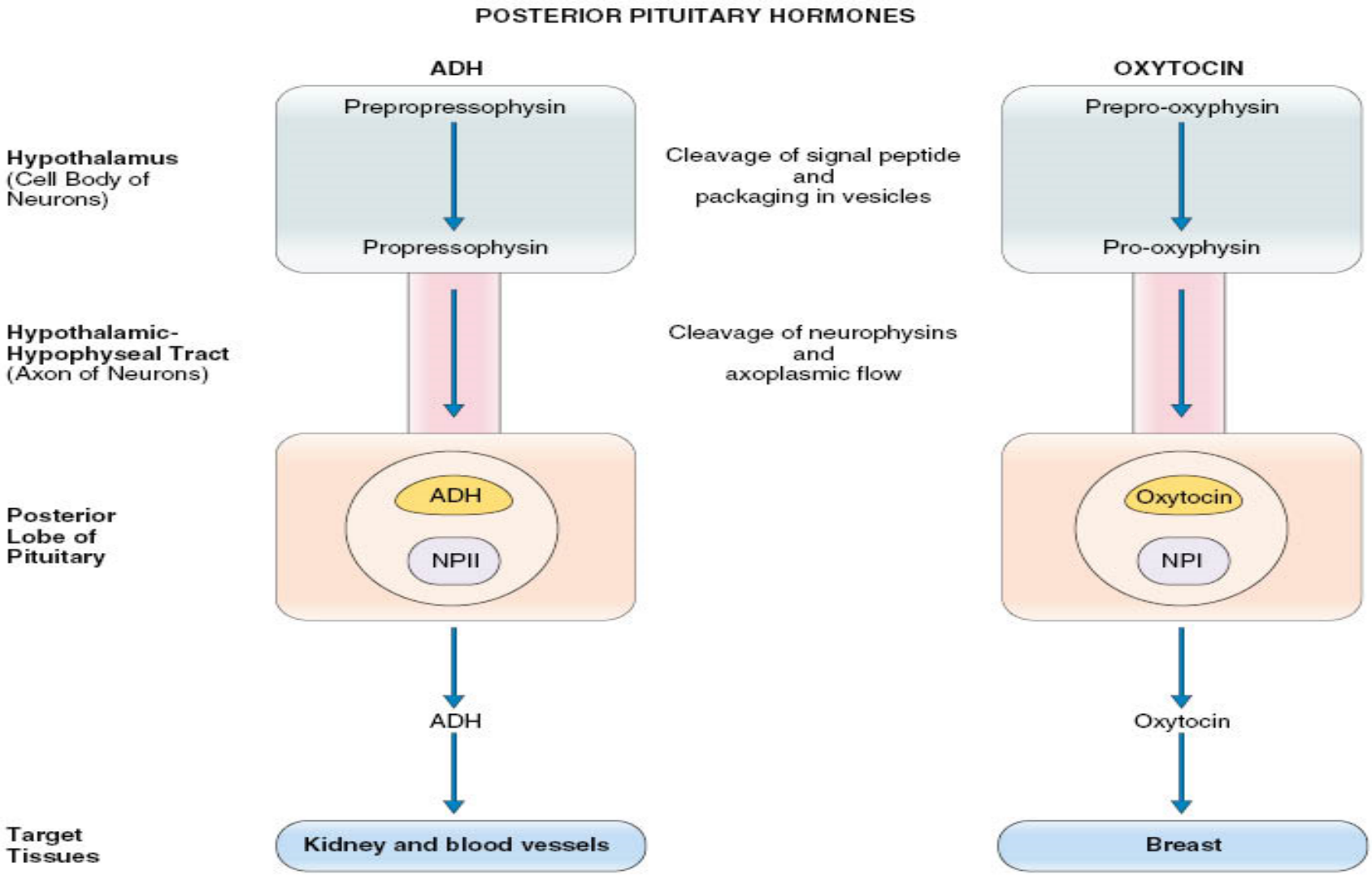


Table 9-7 Factors Affecting Oxytocin Secretion

Stimulatory Factors	Inhibitory Factors
Suckling	Opioids (endorphins)
Sight, sound, or smell of the infant	
Dilation of the cervix	
Orgasm	

ACTION

1- Milk ejection.

2- Uterine contraction.

Inducing labor.

Reducing postpartum bleeding.

ANTERIOR PITUITARY GLAND

ANTERIOR PITUITARY GLAND

- **Hormones:**

1- TSH

2- FSH

3- LH

4- GH

5- PROLACTIN

6- ACTH.

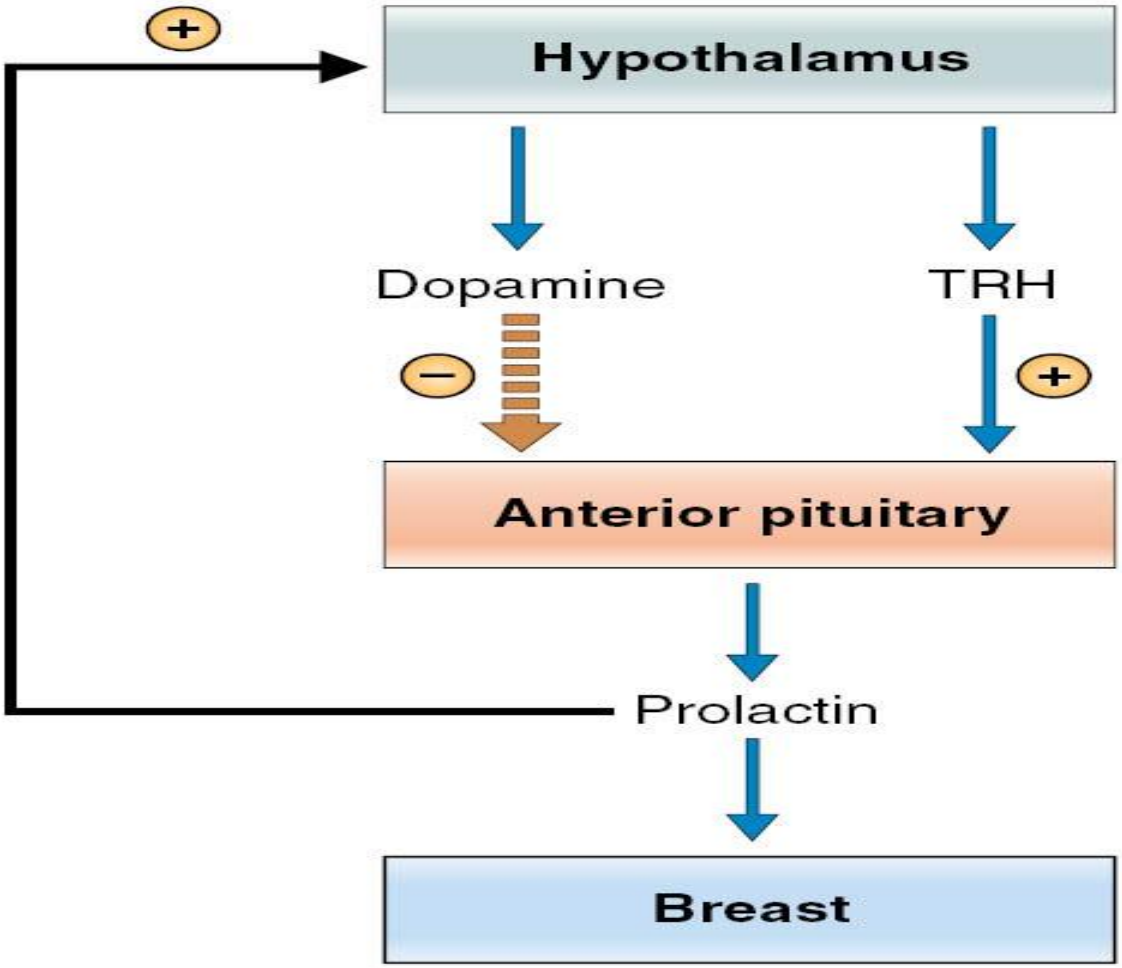
Table 11.6 | Anterior Pituitary Hormones

Hormone	Target Tissue	Principal Actions	Regulation of Secretion
ACTH (adrenocorticotropic hormone)	Adrenal cortex	Stimulates secretion of glucocorticoids	Stimulated by CRH (corticotropin-releasing hormone); inhibited by glucocorticoids
TSH (thyroid-stimulating hormone)	Thyroid gland	Stimulates secretion of thyroid hormones	Stimulated by TRH (thyrotropin-releasing hormone); inhibited by thyroid hormones
GH (growth hormone)	Most tissue	Promotes protein synthesis and growth; lipolysis and increased blood glucose	Inhibited by somatostatin; stimulated by growth hormone-releasing hormone
FSH (follicle-stimulating hormone)	Gonads	Promotes gamete production and stimulates estrogen production in females	Stimulated by GnRH (gonadotropin-releasing hormone); inhibited by sex steroids and inhibin
PRL (prolactin)	Mammary glands and other sex accessory organs	Promotes milk production in lactating females; additional actions in other organs	Inhibited by PIH (prolactin-inhibiting hormone)
LH (luteinizing hormone)	Gonads	Stimulates sex hormone secretion; ovulation and corpus luteum formation in females; stimulates testosterone secretion in males	Stimulated by GnRH; inhibited by sex steroids

PROLACTIN

- Lactotrophs.(15%)
- 198 AA.
- Related to GH.

REGULATION OF SECRETION



SOURCES OF DOPAMINE

1- Dopaminergic neurons in the hypothalamus.

2- Dopaminergic neurons in the posterior pituitary.

3- Nonlactotrophs cells of the anterior pituitary.

Table 9-5 Factors Affecting Prolactin Secretion

Stimulatory Factors	Inhibitory Factors
Pregnancy (estrogen)	Dopamine
Breast-feeding	Bromocriptine (dopamine agonist)
Sleep	Somatostatin
Stress	Prolactin (negative feedback)
TRH	
Dopamine antagonists	

ACTION

1- Breast development.

2- Lactogenesis.
(Lactose, lipid, casein)
Parturition.

3- Inhibition of ovulation.
GnRH

ABNORMALITIES

1- Prolactin deficiency.

Failure to lactate.

2- Prolactin excess.

Galactorrhea.

