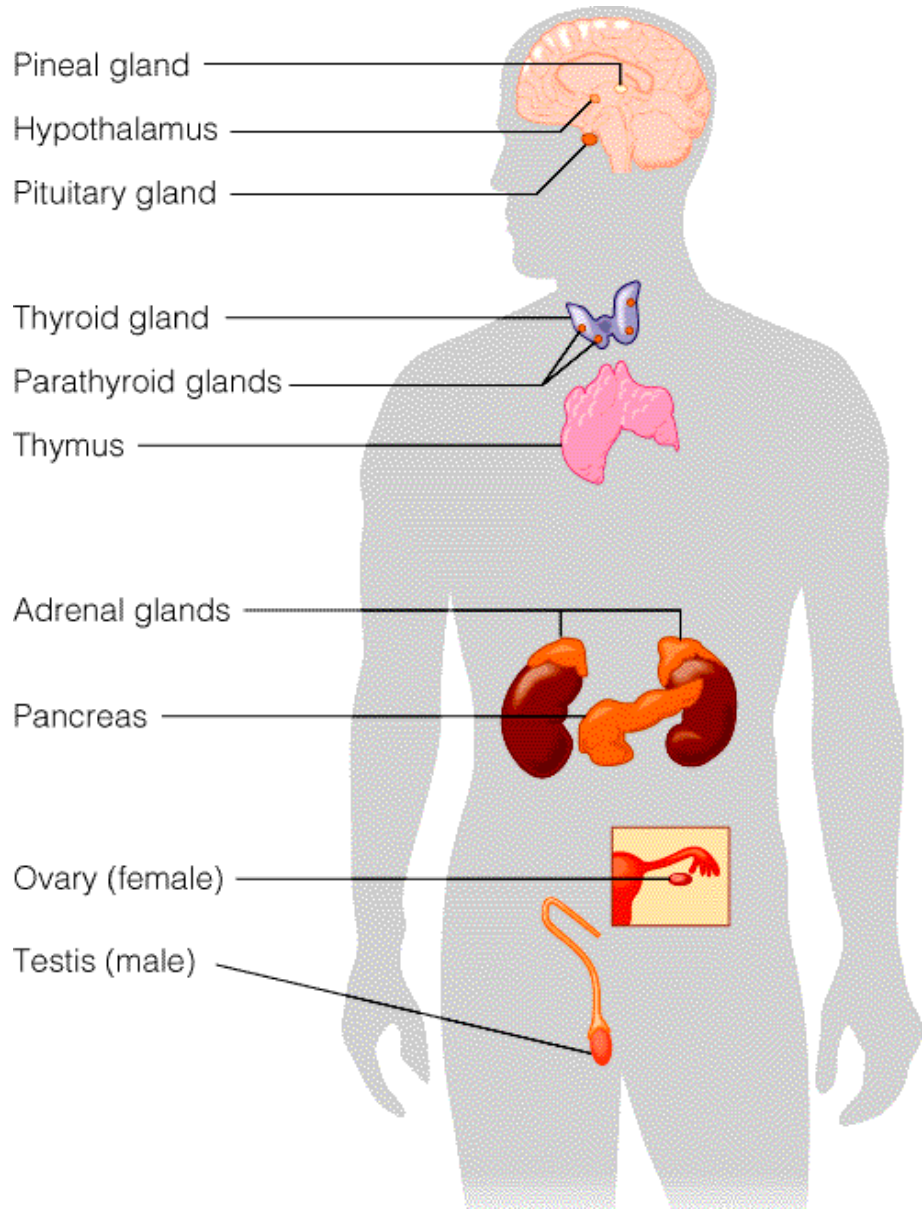
HORMONES

(mainly Chapter 23 Lehninger)

HORMONES AND HORMONAL REGULATION

- Hormonal regulation involves a hierarchy of cell types acting on each other either to stimulate or to modulate the release and action of a hormone.

- Secretion of hormones from endocrine cells is stimulated by chemical signals from regulatory cells that occupy a higher position in this hierarchy.



ENDOCRINE AND NEUROENDOCRINE SYSTEMS

- Nerve (electric) pulse originates in cell bodies and they are rapidly sent, also for long distance, to axon terminals, where a neurotransmitter is released to the target cell that is within a distance of few μm.

- In endocrine system, hormones are secreted to blood flux up to the target tissue that can be more than 1 m distant from the origin point

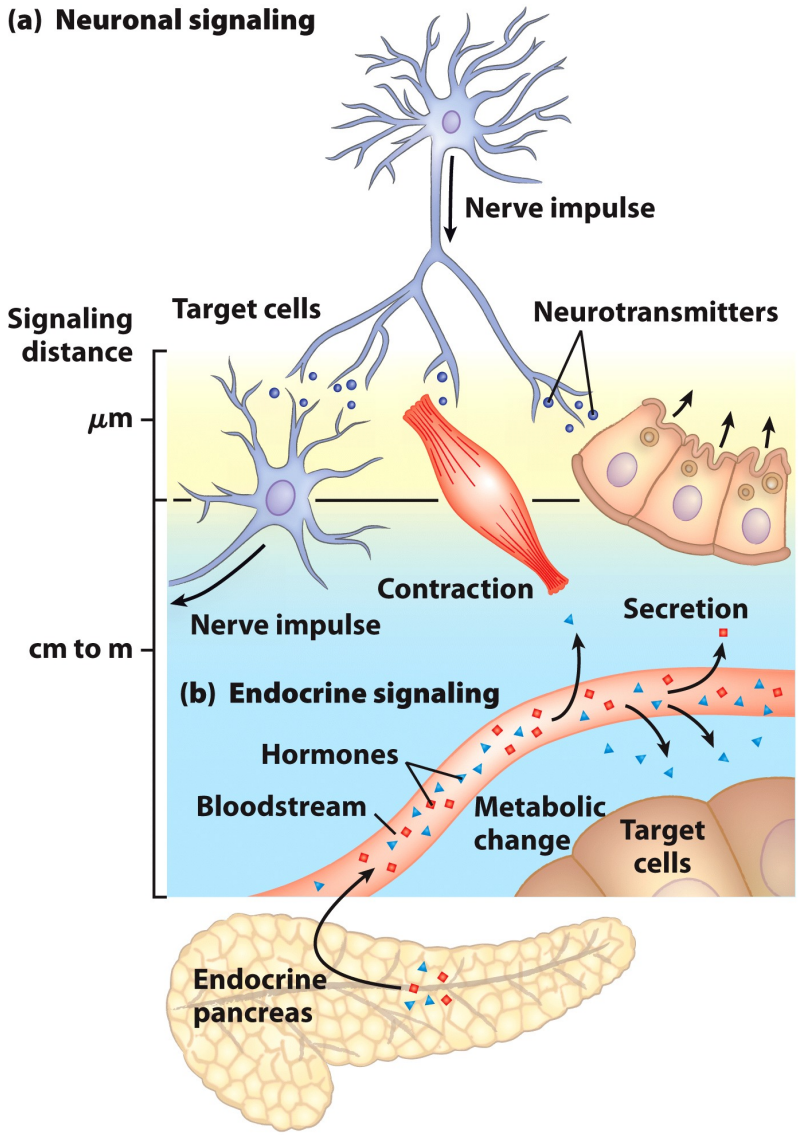


Figure 23-1
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THE HIERARCHICAL NATURE OF HORMONE ACTION

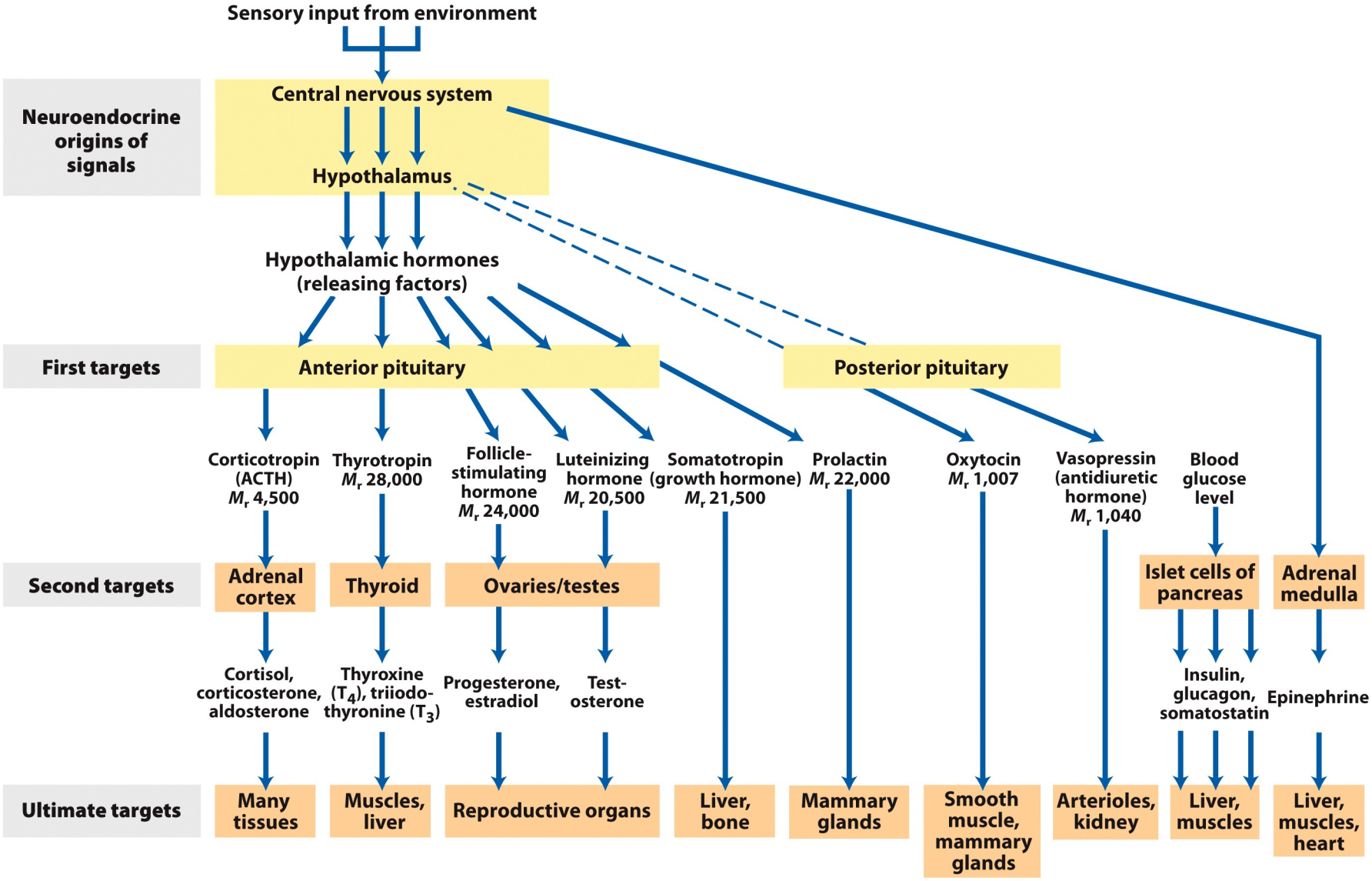

Figure 23-7
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THE HIERARCHICAL NATURE OF HORMONE ACTION

- The hierarchical nature of hormone action can be summarized as follows:

1. Hormonal action is controlled ultimately by the central nervous system, which transmits signals to the hypothalamus. It responds by producing factors that either stimulate (called releasing factors) or inhibit the release of hormones from the pituitary.

2. Pituitary hormones do one of the following:

a. They stimulate other endocrine glands, each of which releases a hormone that acts on a target tissue and elicits a specific metabolic response.

b. Alternatively, they act directly on a target tissue. The action of a hormone sets in motion events that ultimately limit that action.

- Some pituitary hormones stimulate target tissue directly. For example, prolactin stimulates mammary glands to produce milk.

- Most pituitary hormones act on endocrine glands that occupy an intermediate, or secondary, position in the hierarchy, stimulating them to produce hormones that exert the ultimate actions on target tissues. Pituitary hormones that act on other endocrine glands are called **tropic hormones** or **tropins**.

GENERAL MECHANISMS OF HORMONES ACTION

- Peptide and ammine hormones action is more rapid than the one generated by steroid and thyroid hormones.

Intracellular effects of hormones action are:

1. A second messenger (cAMP, cGMP for example) is generated and allosterically regulates one or more enzymes.
2. A tyrosine-kinase receptor is activated by the extracellular hormone.
3. A variation in the membrane potential causes the opening/closing of a ion channel controlled by the hormone.
4. An adhesion receptor on cell surface transfer the information from extracellular matrix to the cytoskeleton
5. A steroid or a steroid-similar molecule causes a variation at trascriptional level of one or more genes through hormonal nuclear receptors.

