ENDOCRINE BIOCHEMISTRY

LECTURE 3

Synthesis and Degradation of Hormones

Slides 1-2 | Synthesis & Degradation: Chemical Classes and Receptor Localization

The discussion of hormone synthesis and degradation now proceeds according to their receptor localization. This organizational framework is essential because the chemical properties that determine receptor location, lipophilicity versus hydrophilicity, also dictate the biosynthetic pathways, storage mechanisms, and degradation routes of each hormone class. Hormones that bind to intracellular receptors, steroids, thyroid hormones, retinoids, are lipophilic, synthesized from **cholesterol** or amino acids, and are generally not stored in large quantities, their synthesis is regulated at the level of enzyme expression.

Hormones that bind to cell surface receptors, peptides, catecholamines, are hydrophilic, synthesized as larger precursors, stored in secretory vesicles, and released rapidly in response to stimuli. This dichotomy has profound clinical implications, influencing drug administration routes, dosing frequency, and therapeutic monitoring. A visual summary of the chemical classification of hormones and their corresponding receptor locations reveals the fundamental organization of endocrinology.

Steroids, thyroid hormones, and retinoids bind to intracellular receptors, nuclear receptors, because they are lipophilic and can cross the plasma membrane. **Nitric oxide**, NO, is a small, gaseous signaling molecule that also acts intracellularly but through a different mechanism activating guanylyl cyclase to produce **cGMP**. Amino acid derivatives are divided into thyroid hormones, intracellular receptors, and catecholamines, cell surface receptors.

Proteins and peptides, including insulin, glucagon, and growth hormone, are hydrophilic and bind to cell surface receptors, activating various signaling pathways. Fatty acid derivatives, eicosanoids, are lipophilic but act through cell surface receptors. This classification has direct therapeutic implications, peptide hormones must be administered by injection and act through rapid, short-lived second messenger pathways, while steroid hormones can be given orally and act through slow, long-lasting gene transcription.

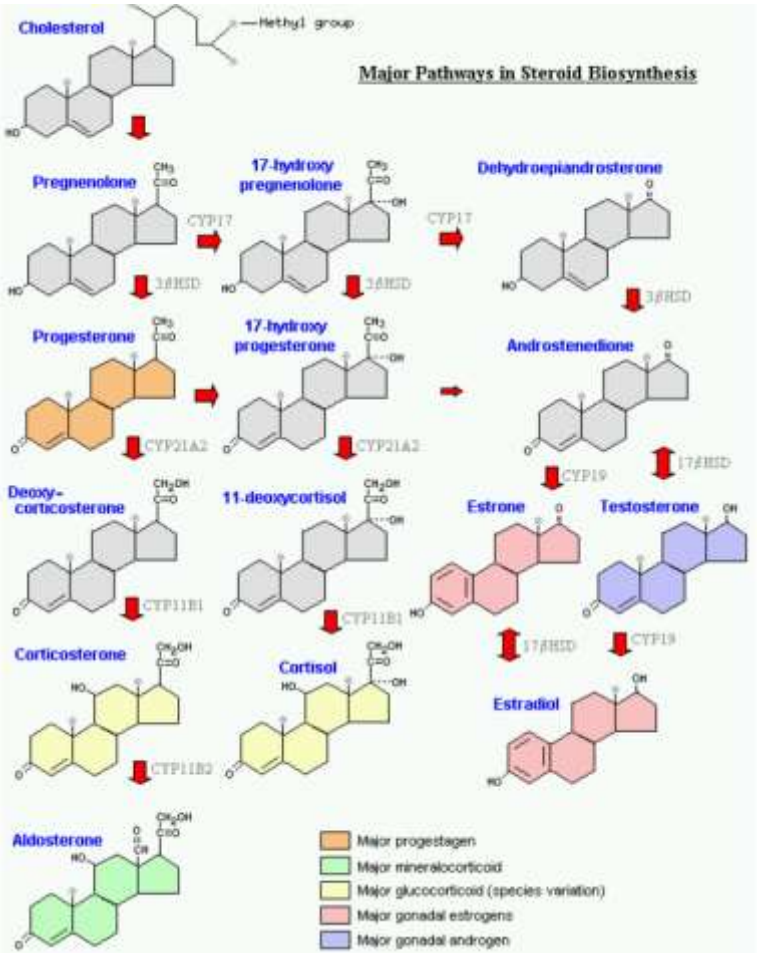
Receptor location determines the signaling mechanism, intracellular receptors directly regulate gene expression, while cell surface receptors trigger second messenger cascades.