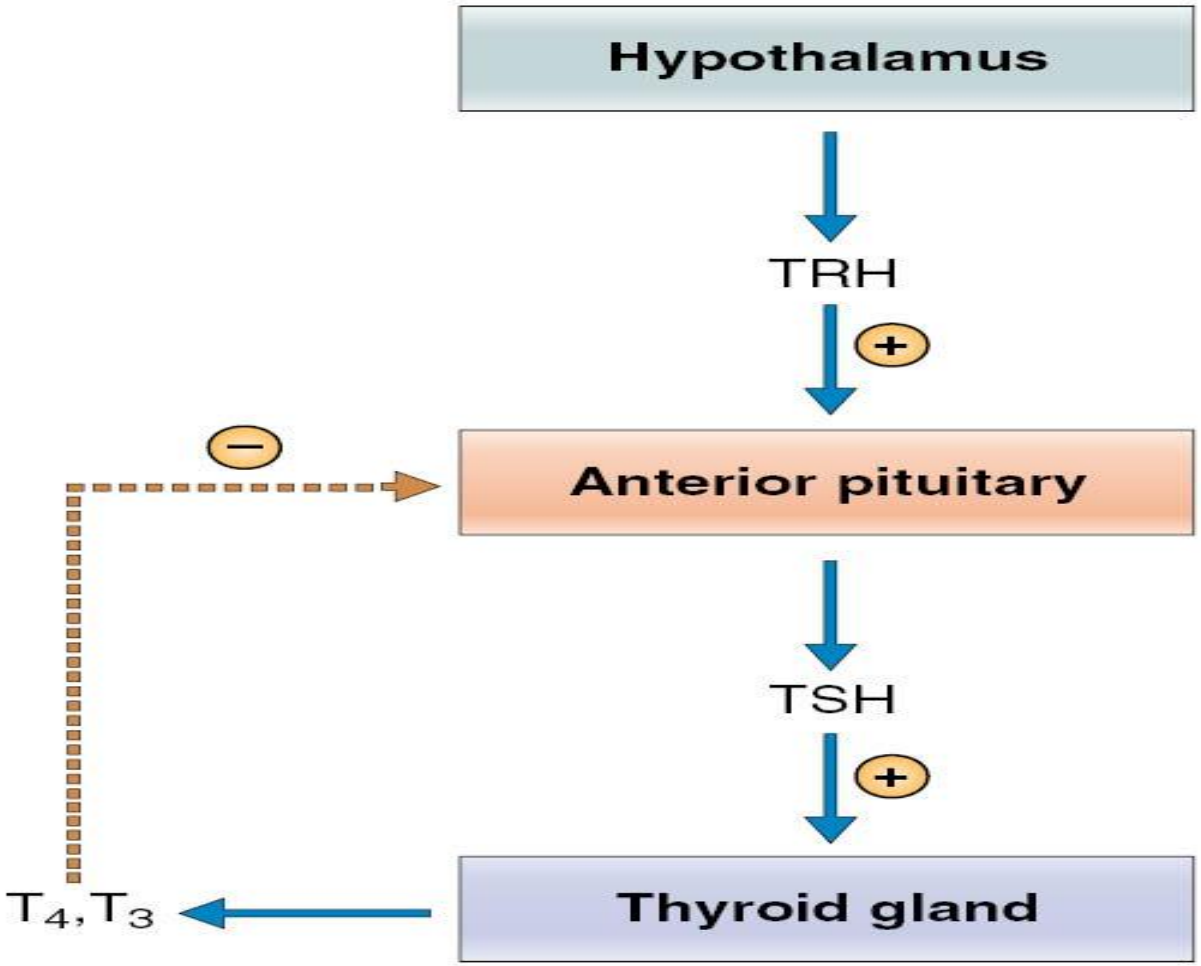
Infertility.

Bromocriptine.

TSH

- Thyrotrophs.(5%)
- Glycoproteins.
- α and β.
- Related to FSH and LH.

REGULATION OF SECRETION



ACTION

1- Increase synthesis and secretion of thyroid hormones.

2- Trophic effect.

Table 9-8 Factors Affecting Thyroid Hormone Secretion

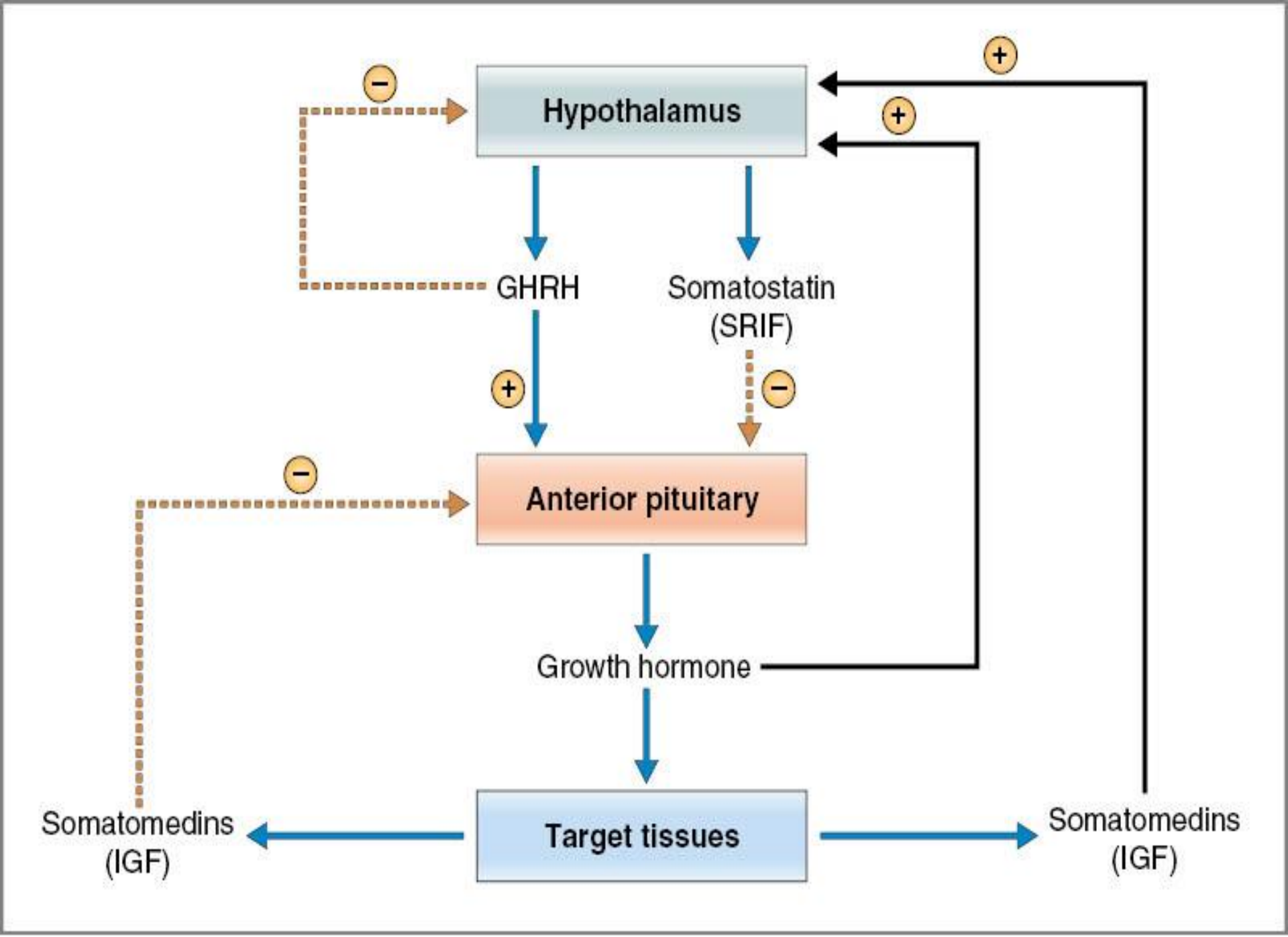
Stimulatory Factors	Inhibitory Factors
TSH	I^- deficiency
Thyroid-stimulating immunoglobulins	Deiodinase deficiency
Increased TBG levels (e.g., pregnancy)	Excessive I^- intake (Wolff-Chaikoff effect)
	Perchlorate; thiocyanate (inhibit Na^+-I^- cotransport)
	Propylthiouracil (inhibits peroxidase enzyme)
	Decreased TBG levels (e.g., liver disease)

ABNORMALITIES

- **Hyperthyroidism.**
- **Hypothyroidism.**

GROWTH HORMONE

- Somatotropic hormone, somatotropin.
- Somatotrophs.(20%)
- 191 AA.
- MW 22000 kD.
- GHRH (ventromedial nucleus).