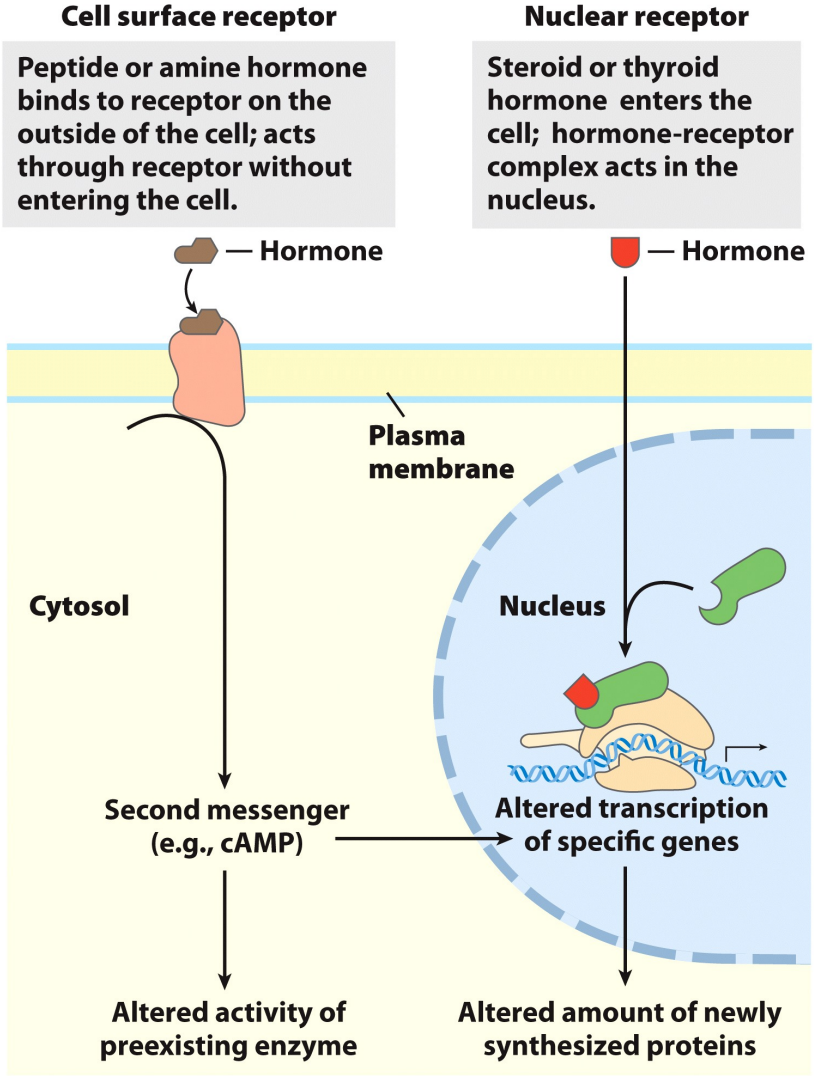

Figure 23-3
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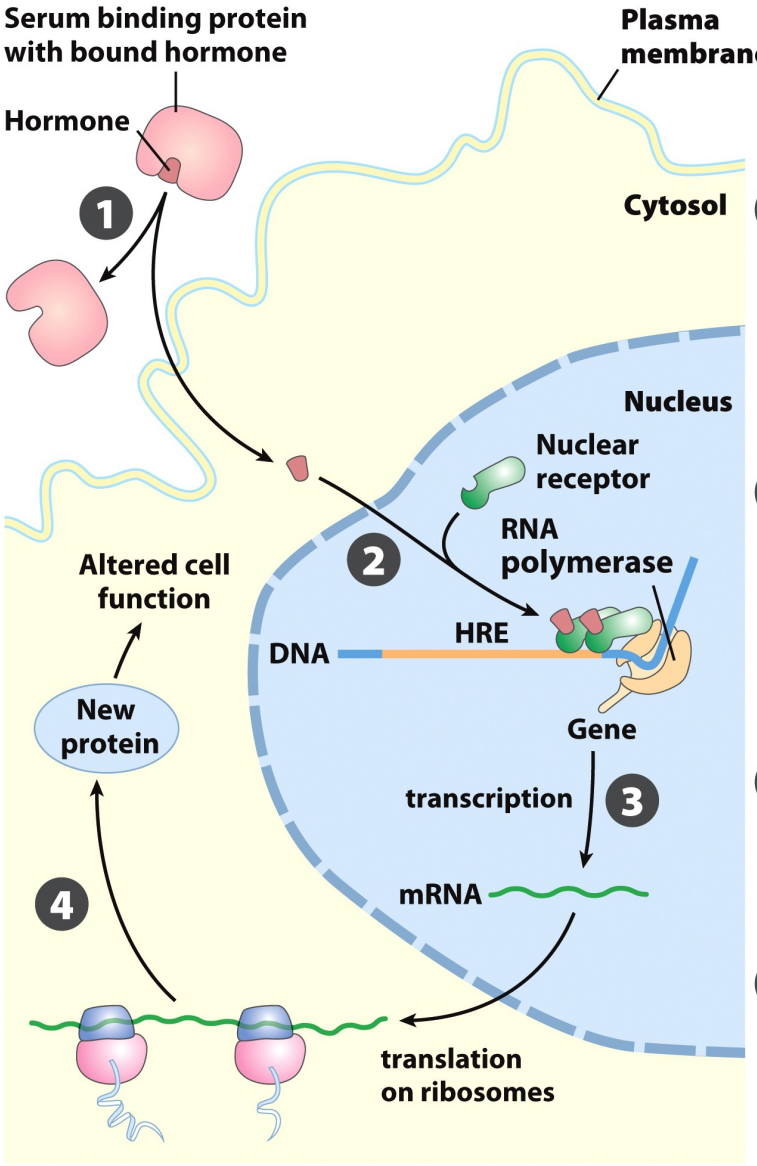
GENERAL MECHANISMS OF HORMONES ACTION



1. Hormone, carried to the target tissue on serum binding proteins, di uses across the plasma membrane and binds to its specific receptor protein in the nucleus.

2. Hormone binding changes the conformation of the receptor; it forms homo- or heterodimers with other hormone-receptor complexes and binds to specific regulatory regions called hormone response elements (HREs) in the DNA adjacent to specific genes.

3. Receptor attracts coactivator or corepressor protein(s) and, with them, regulates transcription of the adjacent gene(s), increasing or decreasing the rate of mRNA formation.

4. Altered levels of the hormone-regulated gene product produce the cellular response to the hormone.

Figure 12-30
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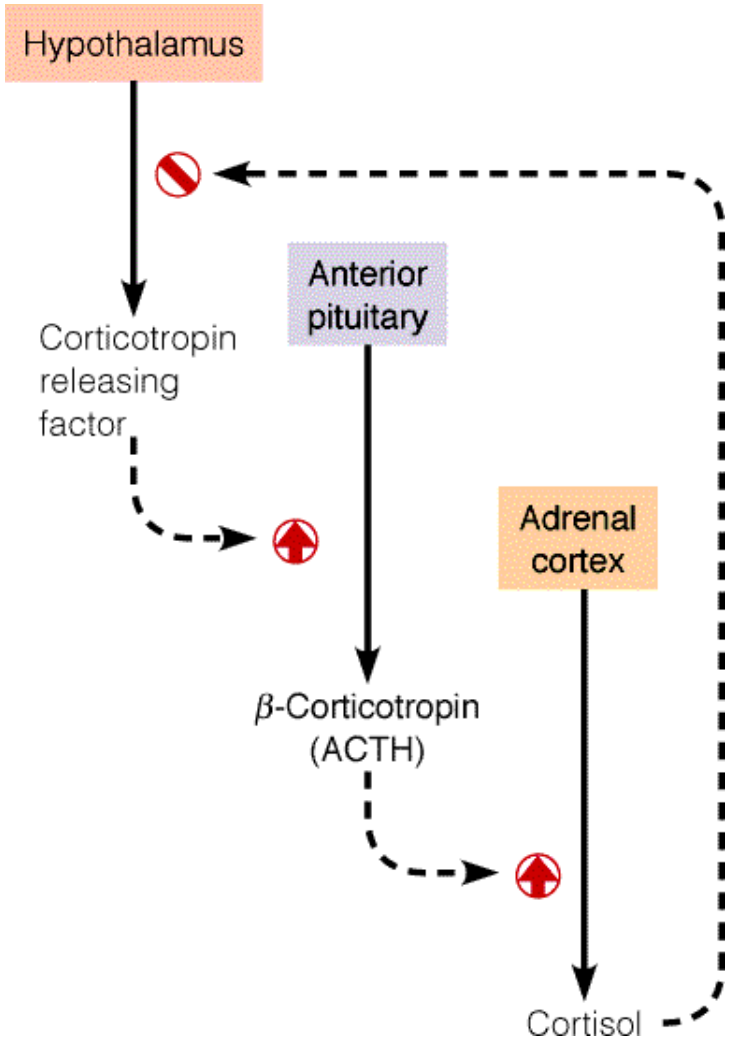
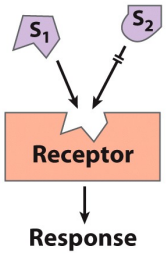
GENERAL MECHANISMS OF HORMONES ACTION

- The action of a hormone is self-limiting because of the existence of feedback loops, in which secretion of a hormone sets in motion a series of events that leads to inhibition of that secretion.

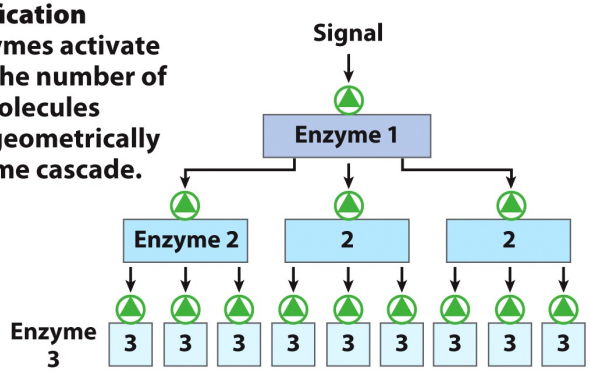


GENERAL MECHANISMS OF HORMONES ACTION

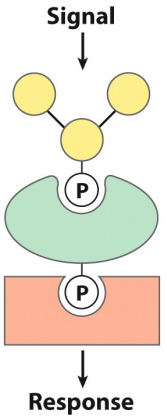
(a) Specificity
Signal molecule fits binding site on its complementary receptor; other signals do not fit.



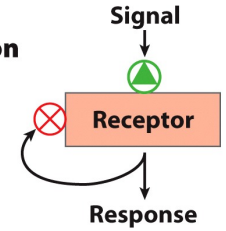
(b) Amplification
When enzymes activate enzymes, the number of affected molecules increases geometrically in an enzyme cascade.



(c) Modularity
Proteins with multivalent affinities form diverse signaling complexes from interchangeable parts. Phosphorylation provides reversible points of interaction.



(d) Desensitization/Adaptation
Receptor activation triggers a feedback circuit that shuts off the receptor or removes it from the cell surface.



(e) Integration
When two signals have opposite effects on a metabolic characteristic such as the concentration of a second messenger X, or the membrane potential V_m, the regulatory outcome results from the integrated input from both receptors.

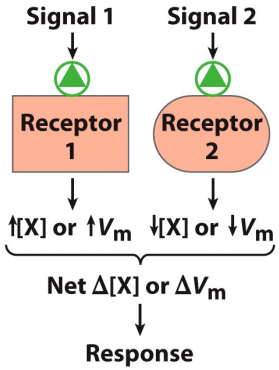


Figure 12-1
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GENERAL MECHANISMS OF HORMONES ACTION

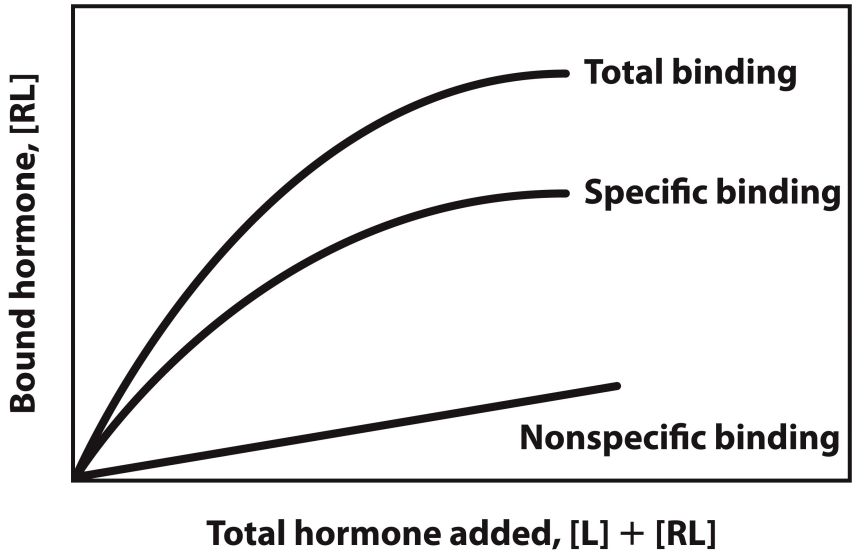
Scatchard Analysis

• The receptor-ligand binding, like the enzyme-substrate binding, depends on the concentration of the interacting components