Slides 3-4 | Steroid Hormone Synthesis

The biosynthesis of C21 steroids, progestins, glucocorticoids, and mineralocorticoids begins with **cholesterol**, the precursor for all steroid hormones. **Cholesterol** is transported into the **mit**ochondria of steroidogenic cells by the steroidogenic acute regulatory protein, STAR, a **rate-limiting step** regulated by tropic hormones such as **ACTH** and LH. Inside the **mit**ochondria, cytochrome P450 site chain cleavage, CYP11A1, cleaves **cholesterol** to produce **pregnenolone**, the first com**mit**ted intermediate, by removing the 6-carbon site chain, C20A-C22.

Pregnenolone then exits to the smooth endoplasmic reticulum, where 3-beta-hydroxysteroid dehydrogenase, 3-beta-HSD, converts it to **progesterone**. **Progesterone** is the precursor for both **cortisol** and **aldosterone**. **Cortisol** synthesis involves 17-alpha-hydroxylation, CYP17A1, 21 -hydroxylation, CYP21A2, and 11-beta-hydroxylation, CYP11B1. **Aldosterone** synthesis involves 21-hydroxylation and 11-beta -hydroxylation, CYP11B2. Deficiencies in these enzymes cause congenital adrenal hyperplasia, CAH, with 21-hydroxylase deficiency being the most common, leading to decreased **cortisol** and **aldosterone** and increased androgens.

Understanding this pathway is essential for diag**nos**ing and managing CAH and for understanding drugs that inhibit steroidogenesis, such as ketoconazole. C19 steroids, androgens, are synthesized from C21 precursors by removal of the 2-carbon site chain, C20A-C21, through the 17-20-lyase activity of the CYP17A1 enzyme complex. This converts 17-alpha-hydroxy**progesterone** to androstenedione, which is then converted to testosterone by 17-beta -hydroxysteroid dehydrogenase, 17-beta-HSD, in the gonads.



In the adrenal cortex, the main product is dehydroepiandrosterone, DHEA, a weaker androgen that can be converted to androstenedione and testosterone in peripheral tissues. C18 steroids, estrogens, are synthesized from C19 steroids by **aromatase**, **CYP19A1**, which removes the C19 methyl group and aromatizes the A ring, converting androstenedione to estrone and testosterone to estradiol.

Aromatase is expressed in the ovaries, adipose tissue, brain, and placenta. Clinically, **aromatase** inhibitors, e.g., anastrozole, letrozole, are used to treat estrogen receptor-positive breast cancer, while **5-alpha-reductase** inhibitors, e.g., finasteride, block conversion of testosterone to dihydrotestosterone, **DHT**, and are used for benign prostatic hyperplasia and male pattern baldness.

Understanding these pathways is essential for understanding disorders of sexual development, such as **5-alpha-reductase** deficiency and **aromatase** deficiency.

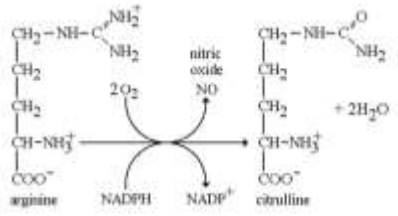
Slide 5 | Steroid Hormone Breakdown

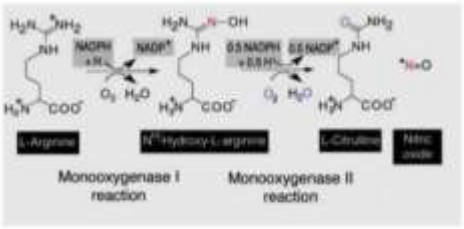
Steroid hormones undergo biotransformation in the liver because the steroid nucleus cannot be completely degraded to CO2 and water. Phase 1 reactions involve hydroxylation by cytochrome P450 enzymes, increasing water solubility. Phase 2 reactions involve conjugation with glucuronic acid or sulfate, producing highly water-soluble metabolites that are excreted in urine or bile. Biliary excretion is important for the enterohepatic circulation of steroids, where conjugates can be deconjugated by intestinal bacteria and reabsorbed.

Measurement of urinary metabolites, 17-ketosteroids, 17-hydroxycorticosteroids, was historically used to assess adrenal function but has been largely replaced by specific serum assays. Clinically, liver disease can impair steroid clearance, leading to accumulation and increased effects, e.g., cirrhosis can cause hyperestrogenism, resulting in gynecomastia and spider angiomas. Drugs that induce hepatic enzymes, e.g., phenytoin, rifampin, can increase steroid clearance, potentially reducing the effectiveness of steroid-based therapies

Slides 6-8 | Nitric Oxide (NO)

that have intracellular receptor localization. **Nitric oxide**, NO, is a unique gaseous signaling molecule with a very short half-life, seconds. It is synthesized by **nitric oxide** synthase, **NOS**, which catalyzes the oxidation of L-arginine to L-citrulline. There are three **NOS** isoforms, neuronal, N**NOS**, inducible, I**NOS**, and endothelial, E**NOS**.