- GHRH → receptor → Gs protein →

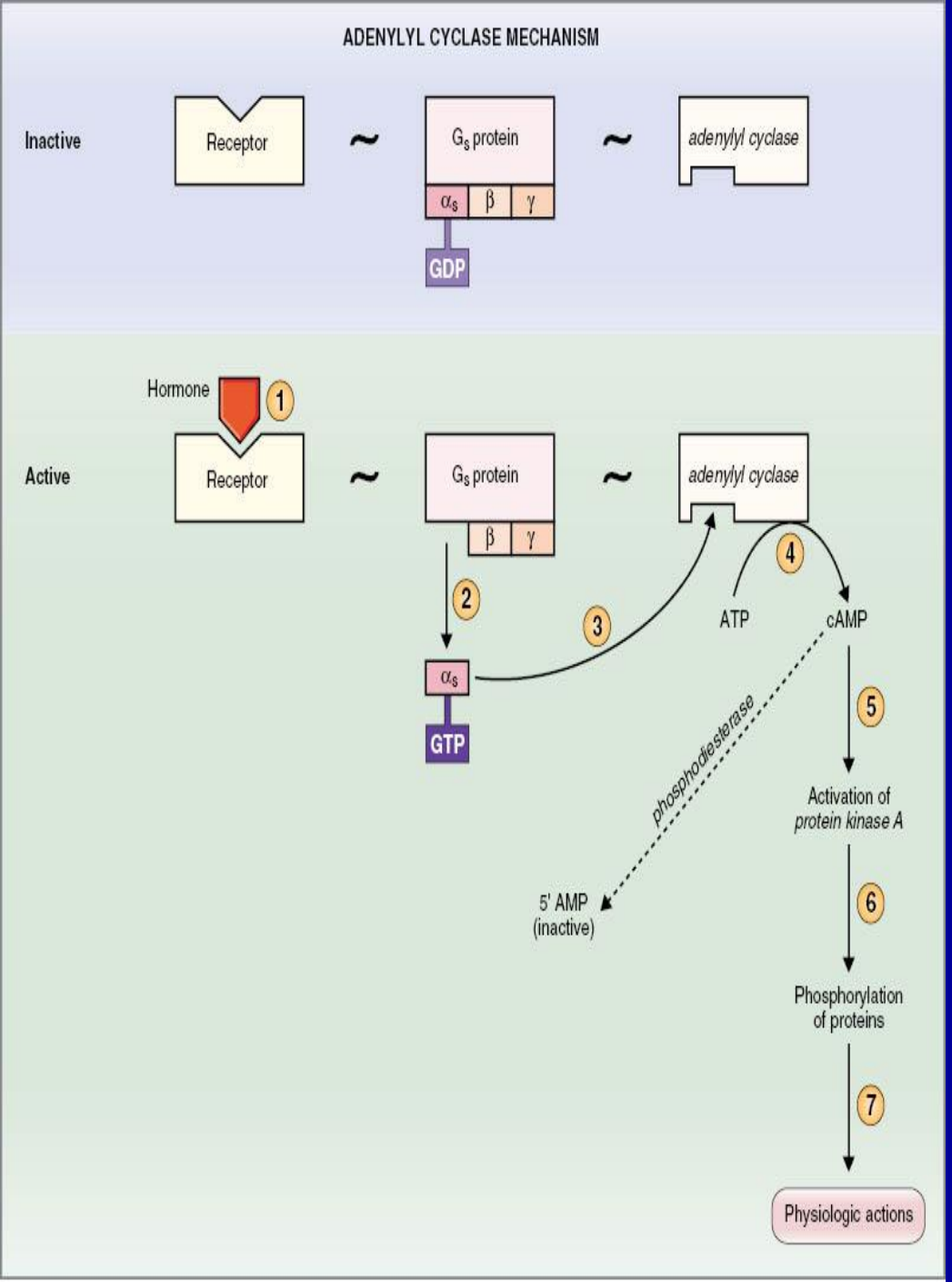
Adenylyl cyclase and phospholipase C →

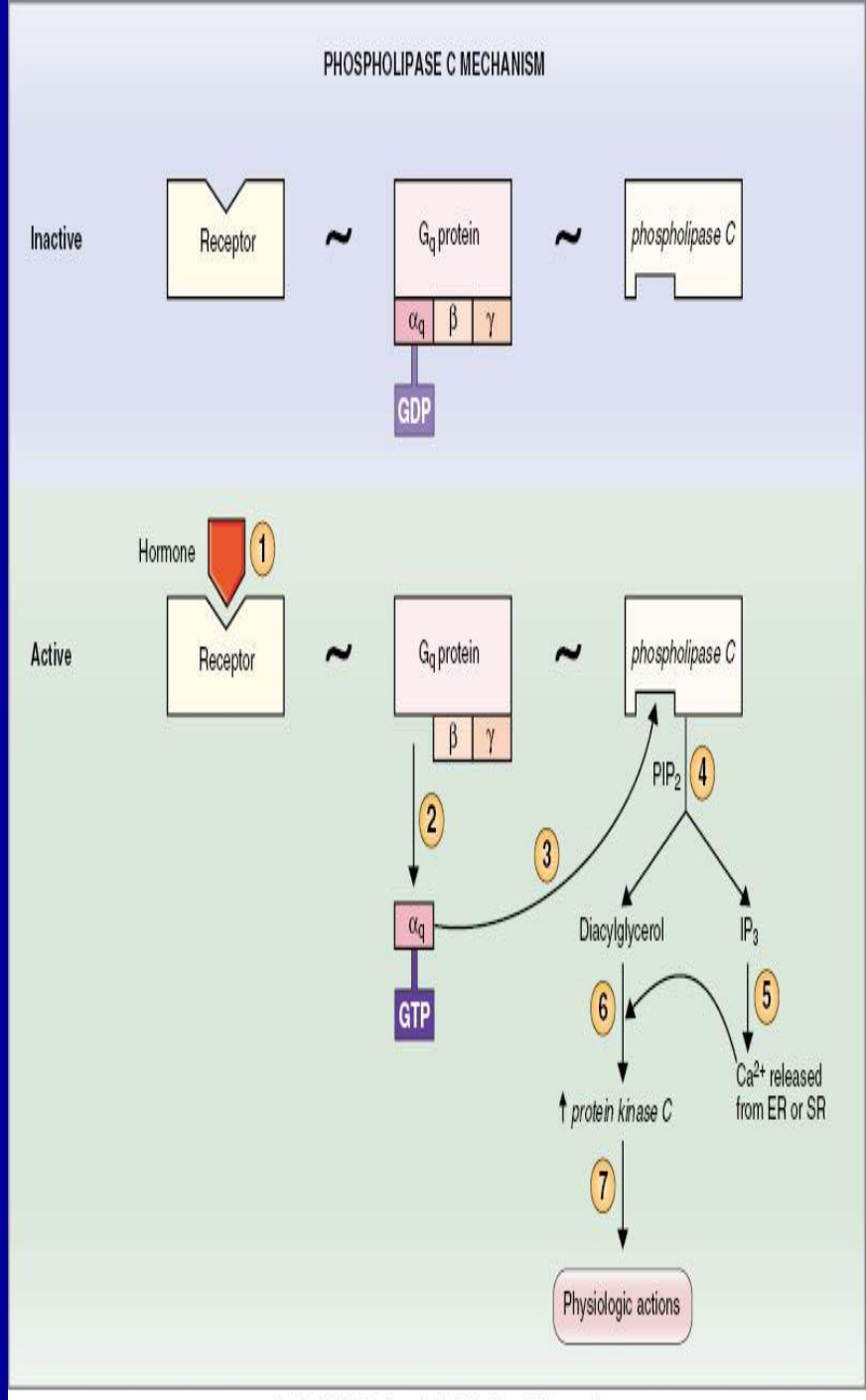
cAMP and IP3/Ca → secretion + synthesis.

- **Somatostatin (SRIF) → receptor**

Gi → inhibit generation of cAMP →

Decrease secretion.





SECRETION

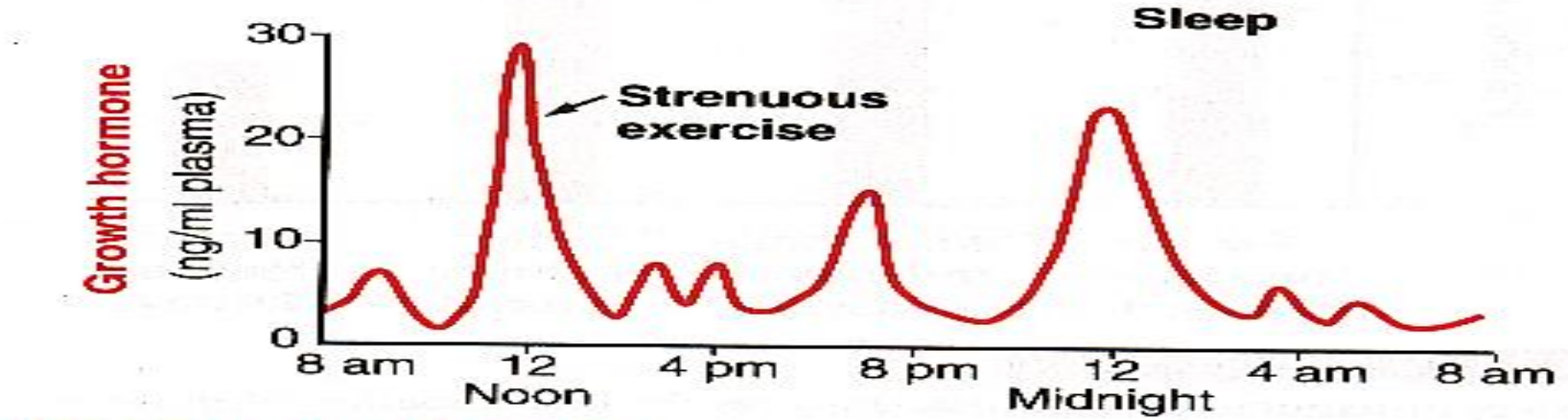


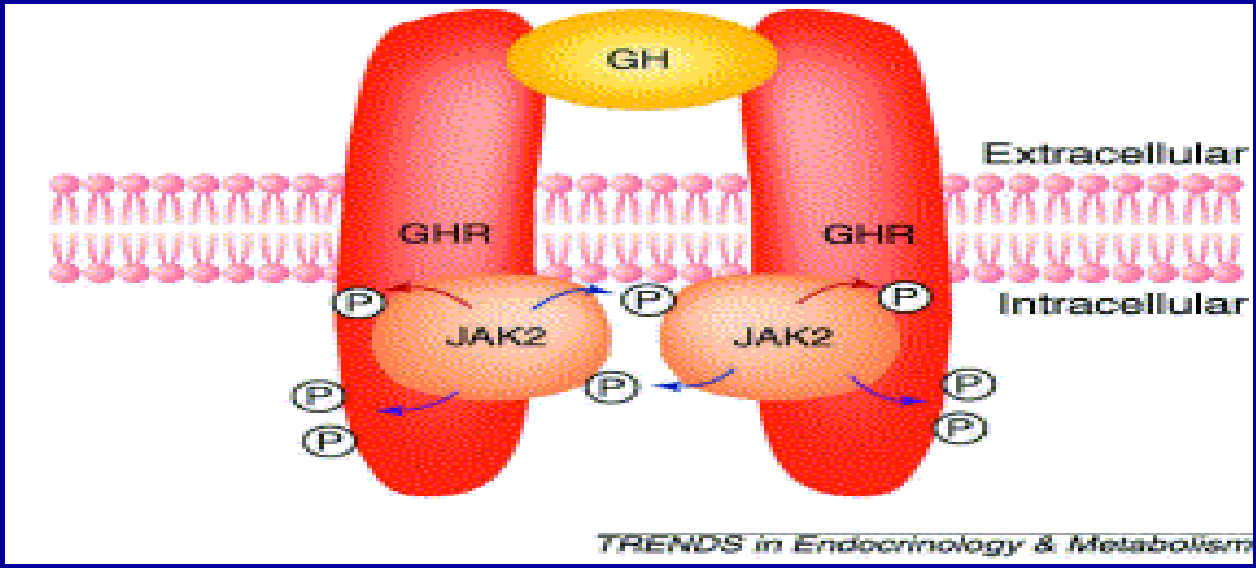
FIGURE 75–6

Typical variations in growth hormone secretion throughout the day, demonstrating the especially powerful effect of strenuous exercise and also the high rate of growth hormone secretion that occurs during the first few hours of deep sleep.