• The resulting equilibrium can be described in term of K**a** (the association constant) or K**d** (the dissociation constant)

$$R + L \underset{k-1}{\overset{k+1}{\rightleftharpoons}} RL$$

$$K_a = \frac{[RL]}{[R][L]} = \frac{k_{+1}}{k_{-1}} = \frac{1}{K_d}$$



Box 12-1 figure 1a
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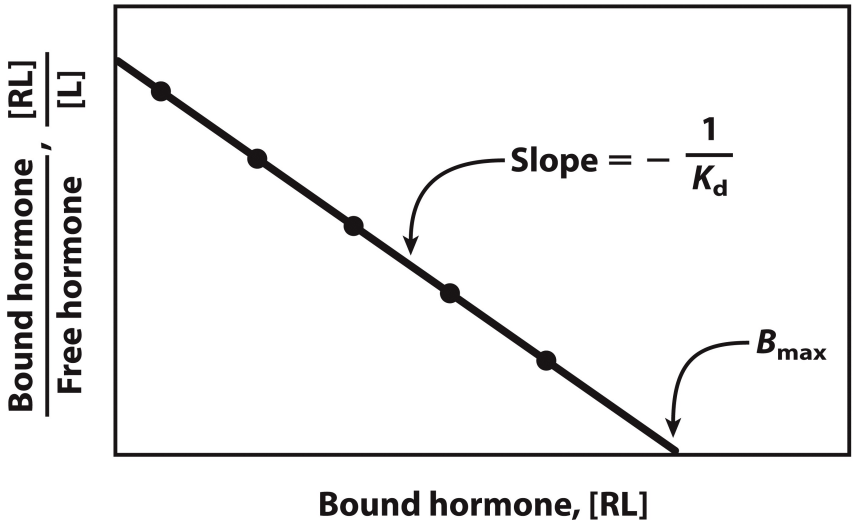

Box 12-1 figure 1b
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HORMONES CLASSIFICATION

TABLE 23–1 Classes of Hormones

Type	Example	Synthetic path	Mode of action
Peptide	Insulin, glucagon	Proteolytic processing of prohormone	Plasma membrane receptors; second messengers
Catecholamine	Epinephrine	From tyrosine	
Eicosanoid	PGE_1	From arachidonate (20:4 fatty acid)	
Steroid	Testosterone	From cholesterol	Nuclear receptors; transcriptional regulation
Vitamin D	1α,25-Dihydroxyvitamin D_3	From cholesterol	
Retinoid	Retinoic acid	From vitamin A	
Thyroid	Triiodothyronine (T_3)	From Tyr in thyroglobulin	
Nitric oxide	Nitric oxide	From arginine + O_2	Cytosolic receptor (guanylyl cyclase) and second messenger (cGMP)

Table 23-1
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PEPTIDES

- Nearly all peptide hormones are synthesized as inactive precursors and then converted to active hormones by proteolytic processing.
- Insulin contains two polypeptide chains, of 21 and 30 residues, with two inter-chain disulfide bridges and one intra-chain bridge.
- The first product of translation of the insulin gene is a 105-residue polypeptide called pre-proinsulin.
- Cleavage from pre-proinsulin of a 24-residue N-terminal "signal sequence" yields proinsulin, an 81-residue polypeptide.
- Proinsulin then undergoes folding, disulfide bond formation, and cleavage to give the two polypeptide chains of the active hormone, insulin.
- The signal sequence that is eventually cleaved from preproinsulin to form proinsulin is needed to transport the protein through membranes.
- All known polypeptide hormones are synthesized in "pre-pro" form, with a signal sequence and additional sequence(s) that are cleaved out during maturation of the hormone.

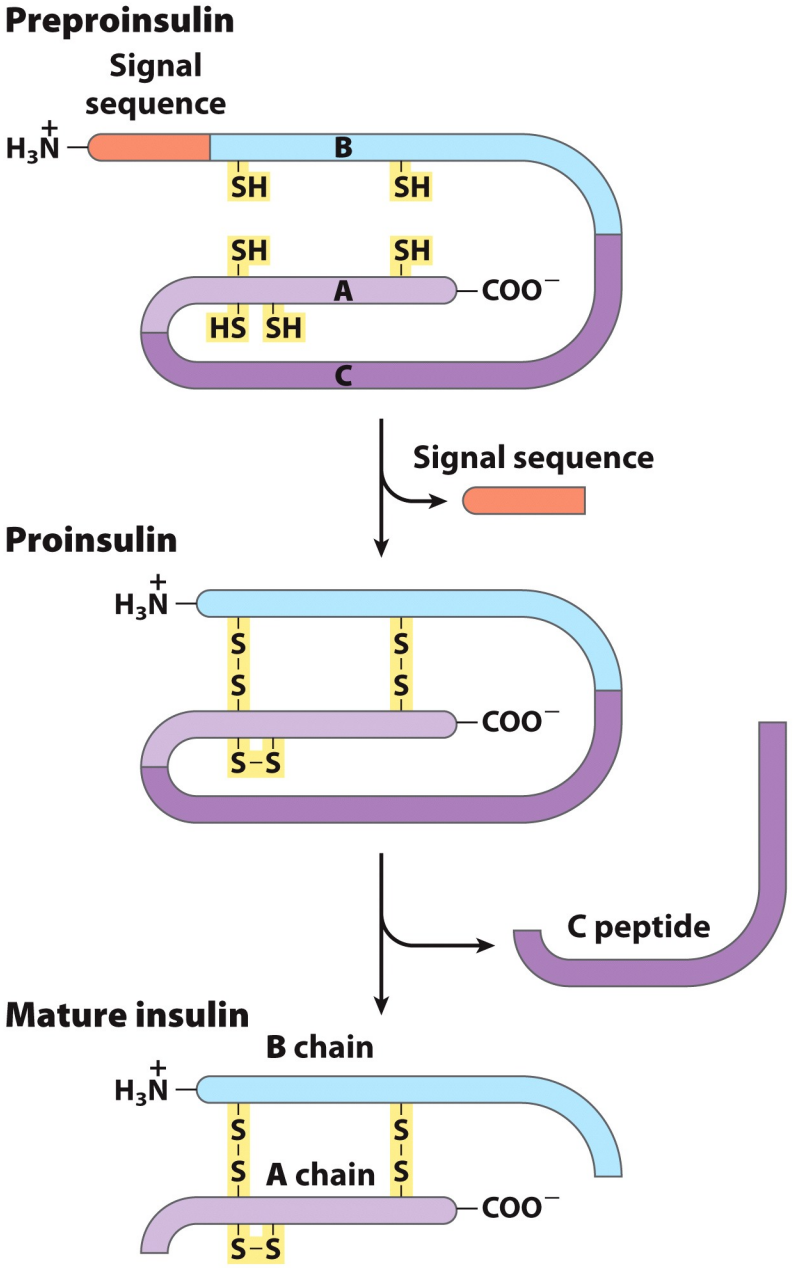

Figure 23-4
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PEPTIDES

- A particularly interesting case is that seen when a single polypeptide sequence contains two or more distinct hormones.
- The most complex example is a pituitary multihormone precursor that contains sequences for β- and γ-lipotropin, α-,β -, and γ-melanocyte-stimulating hormone (MSH), endorphin, enkephalin, and ACTH.
- This precursor, called pro-opiomelanocortin, derives its name from its role as precursor to endogenous opiates, melanocyte-stimulating hormone, and corticotropin.
- A remarkable fact about pro-opiomelanocortin is that it is cleaved at different sites in different cells, so that different cell types produce different ensembles of hormones derived from this one precursor.
- In the anterior pituitary, cleavage generates ACTH and β-lipotropin, and further processing in the central nervous system yields endorphin and enkephalin, among other products.

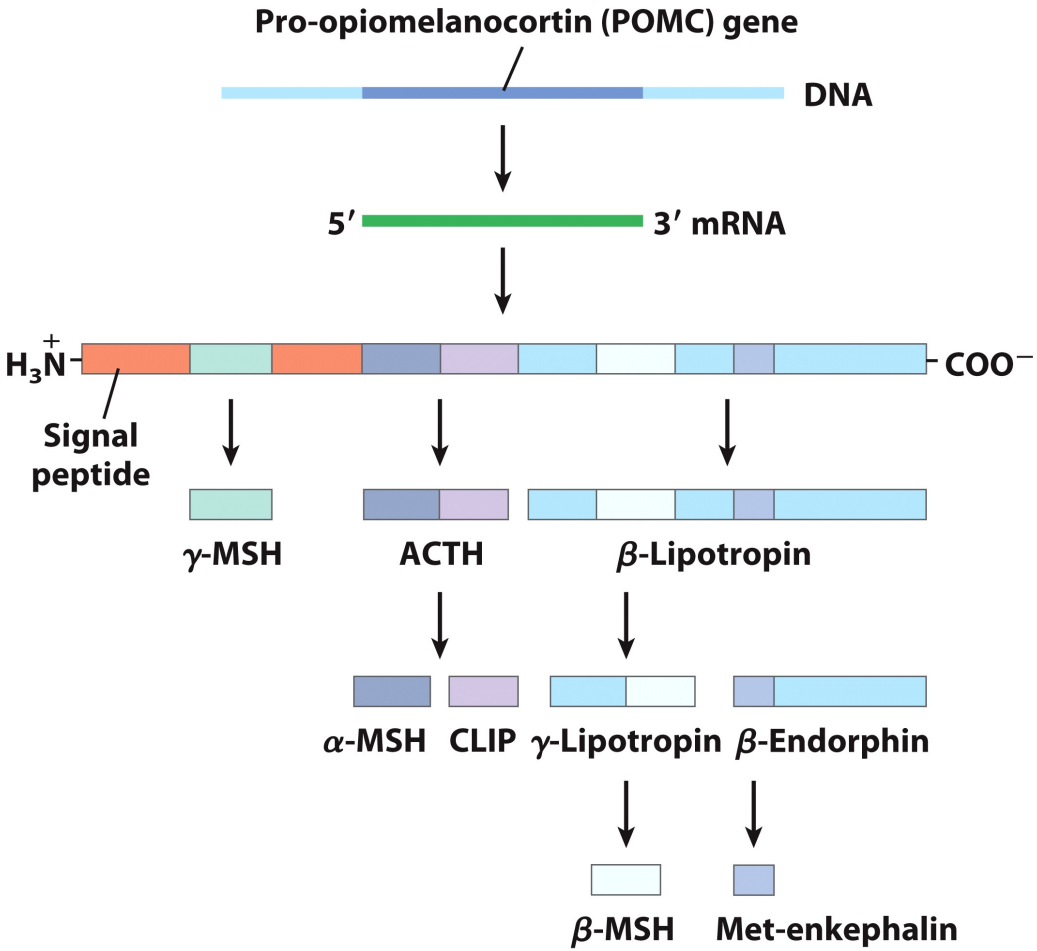

Figure 23-5
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PEPTIDES

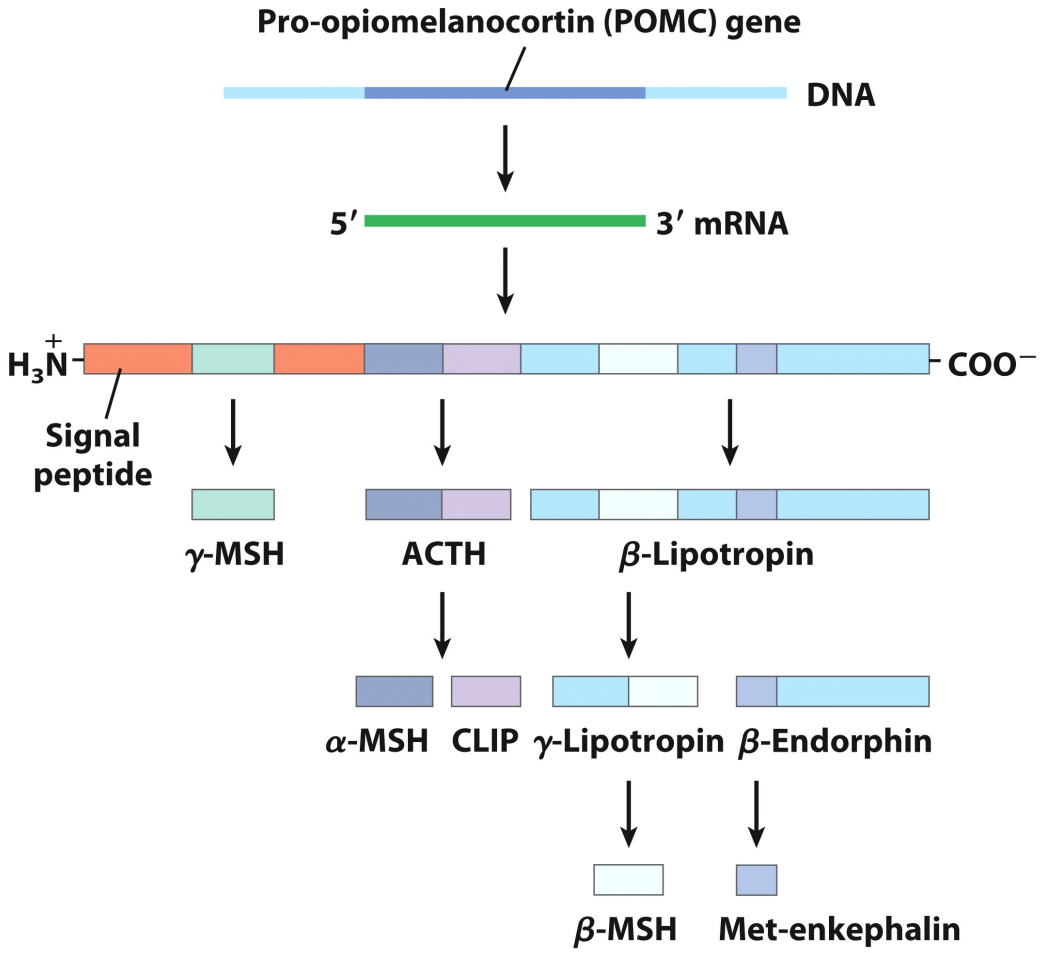

Figure 23-5
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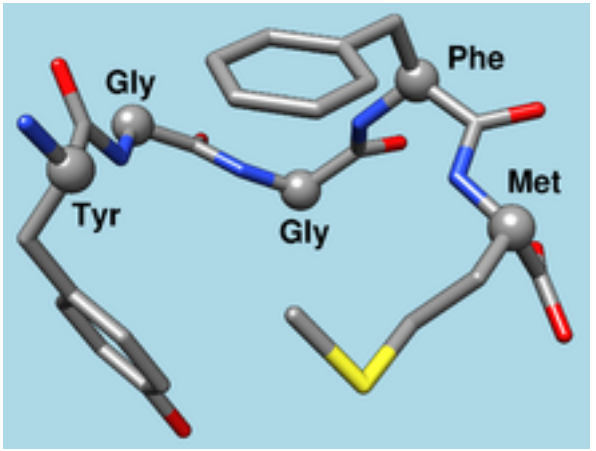
Endogenous opioid peptides

Met-enkephalin is Tyr-Gly-Gly-Phe-Met.