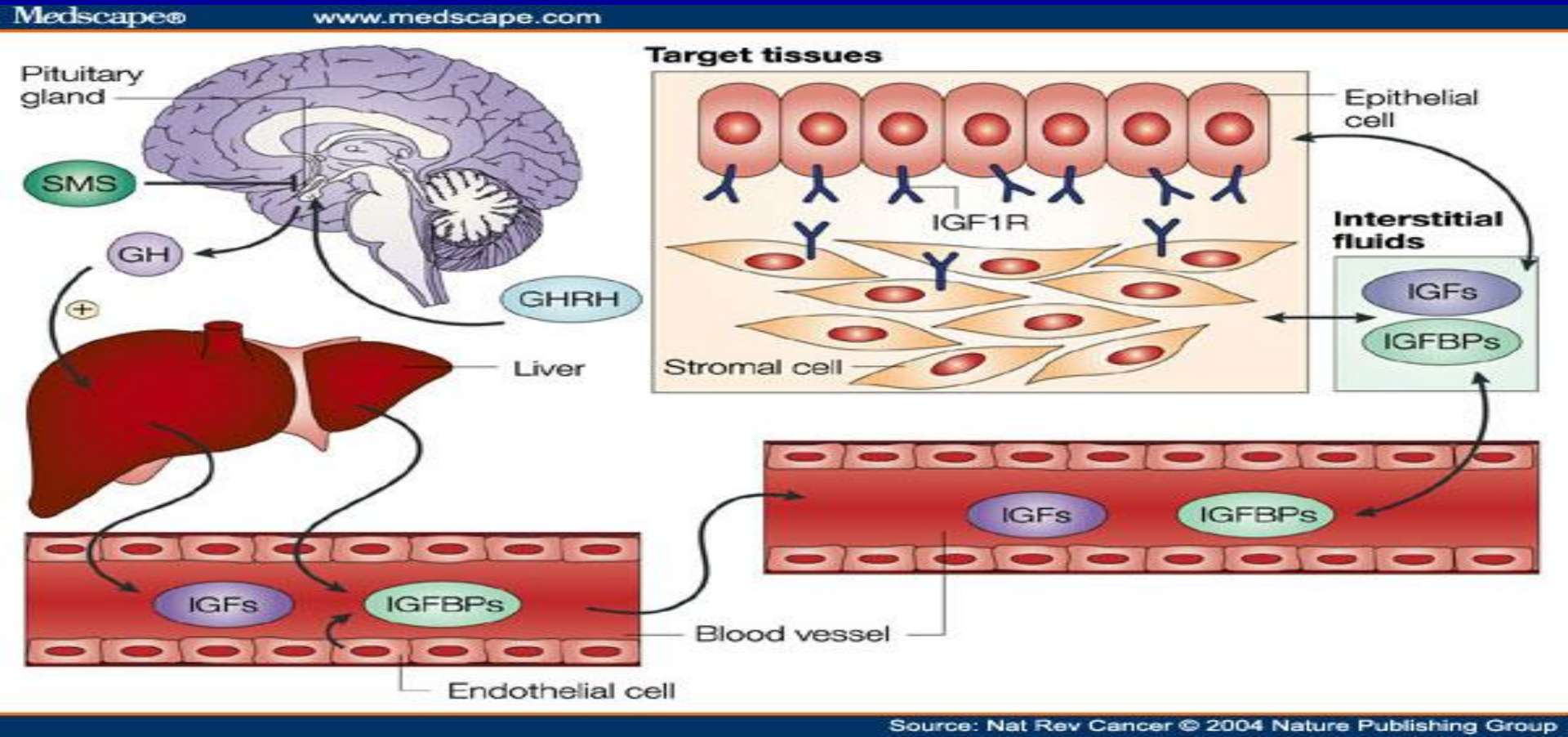
- Pulsatile every 2H.

ACTION OF GROWTH HORMONE

- **Direct.**
- **Skeletal muscles, liver and adipose.**



- **Indirect (somatomedine IGF).**
- **4500-7500 MW.**
- **Somatomedine C.**



EFFECT ON CARBOHYDRATE

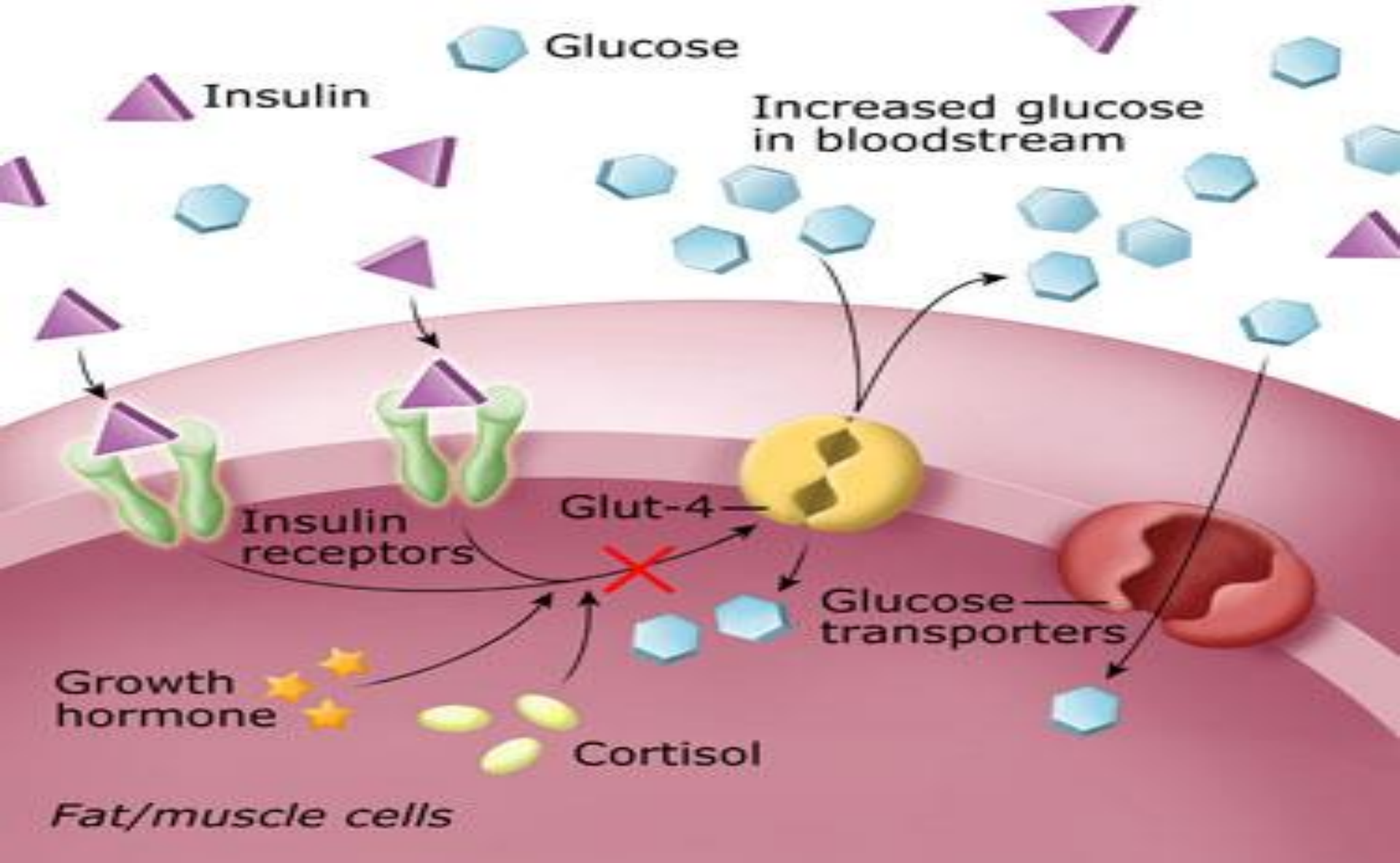
- **Increase blood glucose.(Diabetogenic effect.)**

(↑ gluconeogenesis)

Decrease glucose utilization in energy.

- **Increase in insulin.**

Glucose Counter-regulatory Hormones: Effect on Fat and Muscle Cells



EFFECT ON PROTEIN

- Increase protein synthesis.

a- Increase AA uptake.

b- Increase DNA synthesis.

c- Increase RNA synthesis.

- Decrease protein catabolism.

EFFECT IN FAT

1- Increase FFA.

2- FFA → Acetyl-CoA → energy

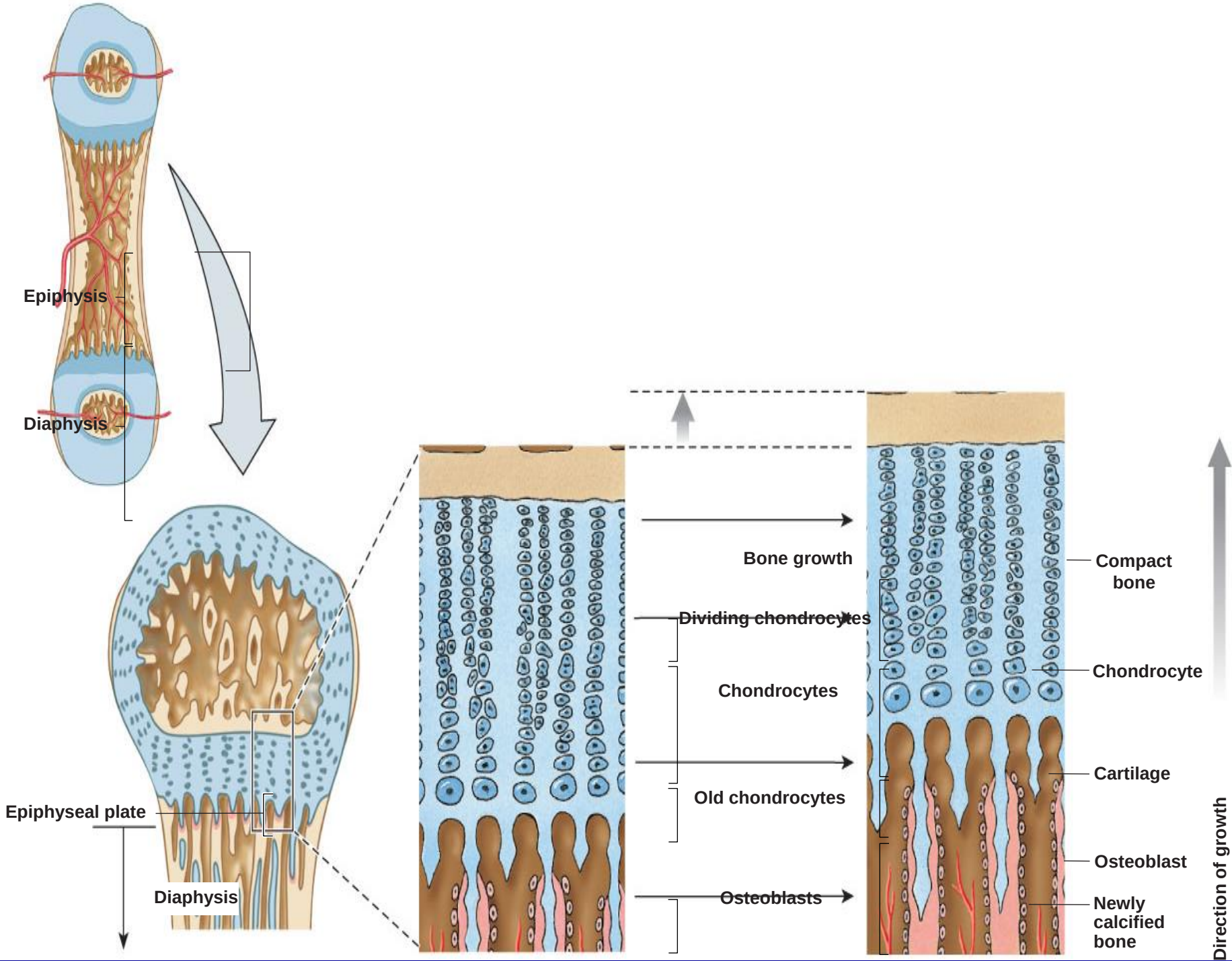
EFFECT IN BONE AND CARTILAGE

1- Increase liner growth.