Leu-enkephalin has Tyr-Gly-Gly-Phe-Leu.



PEPTIDES

- The C-term residue of these two hormones is glycinammide.
- The amidation of small peptide hormones is common
- These two hormones differ in two residues (pink box) but they have completely different effects
- Oxytocin act on smooth muscle of the uterus and mammary gland
- Vasopressin increases water adsorption in kidney and induces blood vessels constriction

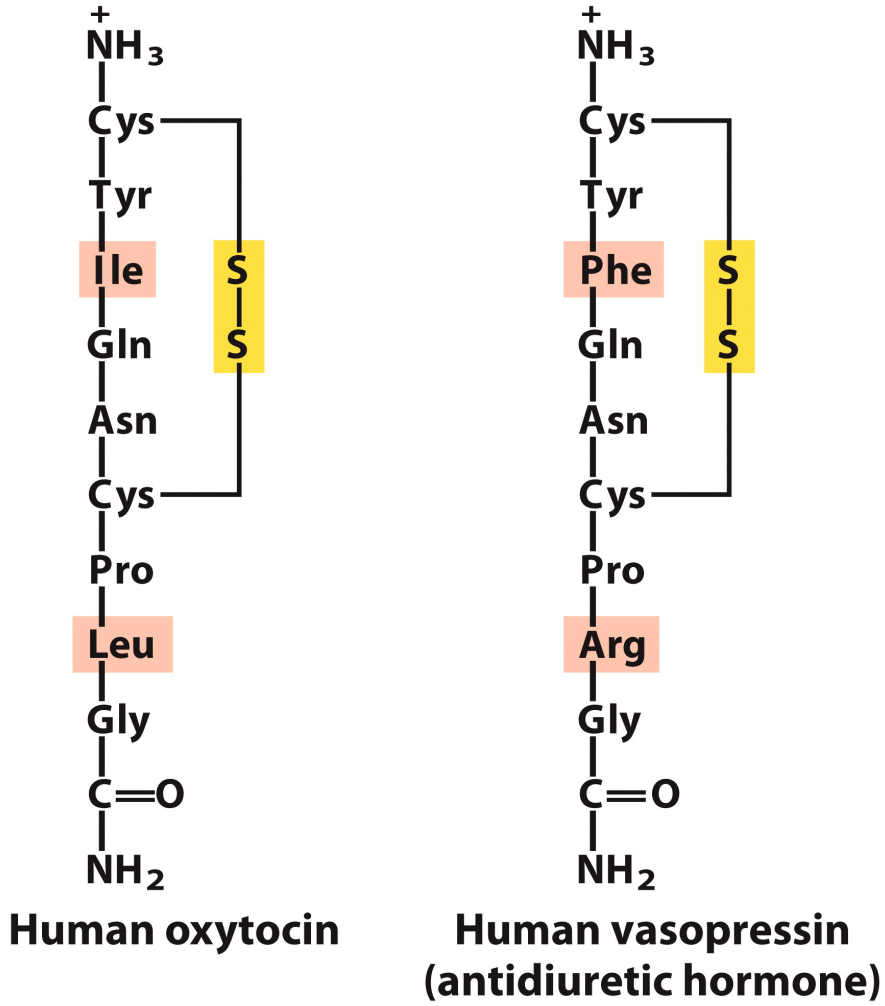


Figure 23-9
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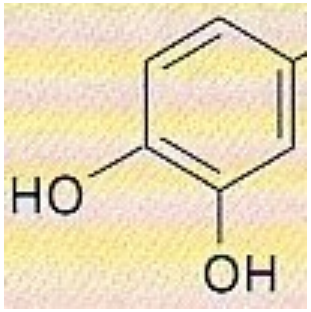
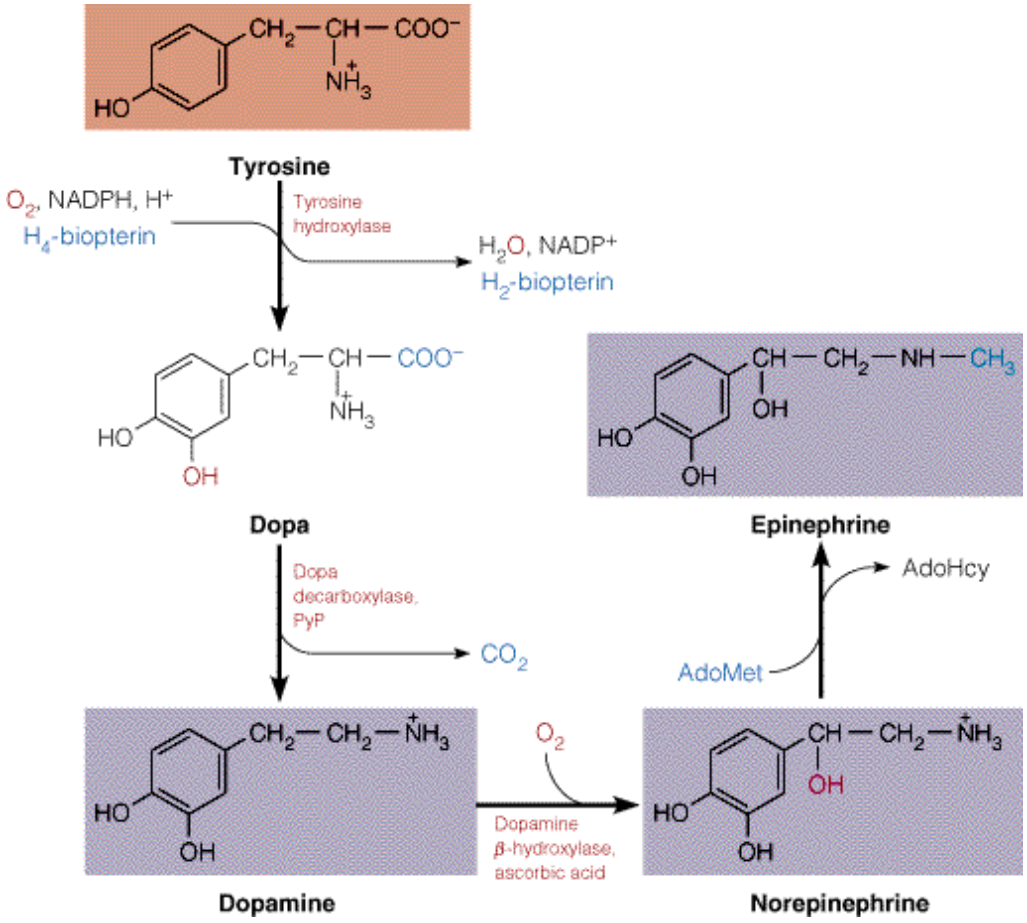
CATECHOLAMINES



Catechol

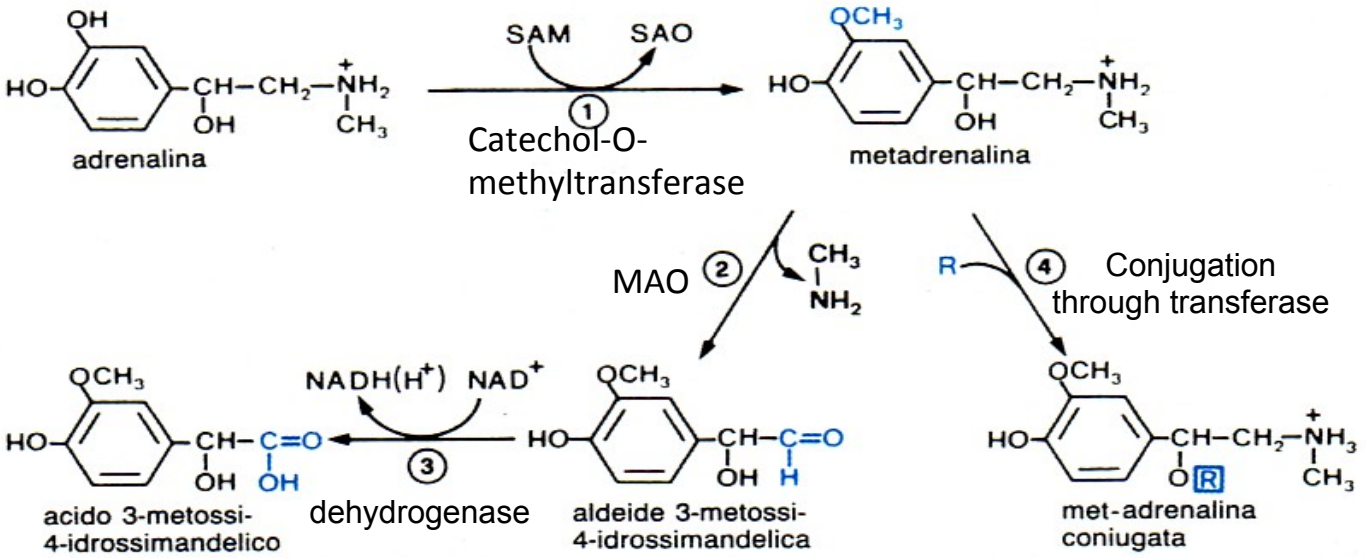


Receptor Class	Target Tissue	Effect of Hormone or Agonist
α_1	Iris of the eye	Contraction
	Intestine	Decreased motility
	Salivary glands	Potassium and water secretion
α_2	Pancreatic B cells	Decreased secretion
	Blood platelets	Aggregation
	Adipocytes	Decreased lipolysis
	Stomach	Decreased motility
α (subtype not identified)	Arterioles in skin, mucosa	Constriction
	Bladder sphincter	Contraction
	Male sex organs	Ejaculation
β_1	Heart	Increased rate, force, and depth of contraction
	Adipocytes	Increased lipolysis
	Intestine	Decreased motility
β_2	Lung	Muscle relaxation
	Liver	Increased glycogenolysis
	Intestine	Decreased motility

Source: Adapted from L. S. Goodman and A. C. Gilman, eds., *Pharmacological Basis of Therapeutics*, 7th ed. (New York: Macmillan, 1985), p. 72. © 1985 McGraw-Hill, Inc.

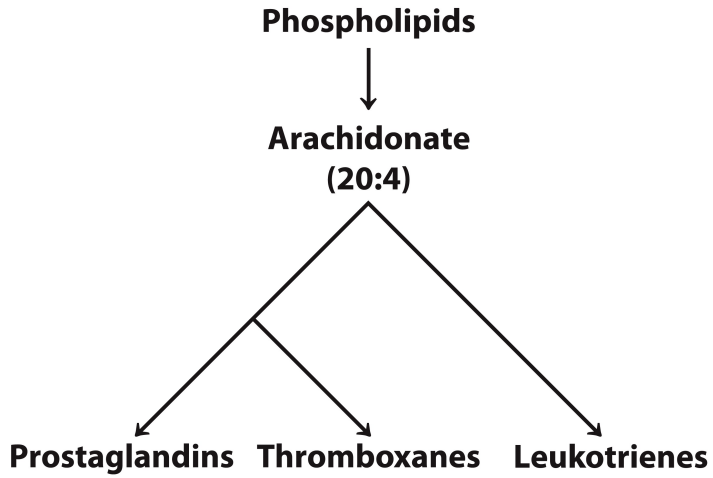
Catecholamines catabolism

- Catabolic process - in the liver
- Catechol-O-methyltransferase
- Use of the new S-adenosylmethionine (SAM) which is converted into S-adenosylhomocysteine (SAO) with methylation of the OH in the aromatic ring
- met-adrenaline is formed (epinephrine O-methylated) that
- or is attacked by the MAO = monoamine oxidase, giving methyl-amine and aldehyde 3-methoxy-4-hydroxymandelic which is dehydrogenated to acid 3-methoxy-4-idrossimandelic
- or it is combined with ac. glucuronic or ac. sulfuric
- all these forms are excreted in the urine



EICOSANOIDS

- Eicosanoids are a class of lipids that include the prostaglandins, thromboxanes, and leukotrienes.
- Eicosanoids derive their name from their common origin, that is, from C20 polyunsaturated fatty acids, the eicosaenoic acids, particularly arachidonic acid (all-cis-5,8,11,14-eicosatetraenoic acid).