2- Increase metabolism in cartilage forming cells.

3- Increase proliferation of condrocytes.

4- Widening of the epiphyseal plate.


Epiphysis
Diaphysis
Epiphyseal plate
Diaphysis
Bone growth
Dividing chondrocytes
Chondrocytes
Old chondrocytes
Osteoblasts
Compact bone
Chondrocyte
Cartilage
Osteoblast
Newly calcified bone
Direction of growth

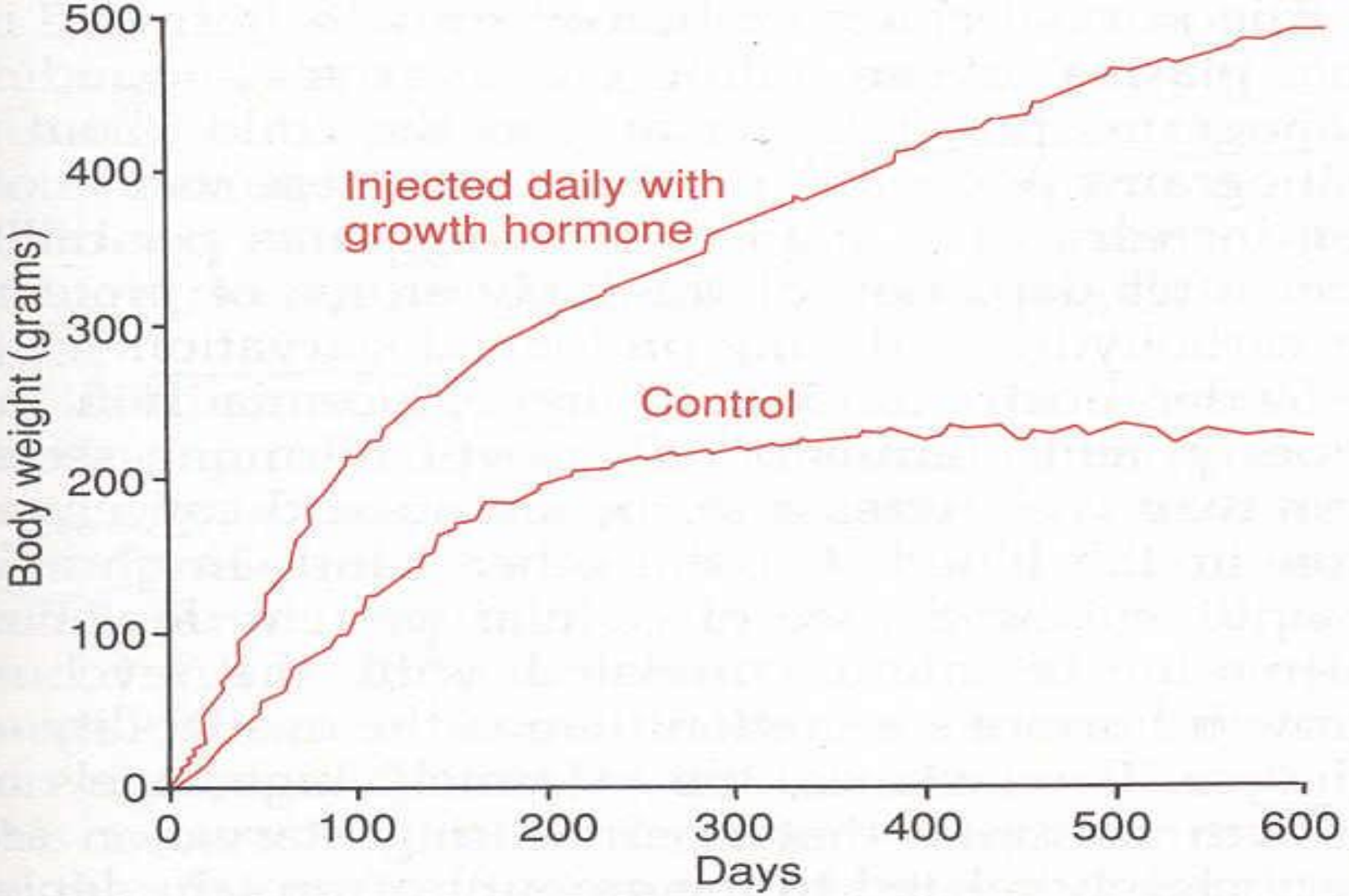


Figure 49–7 Comparison of weight gain of a rat injected daily with growth hormone with that of a normal rat.

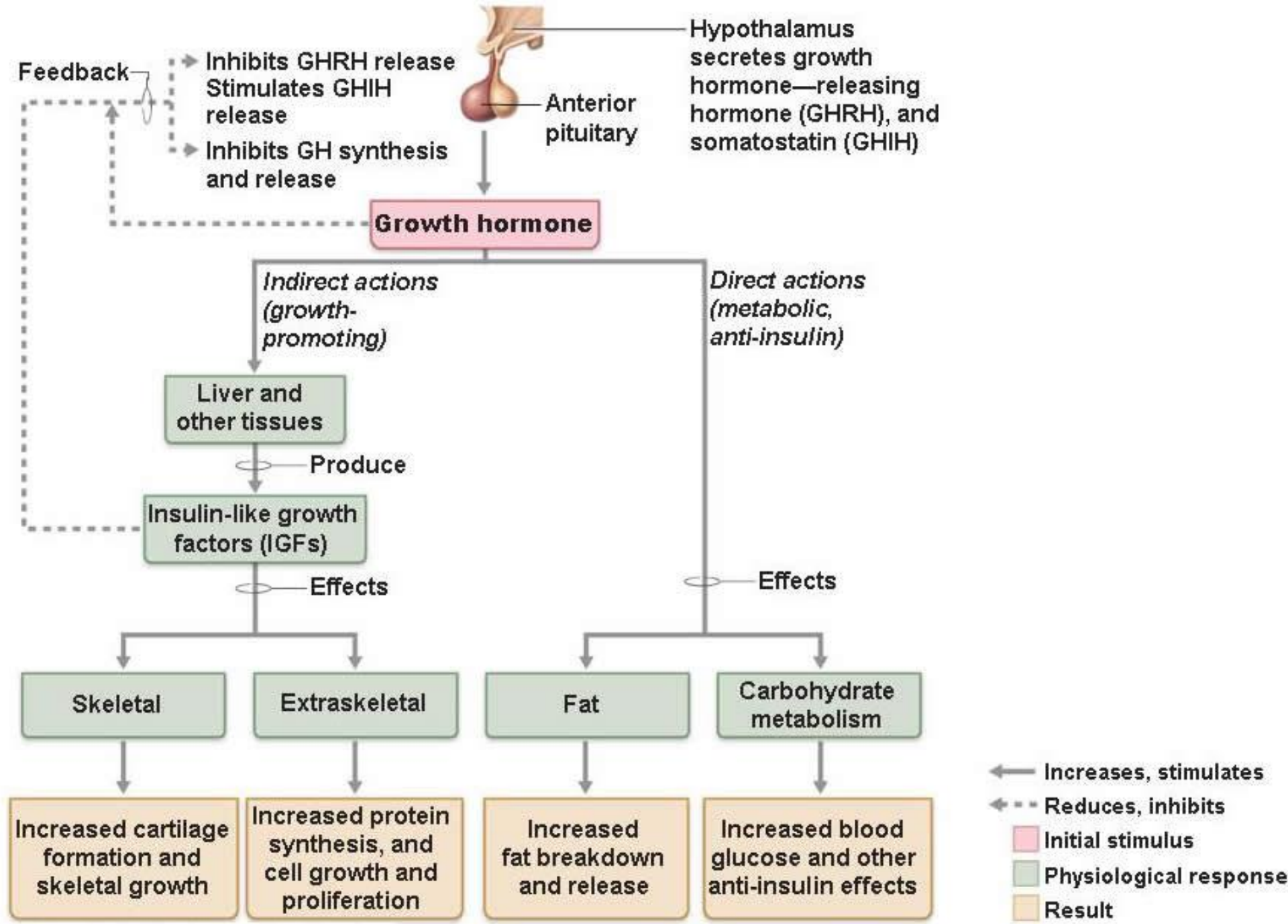


Table 9-4 Factors Affecting Growth Hormone Secretion

Stimulatory Factors	Inhibitory Factors
Decreased glucose concentration	Increased glucose concentration
Decreased free fatty acid concentration	Increased free fatty acid concentration
Arginine	Obesity
Fasting or starvation	Senescence
Hormones of puberty (estrogen, testosterone)	Somatostatin
	Somatomedins
Exercise	Growth hormone
Stress	β-Adrenergic agonists
Stage III and IV sleep	Pregnancy
α-Adrenergic agonists	

TABLE 16.1 Pituitary Hormones: Summary of Regulation and Effects

HORMONE (CHEMICAL STRUCTURE AND CELL TYPE)	REGULATION OF RELEASE	TARGET ORGAN AND EFFECTS	EFFECTS OF HYPOSECRETION ↓ AND HYPERSECRETION ↑
Anterior Pituitary Hormones			
Growth hormone (GH) (Protein, somatotroph)	**Stimulated** by GHRH* release, which is triggered by low blood levels of GH as well as by a number of secondary triggers including hypoglycemia, increases in blood levels of amino acids, low levels of fatty acids, exercise, other types of stressors, and estrogens **Inhibited** by feedback inhibition exerted by GH and IGFs, and by hyperglycemia, hyperlipidemia, obesity, and emotional deprivation via either increased GHIH* (somatostatin) or decreased GHRH* release	 Liver, muscle, bone, cartilage, and other tissues: anabolic hormone; stimulates somatic growth; mobilizes fats; spares glucose Growth-promoting effects mediated indirectly by IGFs	↓ Pituitary dwarfism in children ↑ Gigantism in children; acromegaly in adults

*Indicates hypothalamic releasing and inhibiting hormones:
GHRH = growth hormone–releasing hormone; GHIH = growth hormone–inhibiting hormone

ABNORMALITIES

1- Hyposecretion of GH.

Dwarfism.

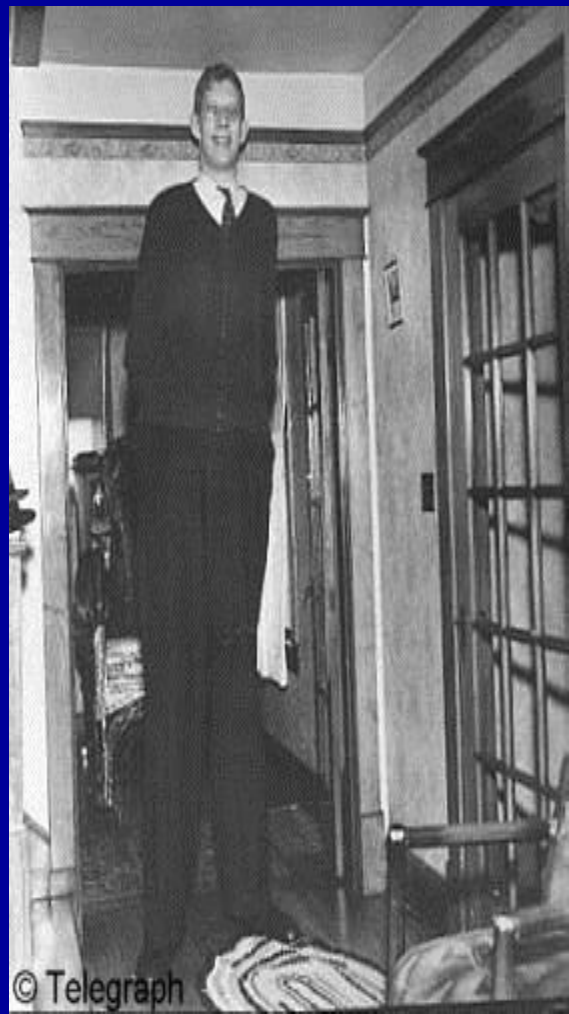
Causes?.

where?

2- Hypersecretion.

- Often associated with tumor.
- Giganitsm.
- Acromegaly.

- Octreotride.



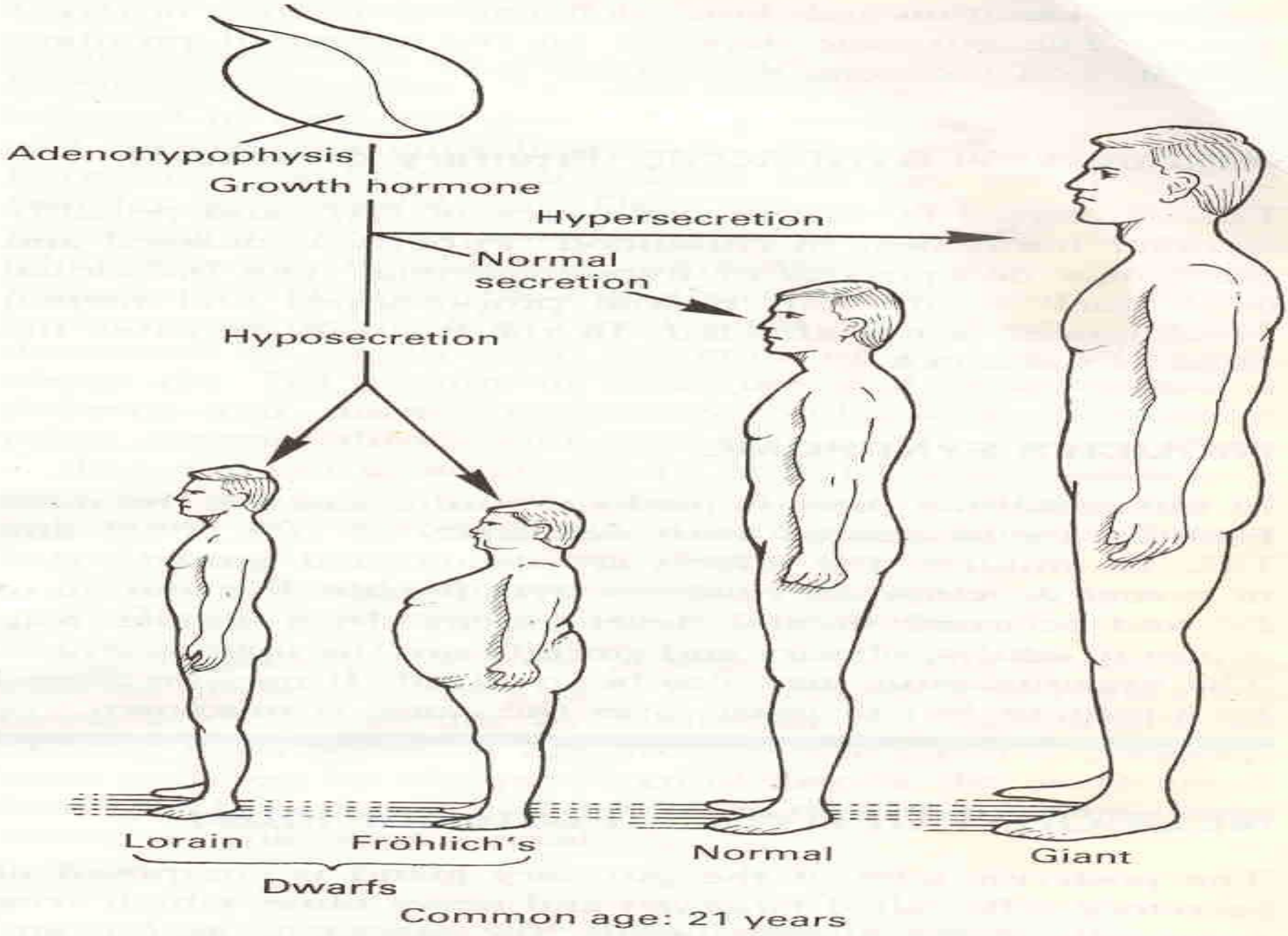


Figure 14:5 Effects of normal and abnormal growth hormone secretion.

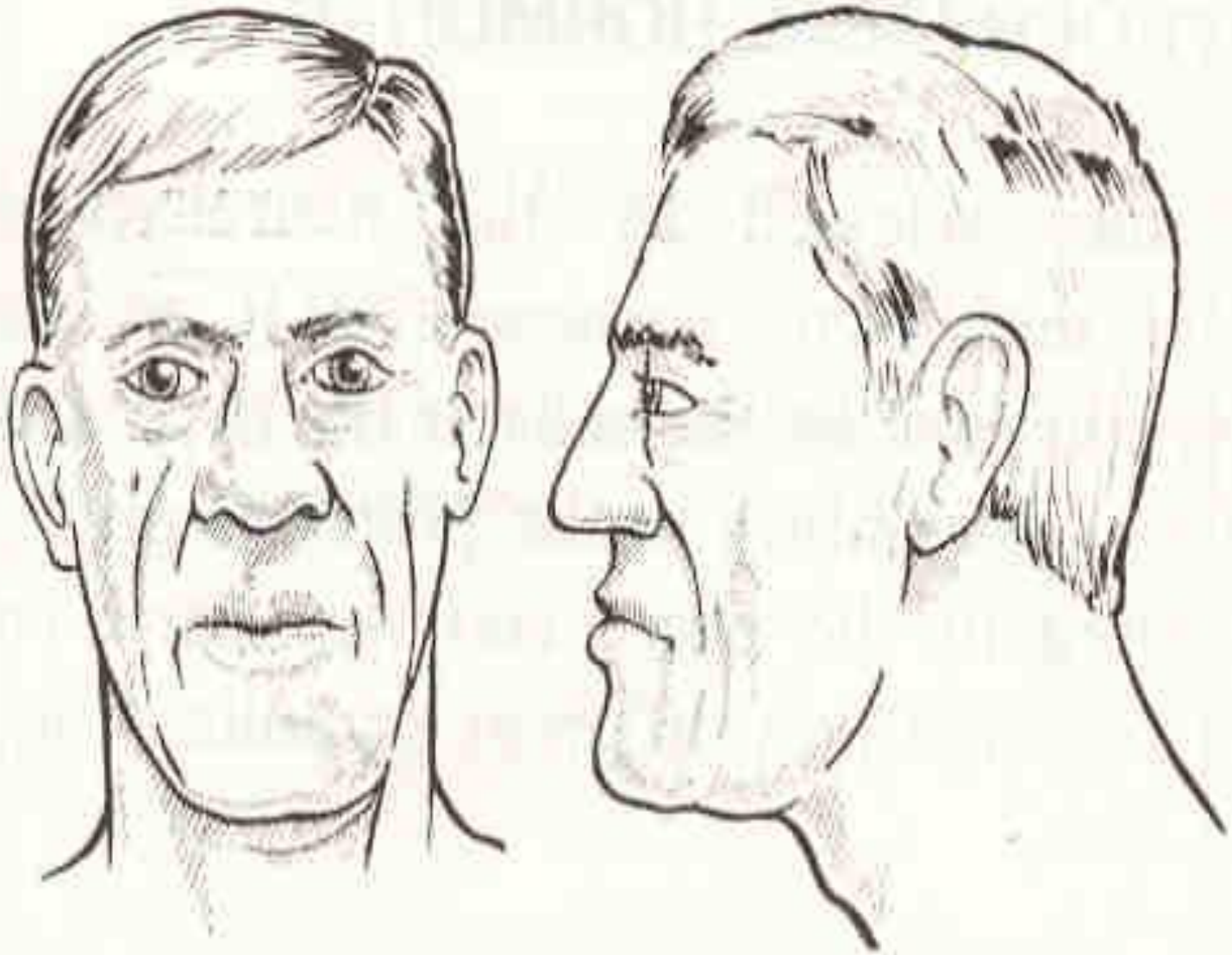


Figure 14:6 Acromegaly.