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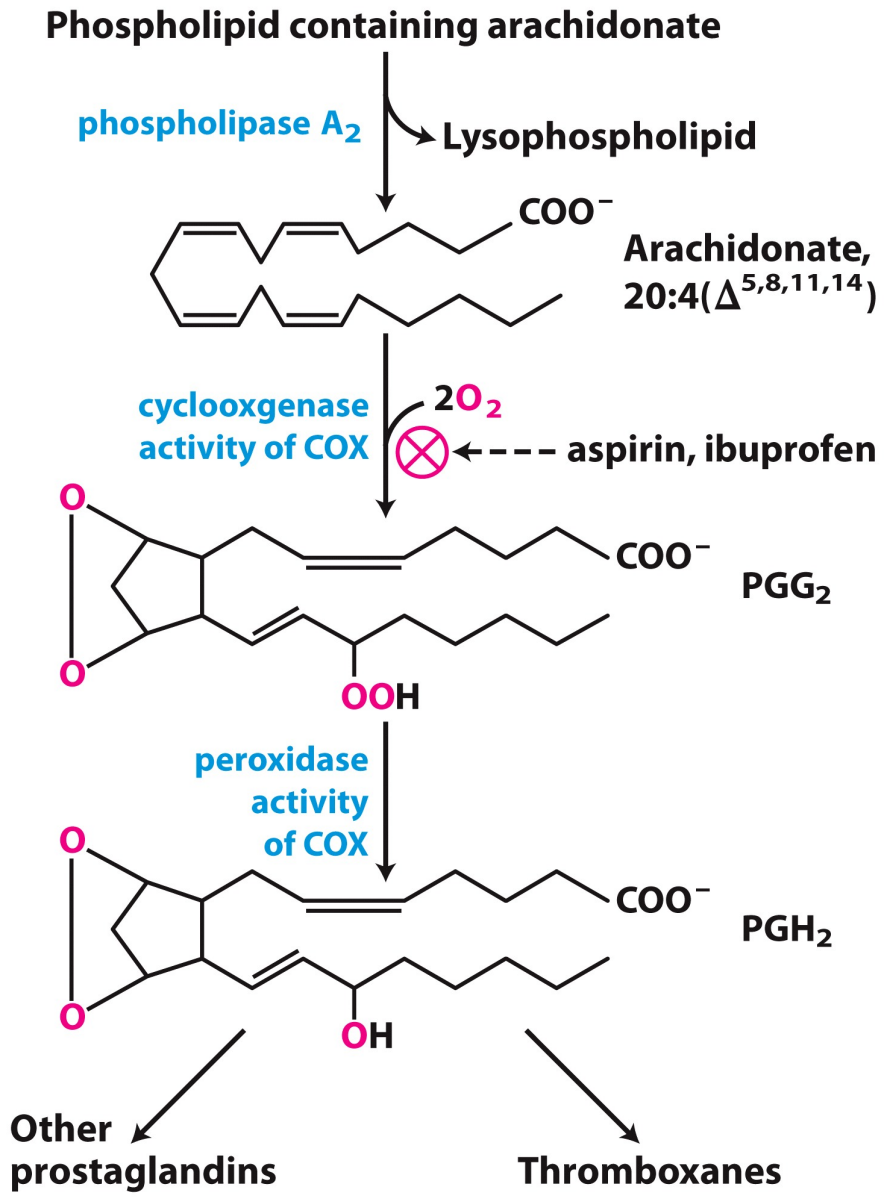

Figure 21-15a
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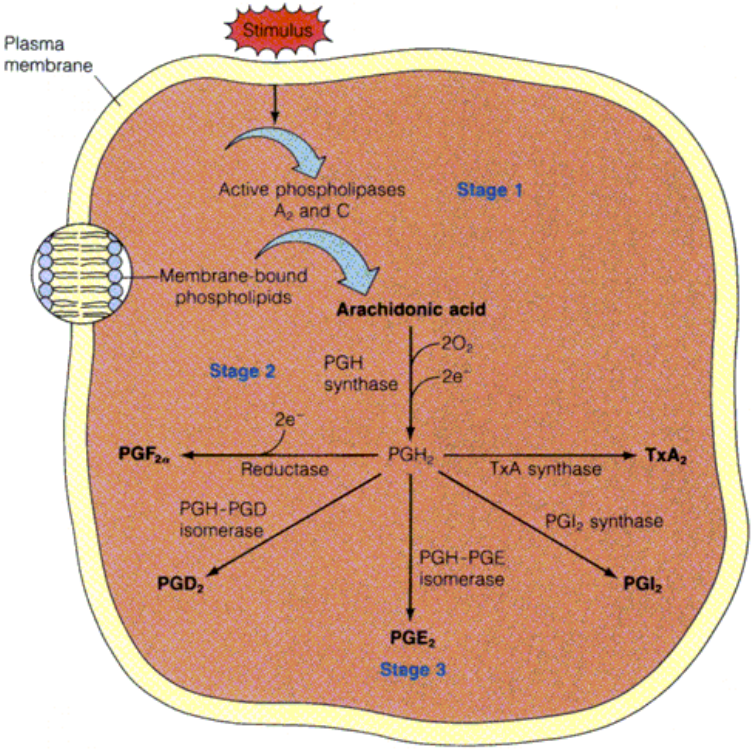
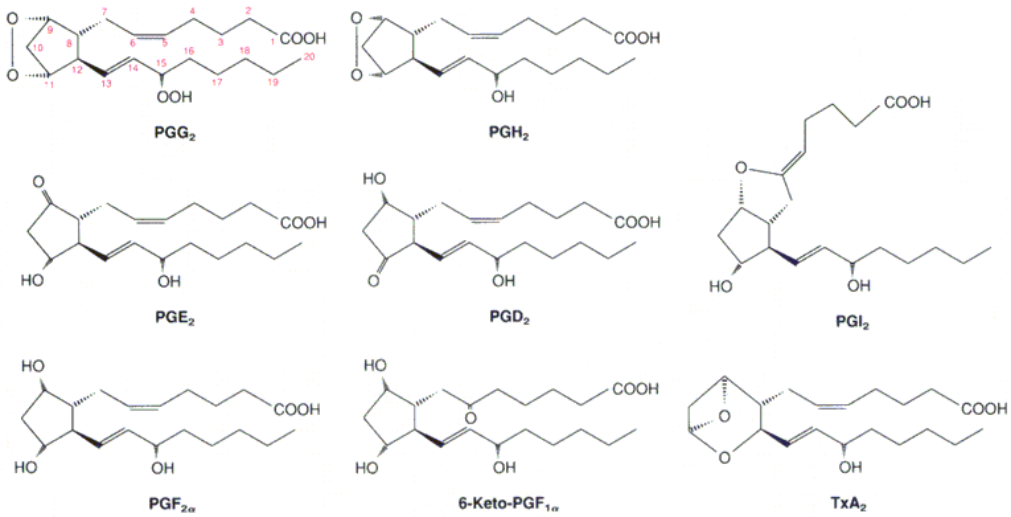
EICOSANOIDS

- Eicosanoids exert short range effects in neighboring cells and tissues
- Eicosanoids (like prostaglandin) exert specific physiological effects on target cells, like hormones
- However, eicosanoids are distinct from most hormones in that they act locally, near their sites of synthesis, and they are catabolized extremely rapidly. Thus, eicosanoids are considered to be locally acting hormones.



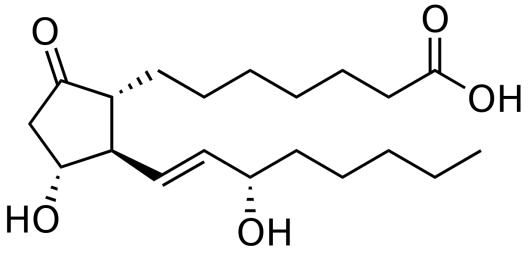
Summary of biosynthetic routes to the major prostaglandins and thromboxane A2.



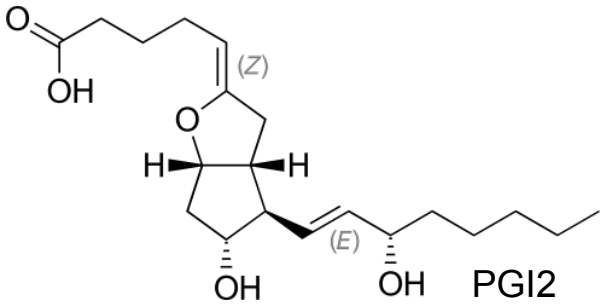
Structures of the major prostaglandins and thromboxane

PROSTAGLANDINES AND THROMBOXANS

- Every prostaglandin contains 20 carbon atoms, including a 5-carbon ring.
- Prostaglandins are powerful locally acting vasodilators and inhibit the aggregation of blood platelets.
- Through their role in vasodilation, prostaglandins are also involved in inflammation.
- They include prostacyclins
- Specific prostaglandins are named with a letter (which indicates the type of ring structure) followed by a number (which indicates the number of double bonds in the hydrocarbon structure).

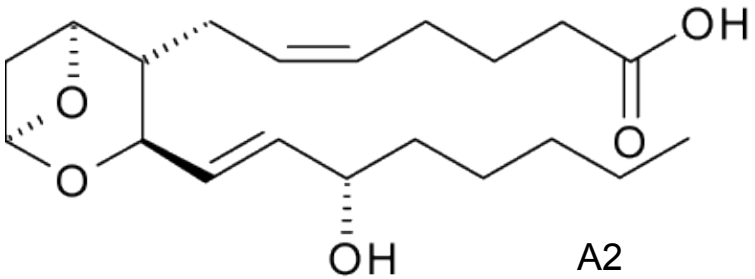


PGE1

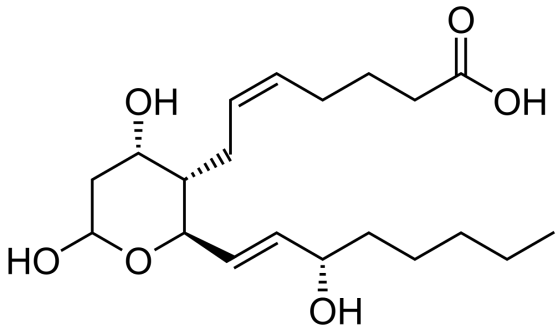


PGI2
(prostacyclin)

- Thromboxane is a member of the family of lipids known as eicosanoids. The two major thromboxanes are thromboxane A2 and thromboxane B2.
- The distinguishing feature of thromboxanes is a 6-membered ether-containing ring.
- Thromboxane is named for its role in clot formation (thrombosis).
- Thromboxane is a vasoconstrictor and a potent hypertensive agent, and it facilitates platelet aggregation.



A2



B2

Cycloxygenase (COX)

- In mammals, two isoforms of COX exist (COX1 and COX2).
- They have similar sequences (60-65% identity), similar reaction mechanisms but different roles
- COX1 is responsible for the synthesis of prostaglandins that regulates secretion of the gastric mucosa
- COX2 is responsible for the biosynthesis of prostaglandins that mediate inflammation, pain and fever

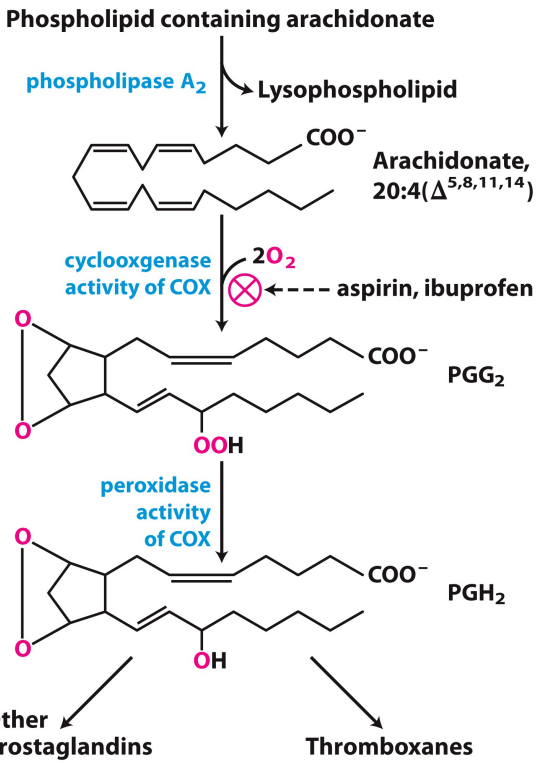


Figure 21-15a
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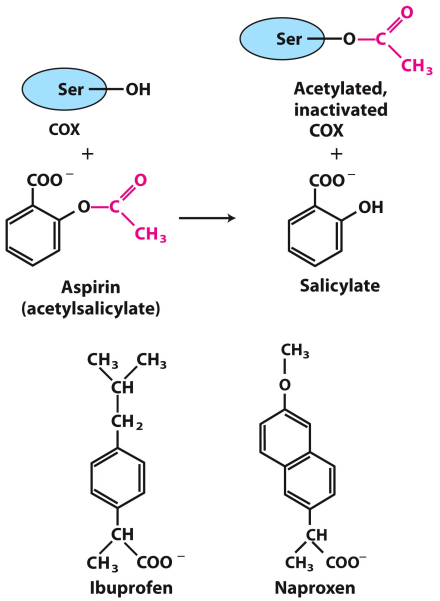

Figure 21-15b
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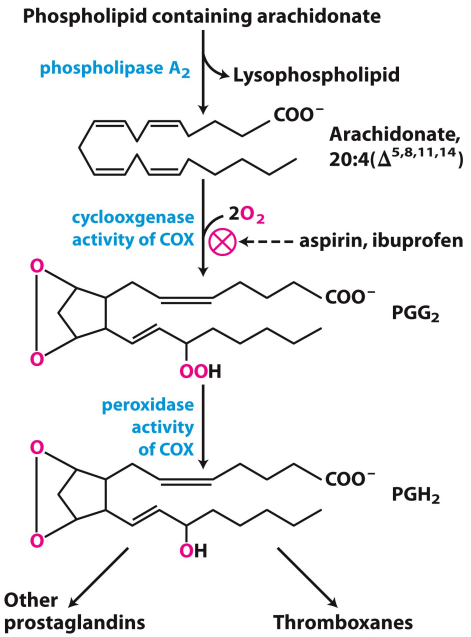
- Aspirin, an inhibitor of both COX1 and COX2, was introduced into the market in 1899
- The drug was able to acetylate a serine residue blocking the active site
- Ibuprofen (a FANS) inhibits both COX1 and COX2