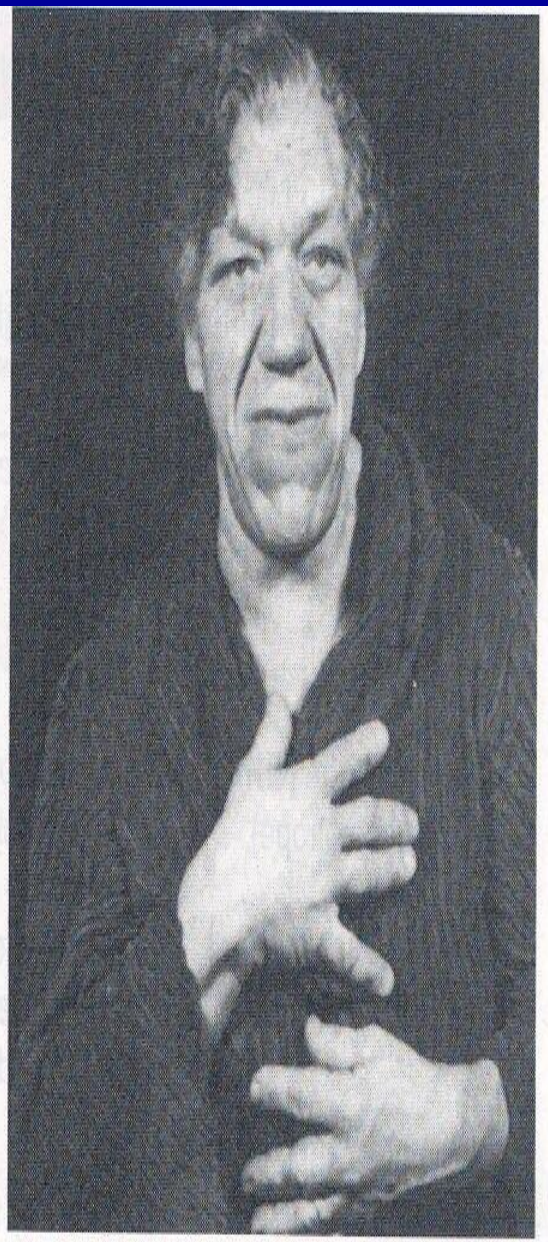
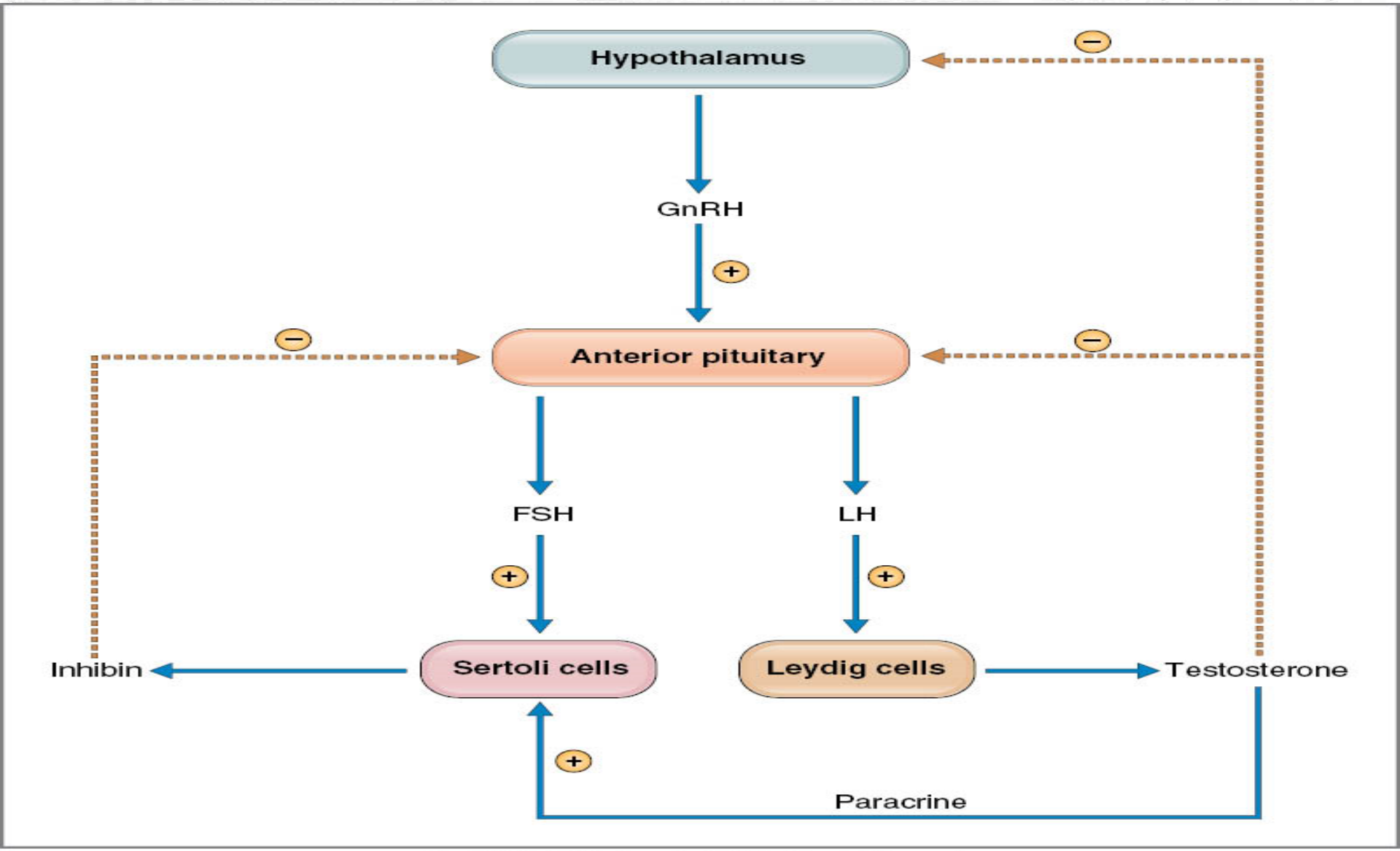


Figure 49–8 An acromegalic patient. (Courtesy of Dr. Herbert Langford.)

FSH AND LH

- Glycoproteins.
- Gonadotrophs (15%)
- α and β.
- Related to TSH.

SECRETION



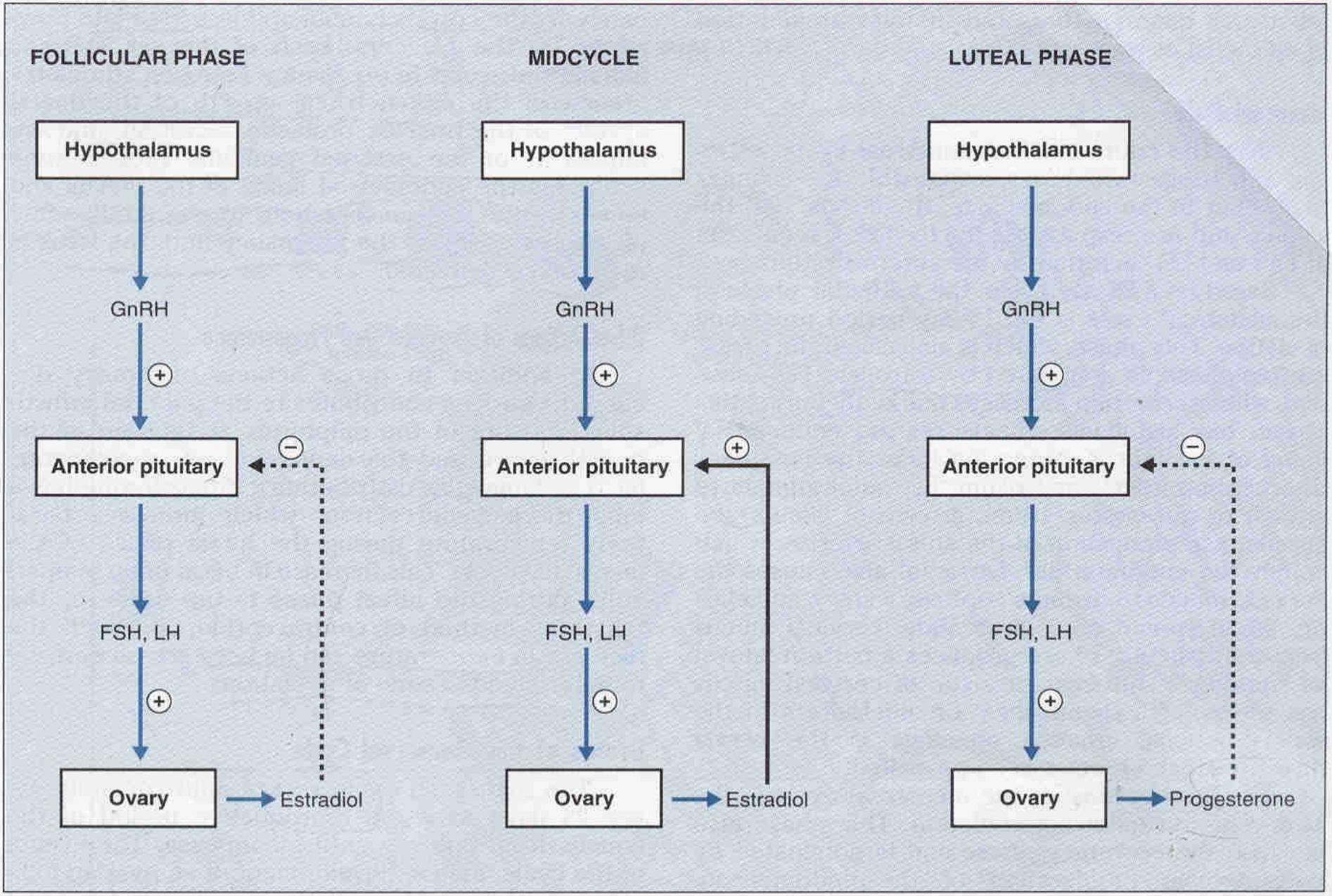
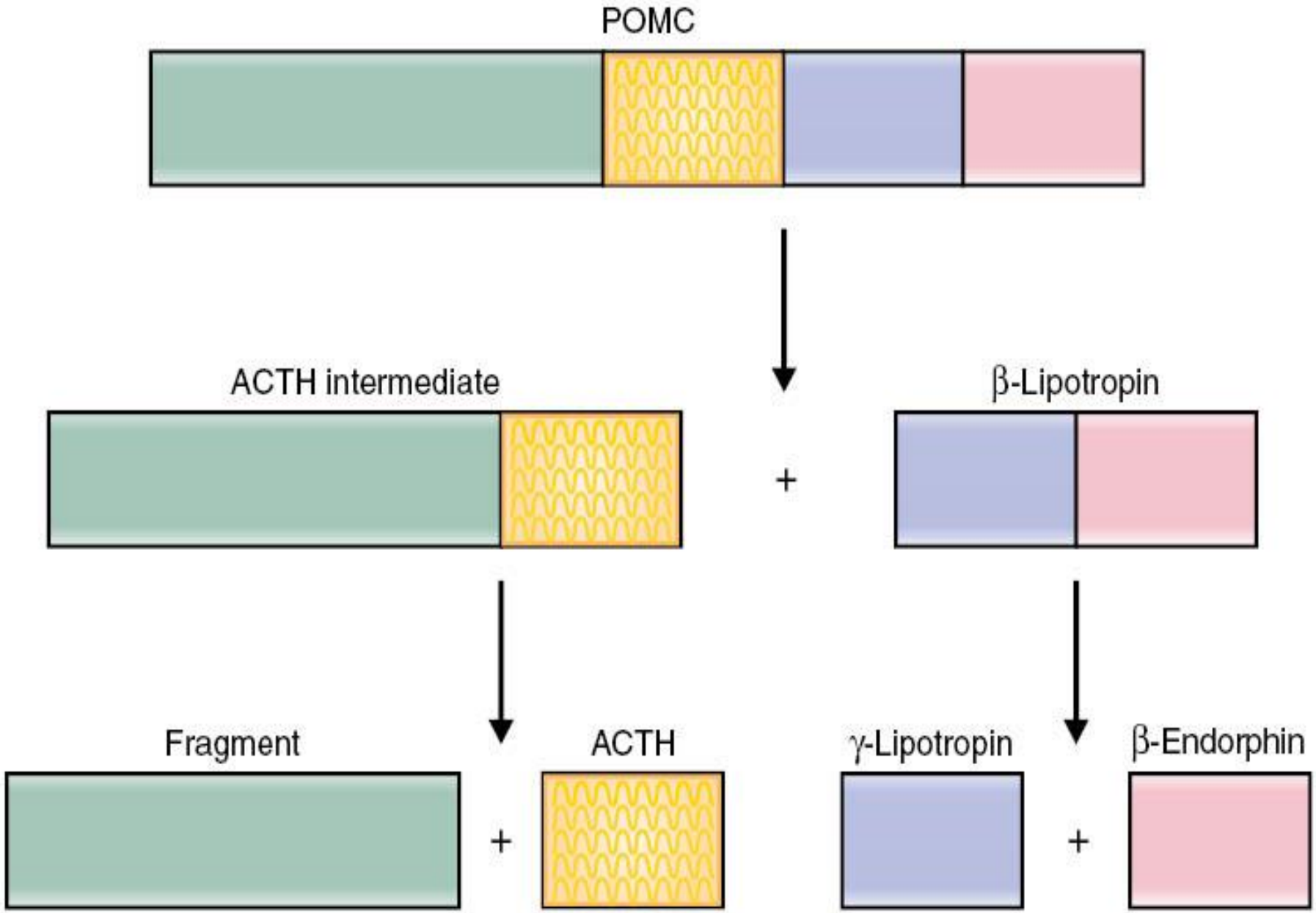


FIGURE 10–9. Control of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion in females during the menstrual cycle. The follicular and luteal phases are characterized by negative feedback of estradiol and progesterone, respectively, on the anterior pituitary. Midcycle is characterized by positive feedback of estradiol on the anterior pituitary. GnRH, gonadotropin-releasing hormone.

ACTH

- Cortictrophs.(15%)
- ACTH, MSH, β-endorphin.
- Preproopiomelanocortin (POMC).



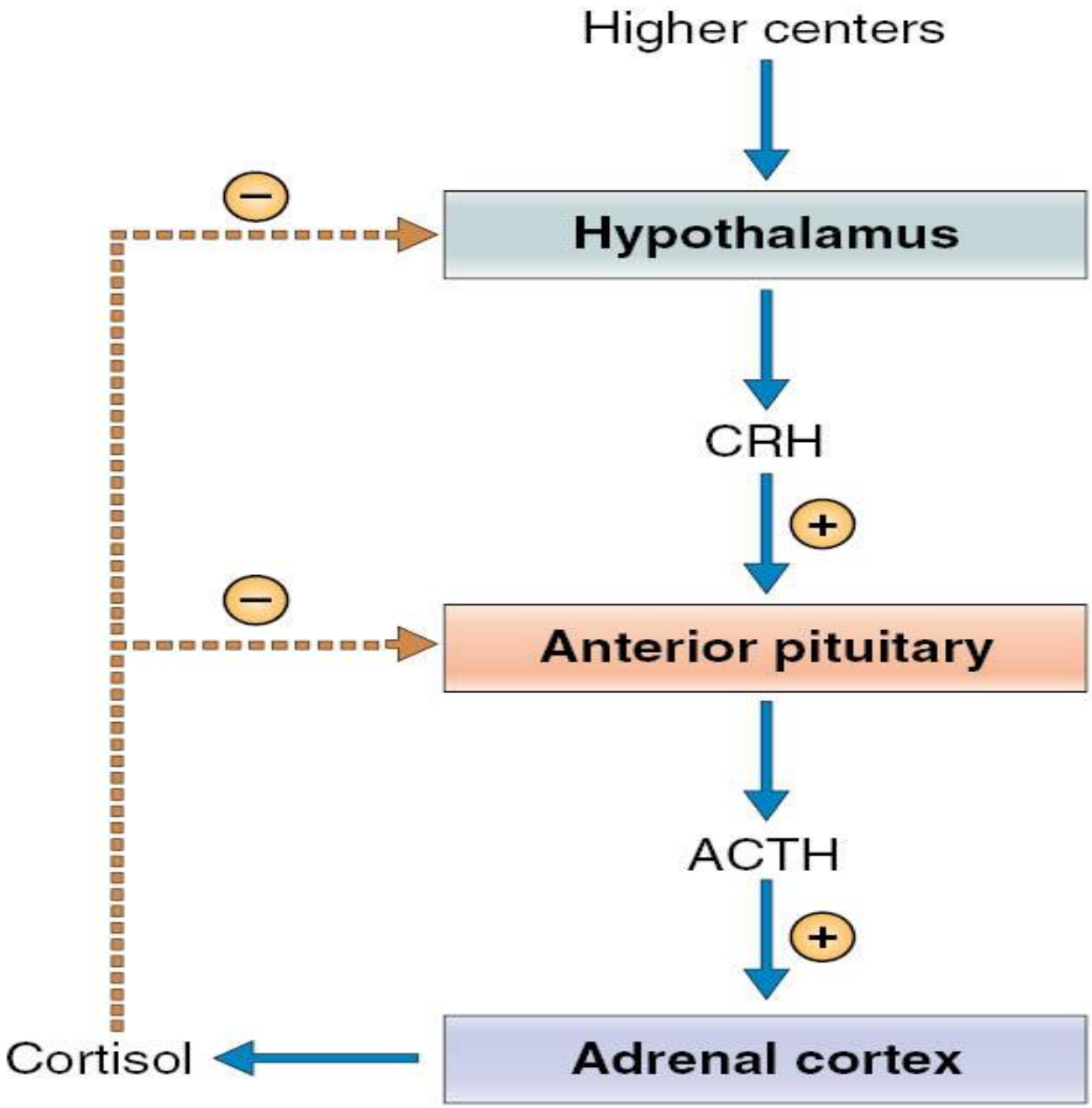


Table 9-10 Factors Affecting ACTH Secretion

Stimulatory Factors	Inhibitory Factors
Decreased blood cortisol levels	Increased blood cortisol levels
Sleep-wake transition	Opioids
Stress; hypoglycemia; surgery; trauma	Somatostatin
Psychiatric disturbances	
ADH	
α-Adrenergic agonists	
β-Adrenergic antagonists	
Serotonin	

ACTION

- **Stimulate synthesis and secretion of adrenal cortical hormones.**

Table 20.19 Hormones in serum

Hormone	Reference range	Units
Adrenocorticotrophic hormone (ACTH) (plasma)	7–51 (07:00–10:00 h)	ng/l
Cortisol	150–550 (at 08:00 h) < 200 (at 22:00 h)	nmol/l
Follicle-stimulating hormone (FSH)		
Male	1.5–9.0	U/l
Female*	3.0–15 (early follicular) Up to 20 (mid-cycle) > 30 (post-menopausal)	U/l
Gastrin (plasma)	Up to 120	ng/l
Growth hormone (GH)	Very variable, usually less than 2, but may be up to 50 with stress	mU/l
Insulin	Highly variable and interpretable only in relation to plasma glucose and body habitus	mU/l
Luteinising hormone (LH)		
Female*	2.5–9.0 (early follicular) Up to 90 (mid-cycle) > 20 (post-menopausal)	U/l
Male	1.5–9.0	U/l
Oestradiol-17β		
Female	110–180 (early follicular) 550–1650 (mid-cycle) 370–770 (luteal) < 150 (post-menopausal)	pmol/l
Male	< 200	pmol/l
Parathyroid hormone (PTH)	10–65	ng/l
Progesterone		
Male	< 2.0	nmol/l
Female	< 2.0 (follicular) > 15 (mid-luteal) < 2.0 (post-menopausal)	nmol/l
Prolactin (PRL)	60–390	mU/l
Testosterone		
Male	10–30	nmol/l
Female	0.4–2.8	nmol/l
Thyroid-stimulating hormone (TSH)	0.15–3.5	mU/l
Thyroxine (free) (free T_4)	10–27	pmol/l
Tri-iodothyronine (T_3)	1.0–2.6	nmol/l
TSH receptor antibodies (TRAb)	< 7	U/l

* Luteal phase values similar to follicular phase.

Notes

1. A number of hormones are unstable, and collection details are critical to obtaining a meaningful result. Refer to local hospital handbook.
2. Values in the table are only a guideline; hormone levels can often only be meaningfully understood in relation to factors such as sex (e.g. testosterone), age (e.g. FSH in women), time of day (e.g. cortisol) or regulatory factors (e.g. insulin and glucose, PTH and [Ca^{++}]). Also, reference ranges may be critically method-dependent.