Cycloxygenase (COX)

- COX1 inhibition has major side effects such as stomach irritation
- In 90s, the crystal structure resolution of COX1 gave the opportunity to design inhibitors more specific for COX2 for pain therapy
- Three drugs were designed and introduced in the market (rofecobix, valdecobix and colecobix)
- Different studies showed a correlation between the use of these drugs and heart attack and stroke so that these drugs were withdrawn from the market
- Most probably, they alter the balance between prostacyclin and thromoboxanes, that control blood coagulation



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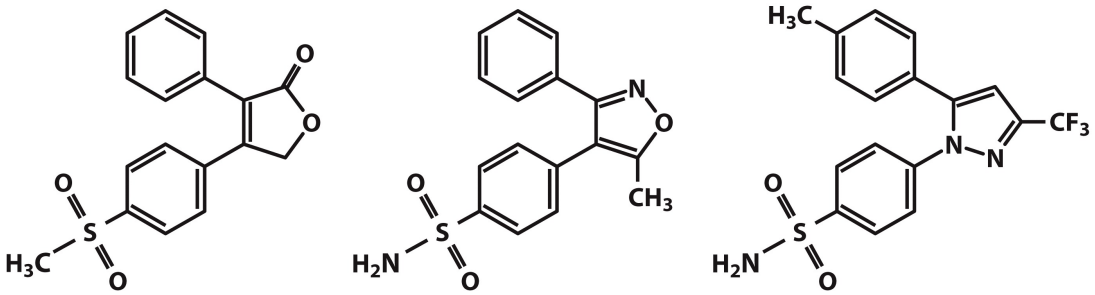

Figure 21-15c
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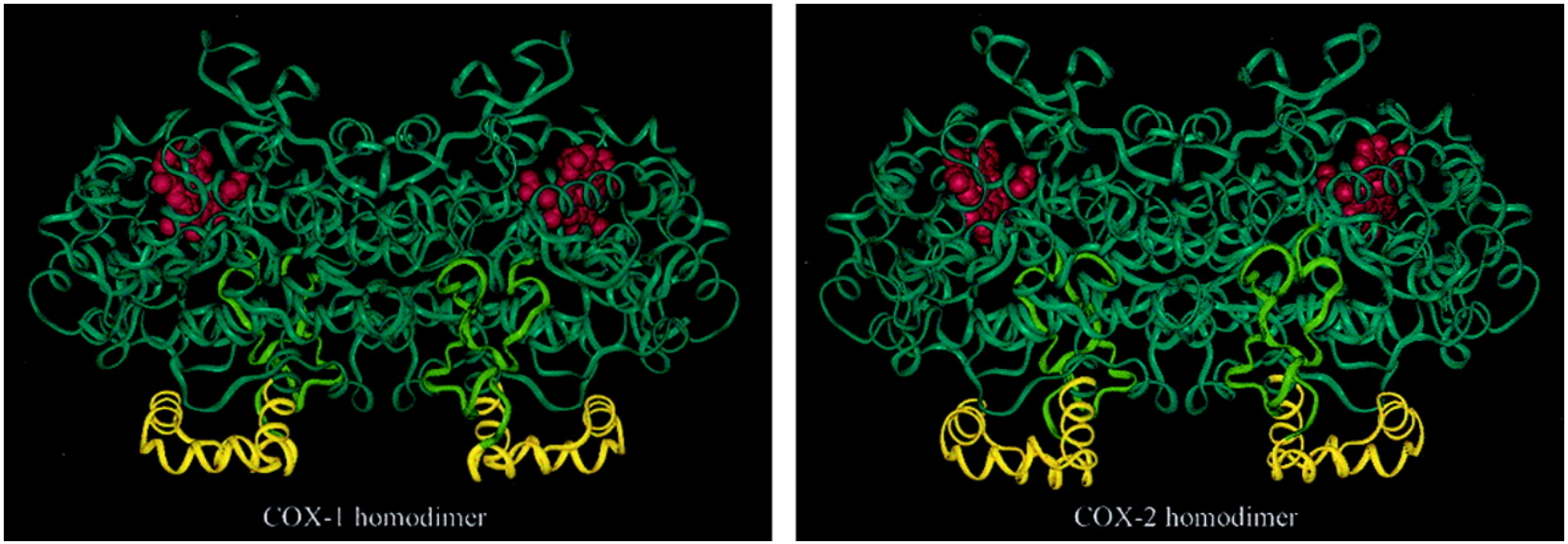
Cycloxygenase (COX)

- COX is a heme-containing bifunctional protein that sequentially catalyzes two reactions.
- The first reaction involves cyclooxygenation of the endogenous substrate arachidonic acid to yield the hydroperoxy endoperoxide PGG2.
- Subsequent reduction of the hydroperoxyl moiety of PGG2 results in formation of PGH2.
- The latter peroxidase reaction occurs in an adjacent, but spatially distinct, site within the COX catalytic domain. Contains two separate active sites for prostaglandin synthase
- One side contains the cyclooxygenase active site
- The opposite side contains the peroxidase active site which is involved in activating the heme group necessary for cyclooxygenase reaction
- Complex composed of identical dimers (2 cyclooxygenase sites and 2 peroxidase active sites)
- Each subunit has a carbon rich knob involved in anchoring the complex to the ER
- Knobs contain funnels to active sites responsible for guiding arachidonic acid from the ER to the enzyme



Crystallographic structures of ovine COX-1 (left) and murine COX-2 (right) homodimers. Functional domains: 1) membrane binding domain (yellow); 2) dimerization domain (light green); catalytic domain (green) heme (red). The open cleft of the peroxidase active site is observable at the top of each monomer. Glycosyl residues are not shown.

EICOSANOIDS: LEUKOTRIENS

- Leukotrienes are eicosanoids that were originally isolated from leukocytes and contain three double bonds, which explains how they were named.
- Leukotrienes are formed by a pathway independent of that of forming the prostaglandins and thromboxanes.
- The pathway to leukotrienes starts by attack on arachidonate of a lipoxygenase, which adds O_2 to C-5, giving 5-hydroperoxyeicosatetraenoic acid (5-HPETE).

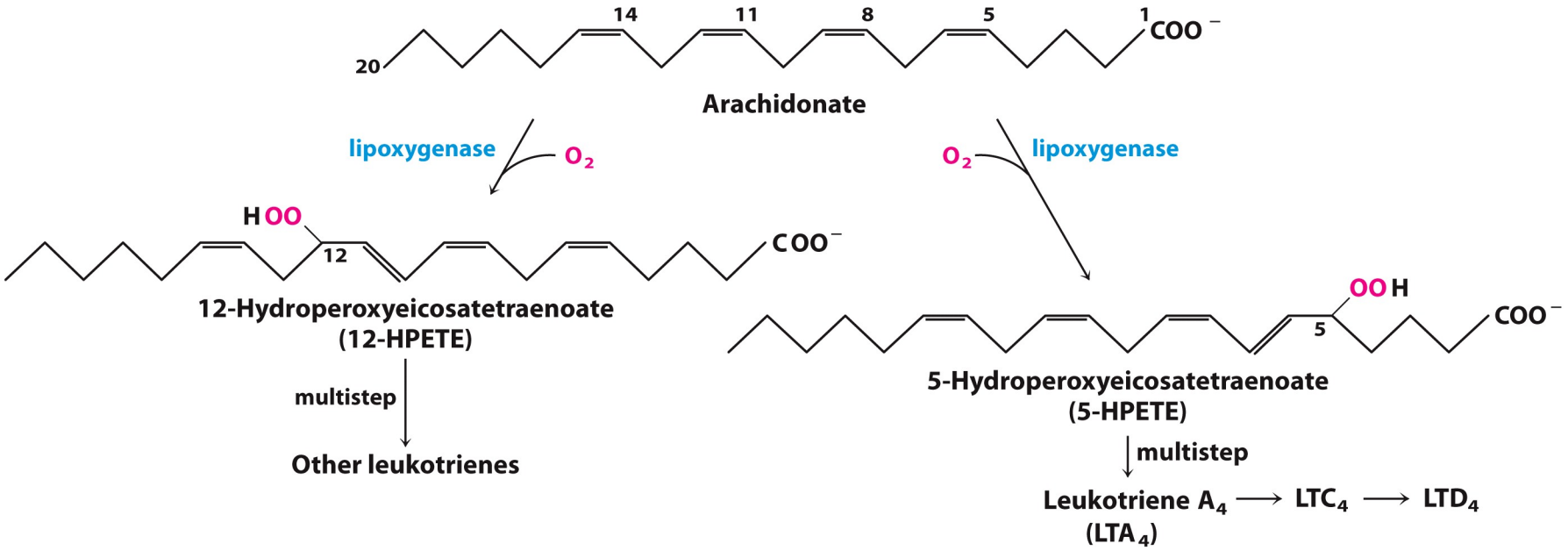


Figure 21-16
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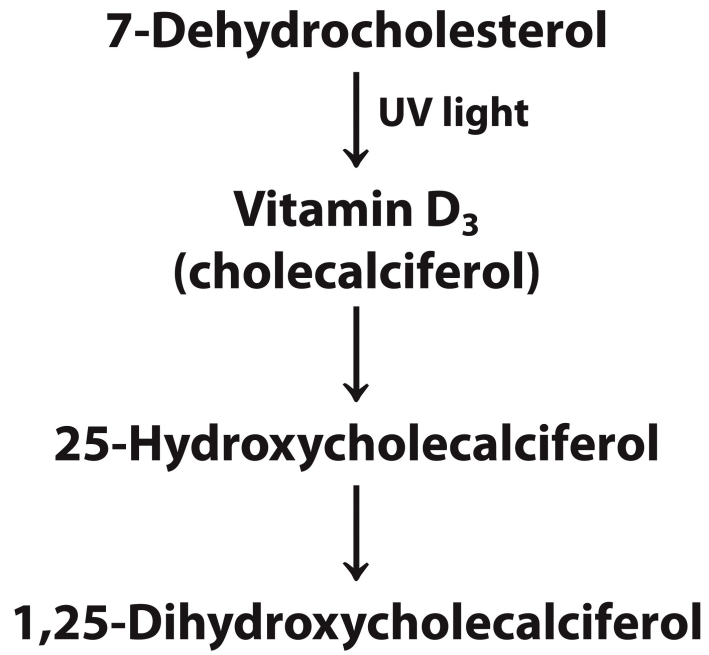
VITAMIN D DERIVATIVE: CALCITRIOL

- The most abundant form of vitamin D is D_3, called cholcalciferol.
- Vitamin D is not technically a vitamin, because it is not required in the diet.
- It arises from UV-photolysis of 7-dehydrocholesterol, an intermediate in cholesterol biosynthesis
- D3 undergoes two successive hydroxylations catalyzed by mixed-function oxidases. The first occurs at carbon 25 in liver.
- When calcium levels are low, hydroxylation occurs at carbon 1, yielding the active form, **1,25(OH)D_3**, which stimulates osteoblasts to take up calcium.
- In the intestine, **1,25(OH)D_3** stimulates transcription of a protein that stimulates calcium absorption into the bloodstream.
- When calcium levels are adequate, hydroxylation occurs instead at carbon 24, yielding the inactive 24,25(OH)D_3 form.



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VITAMIN D DERIVATIVE: CALCITRIOL

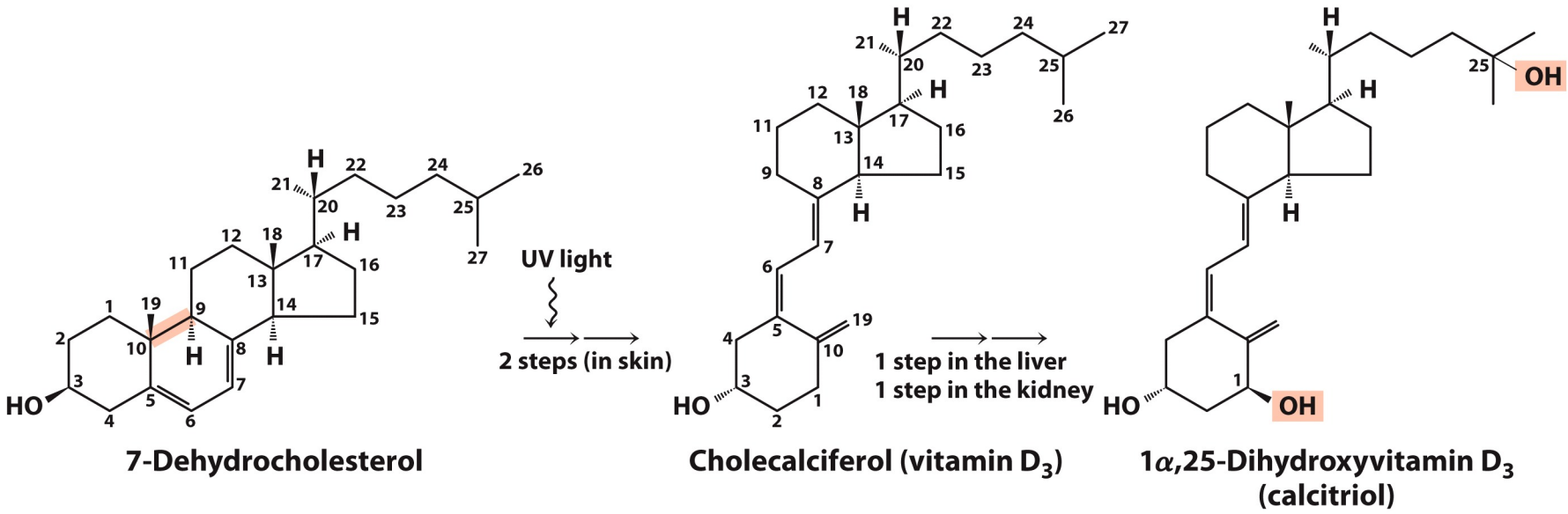

Figure 10-20a
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RETINOID HORMONES

- Retinoids are potent hormones that regulate cell growth, survival and differentiation through nuclear receptors
- Retinol is the pro-hormone and it is synthetised mainly in the liver from beta-carotene, whereas other tissues convert retinol into retinoic acid

β-Carotene

↓

Vitamin A_1 (retinol)

↓

Retinoic acid

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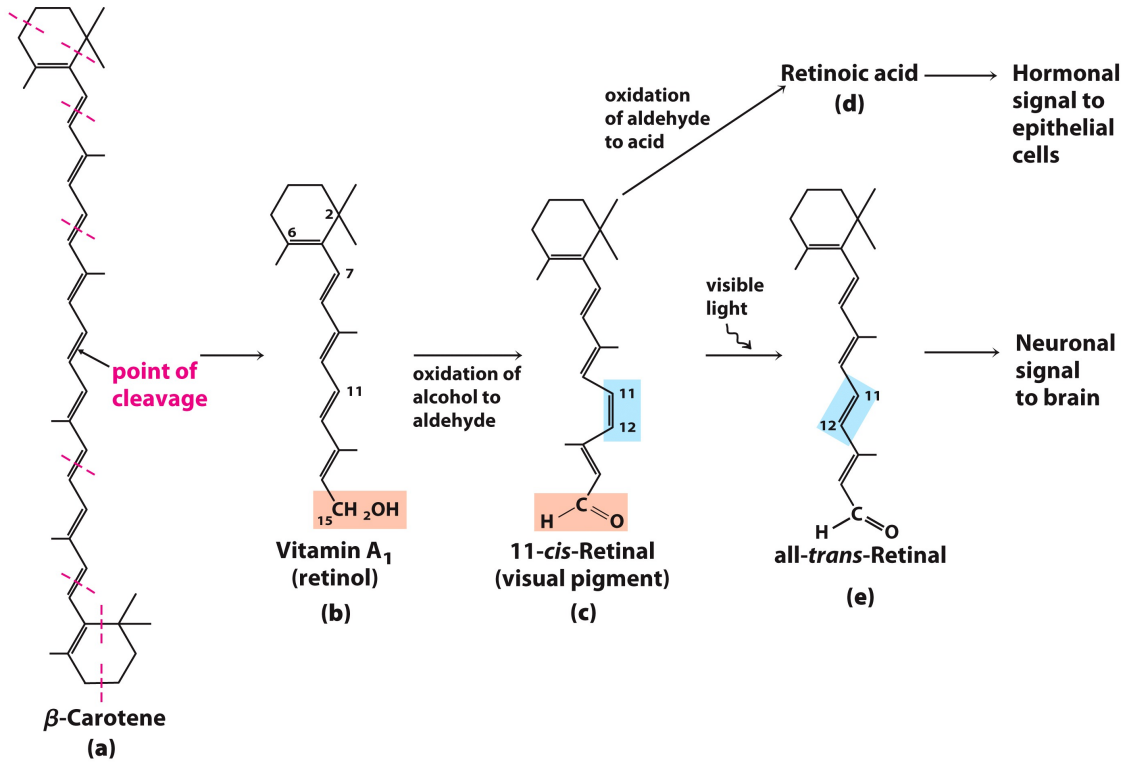

Figure 10-21
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THYROID HORMONES

- Thyroid hormones triiodothyronine (T3) and thyroxine (T4) are synthetised in thyroid gland from the protein precursor tyroglobulin (Mr 660,000).
- Up tp 20 Tyr residues of the protein are enzymatically iodinated in thyroid gland
- Then, 2 residues of di-iodo-iodotyrosine condensate to form the precursor of thyroxine
- A proteolytic cleavage makes thyroxine available when required
- The condensation of a mono-iodo-tyrosine residue with a di-iodo-tyrosine give rise to triiodothyronine (T3)
- The condensation of two di-iodo-tyrosines give rise to thyroxine (T4)

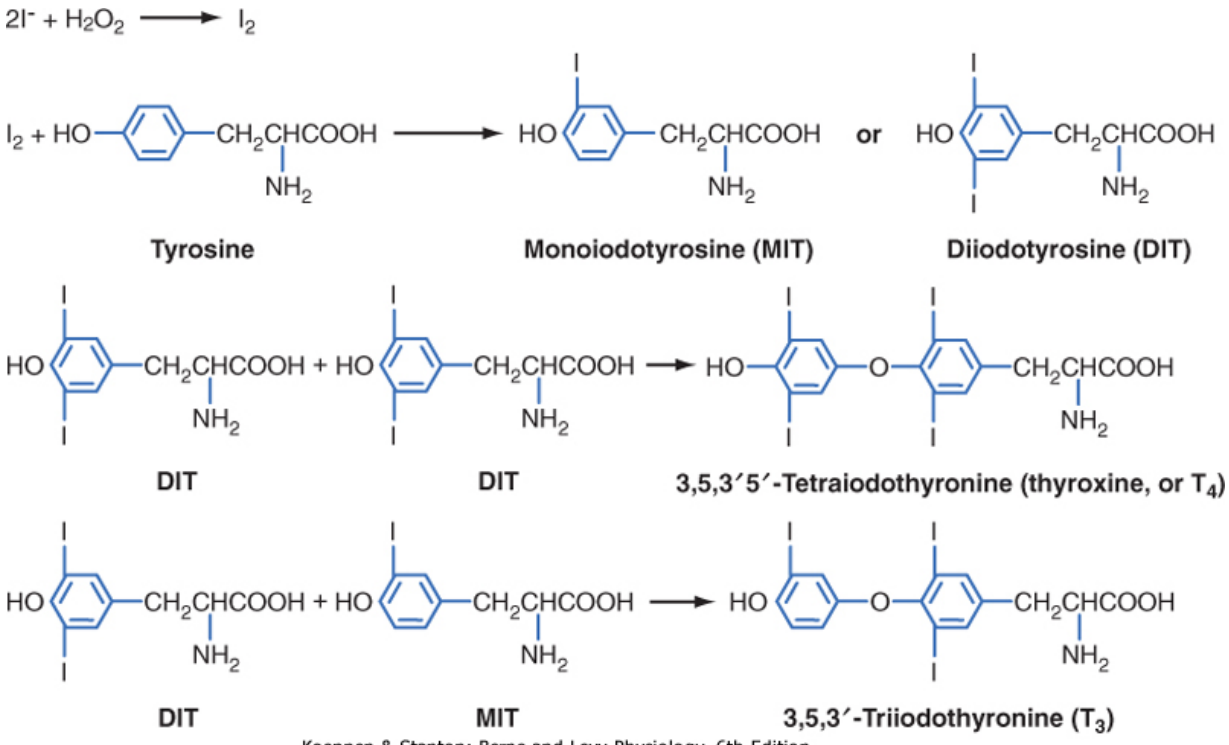
Thyroglobulin – Tyr

↓

Thyroglobulin – Tyr – I (iodinated Tyr residues)

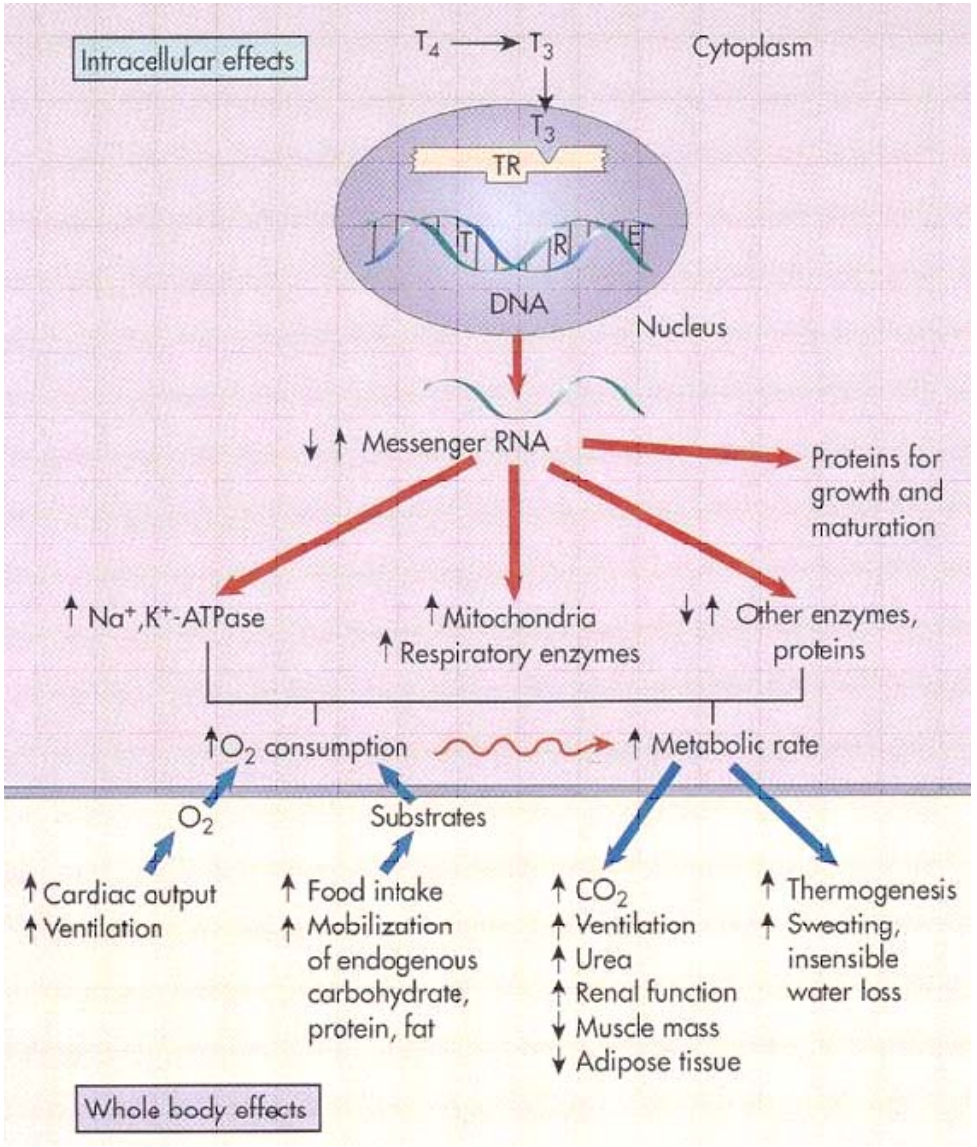
↓ **proteolysis**

Thyroxine (T_4), triiodothyronine (T_3)



THYROID HORMONES

- Thyroid hormones triiodothyronine (T3) and thyroxine (T4) stimulates energy producing metabolism in liver and muscle
- They act through nuclear receptors activating genes coding for catabolic key enzymes



IODINE METABOLISM

- Thyroid hormones are unique biological molecules in that they incorporate iodine in their structure.

- Thus, adequate iodine intake (diet, water) is required for normal thyroid hormone production.

- Dietary iodine is absorbed in the gastrointestinal tract, then taken up by the thyroid gland (or removed from the body by the kidneys).

- The transport of iodide into follicular cells is dependent upon a Na^+/I^- cotransport system.

- Iodide taken up by the thyroid gland is oxidized by peroxide in the lumen of the follicle:

$$I^- \xrightarrow[\text{peroxidase}]{} I^+$$

- Oxidized iodine can then be used in production of thyroid hormones.

PRODUCTION OF THYROGLOBULIN

- Pituitary produces TSH, which binds to follicle cell receptors.
- The follicle cells of the thyroid produce thyroglobulin.
- Thyroglobulin is a very large glycoprotein.
- Thyroglobulin is released into the colloid space, where it's tyrosine residues are iodinated by I^+.
- This results in tyrosine residues which have one or two iodines attached (monoiodotyrosine or diiodotyrosine).

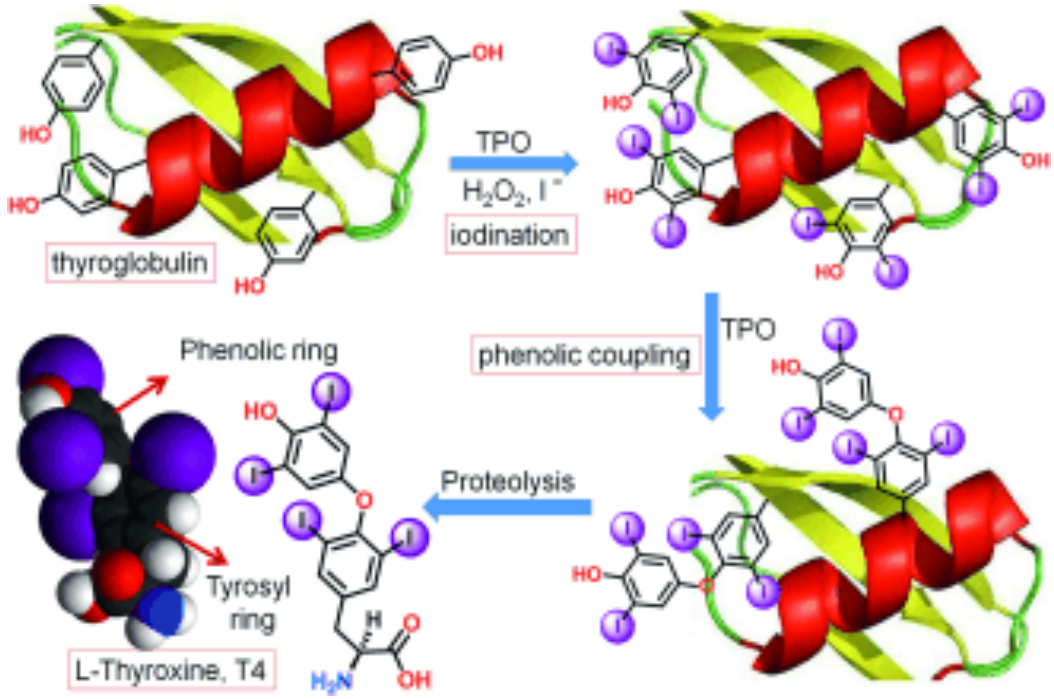


Figure 1: A) Biosynthesis of L-thyroxine (T4) from thyroglobulin by thyroid peroxidase (TPO) in the presence of H_2O_2 and iodide. The space-filling model indicates the relative orientation of the two iodinated phenyl rings in T4.

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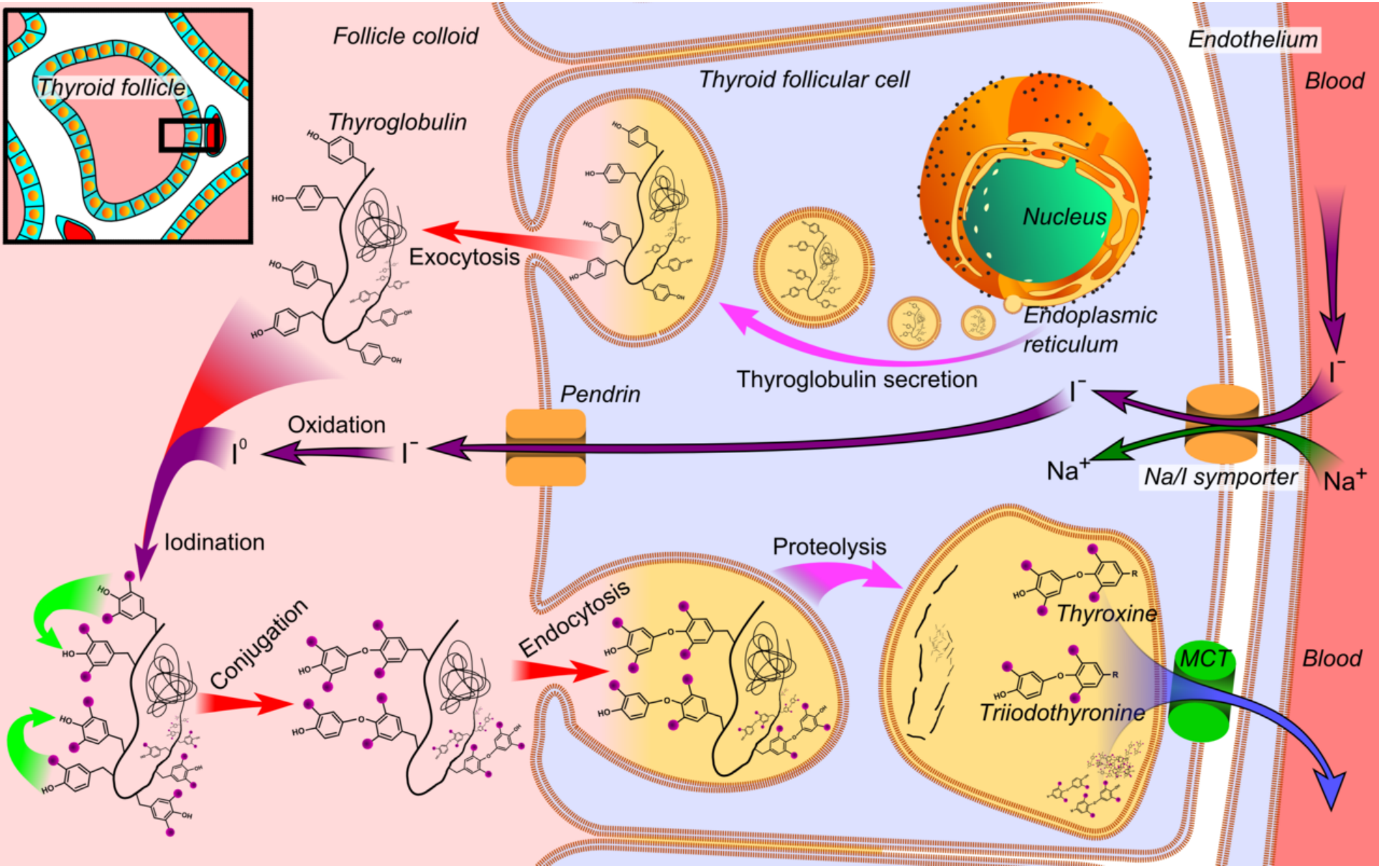
THYROID HORMONES: SUMMARY



Häggström, Mikael. "Medical gallery of Mikael Häggström 2014". Wikiversity Journal of Medicine 1 (2). DOI:10.15347/wjm/2014.008. ISSN 20018762.

TRANSPORT OF THYROID HORMONES

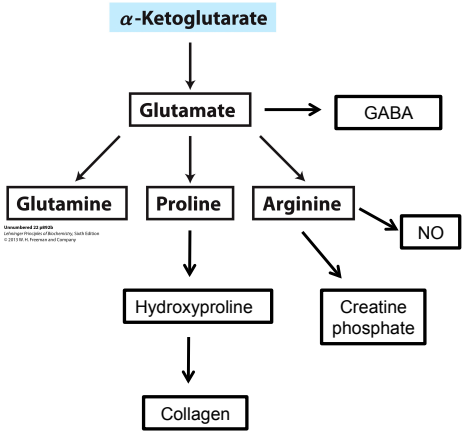
- Thyroid hormones are not very soluble in water (but are lipid-soluble).
- They leave the follicol cells through a monocarboxylate transporter (MCT)
- Thus, they are found in the circulation associated with binding proteins:

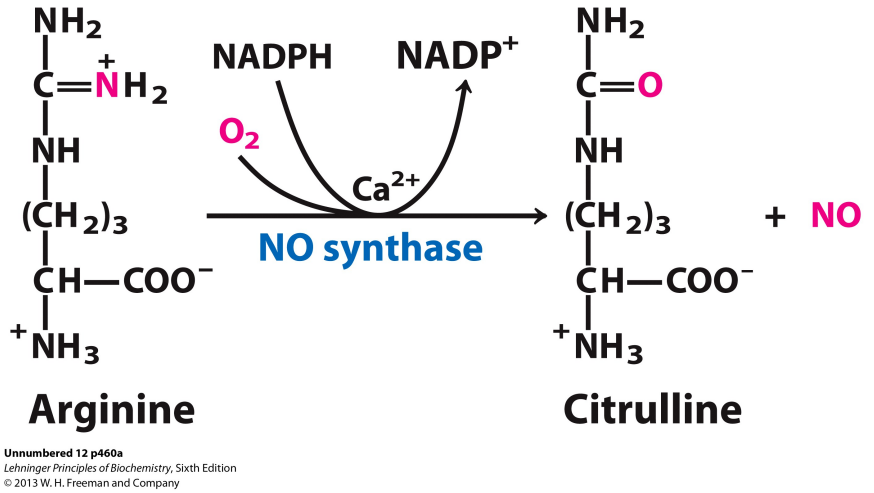
  - Thyroid Hormone-Binding Globulin (~70% of hormone)

  - Pre-albumin (transthyretin), (~15%)

  - Albumin (~15%)
- Less than 1% of thyroid hormone is found free in the circulation.
- Only free and albumin-bound thyroid hormone is biologically available to tissues.

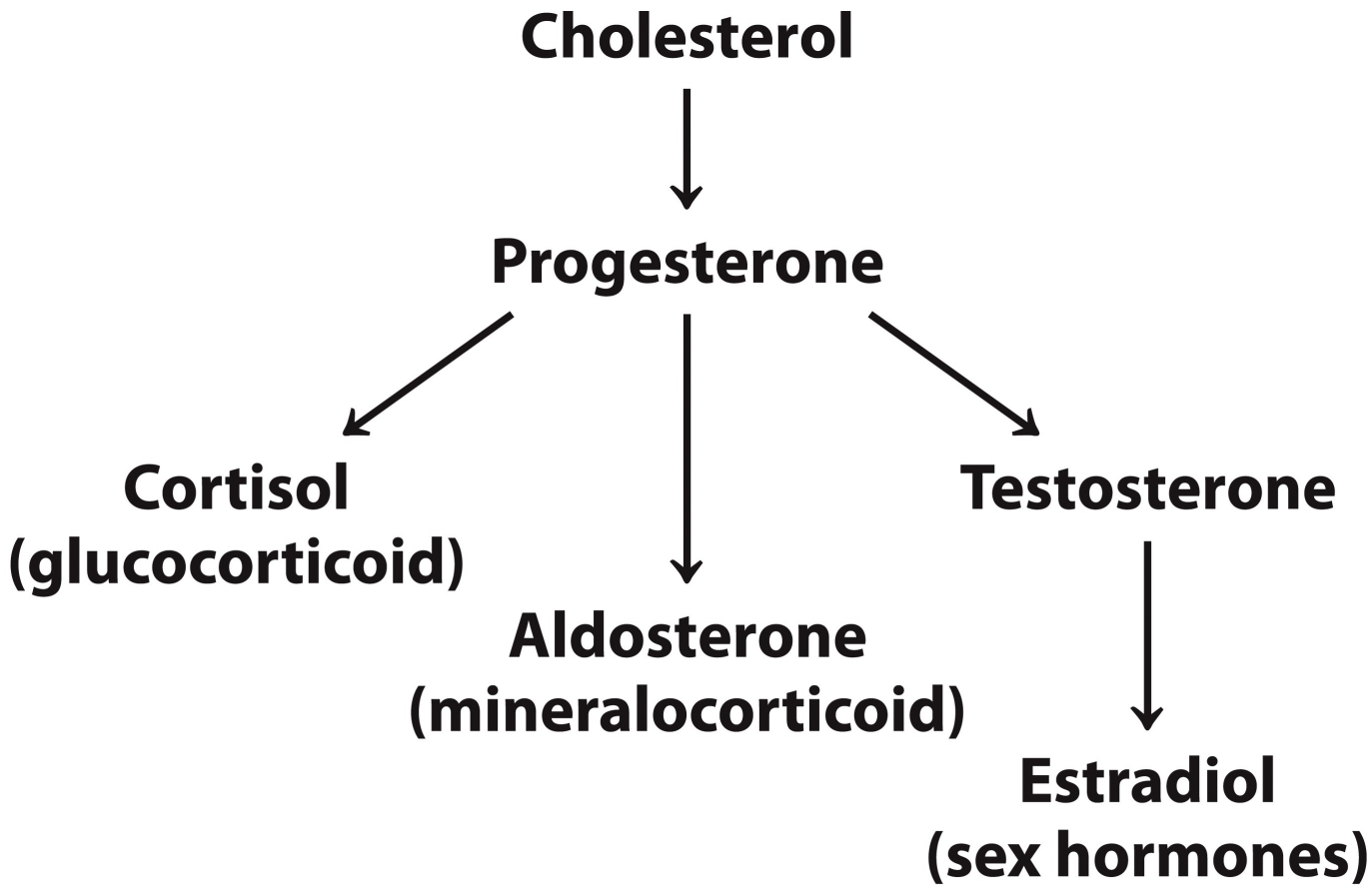
ARGININE IS THE PRECURSOR OF THE SECOND MESSENGER NO





- Nitric oxide is produced from <u>arginine</u> in an unusual five-electron oxidation that also yields <u>citrulline</u>
- The enzyme catalyzing the reaction, nitric oxide synthase, contains bound FMN, FAD, non-heme iron, and tetrahydrobiopterin.
- Nitric oxide, is is a **signal-transducing agent in the vasodilation of endothelial vascular cells and underlying smooth muscle.**
- It is also involved in signaling decreases in blood pressure, and inhibiting platelet aggregation.
- In the inflammatory and immune responses, an inducible form of nitric oxide synthase produces nitric oxide at levels sufficient to be toxic to pathogenic organisms.
- It can act in neurotransmission in the central nervous system and stimulate erection of the penis.
- Nitric oxide is a gas so it can diffuse rapidly into neighboring cells and control their metabolism.
- It is also unstable, with a half-life of 1 to 5 seconds, so its effects are short-lived. In the cell, nitric oxide acts primarily by **stimulating cyclic GMP synthesis**.
- The drug, Viagra, acts by inhibiting cyclic GMP breakdown, thereby prolonging the effect of nitric oxide.

STEROIDS



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SEE LECTURE 9