NO does not bind to a classical receptor, instead, it diffuses across cell membranes and activates guanylyl cyclase by binding to its heme group, increasing **cGMP** production. The primary physiological effects of NO are vasovilation, via vascular smooth muscle relaxation, neurotransmission, and immune defense. Clinically, NO is best known for cardiovascular regulation.

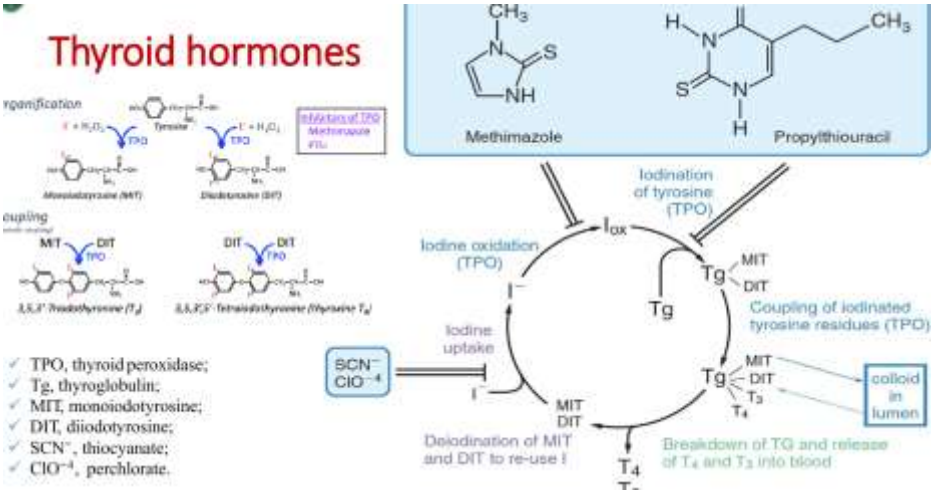
Nitroglycerin and organic nitrates used for angina are converted to NO, relaxing coronary arteries. The discovery of the NO pathway earned the 1998 Nobel Prize. In septic shock, I**NOS** is massively upregulated, producing excessive NO that causes widespread vasovilation and refractory hypotension. Understanding NO is essential for managing both cardiac conditions and critical care.

The three isoforms of **nitric oxide** synthase have distinct localization and functions. **NOS**A, neuronal N**NOS**, is calcium-dependent and constitutively expressed in neurons, involved in neurotransmission, synaptic plasticity, and long-term potentiation, as well as gastrointestinal motility and penile erection. **NOS**2, inducible I**NOS**, is calcium-independent and induced by inflammatory stimuli, LPS, IF and gamma.

It produces large amounts of NO as part of host defense, but excessive NO contributes to tissue damage and vasovilation in septic shock. **NOS**3, endothelial I**NOS**, is calcium-dependent and constitutively expressed in endothelial cells, producing NO that diffuses to vascular smooth muscle to cause relaxation. It is regulated by shear stress, hormones, insulin, estrogens, and pharmacological agents.

Clinically, nitrates treat angina by releasing NO, while in septic shock, excessive INOS-derived NO causes vasovilation requiring high-dose vasopressors. Understanding NOS isoforms is important for pharmacology, as drugs that selectively inhibit INOS are being investigated as anti-inflammatory agents.

Slides 9-12 | Thyroid Hormones: Synthesis and Degradation



Thyroid hormones are unique as the only biologically active molecules that contain iodine. Synthesis involves active transport of iodide into thyroid follicular cells by the sodium iodide's importer, NIS. Iodide is oxidized to iodine by thyroid peroxidase, **TPO**, and attached to tyrosine residues on **thyroglobulin**, TG, in a process called iodination.

TPO then catalyzes the coupling of two diodotyrosine, DiA, molecules to form thyroxine, **T4**, and one diionine-one monoiodotyrosine, MID, to form triiodothyronine, **T3**. These iodinated **thyroglobulin** molecules are stored in the follicular colloid. When TSH stimulates the thyroid, **thyroglobulin** is endocytosed and digested by lysosomal proteases to release **T4** and **T3**.

T4 accounts for 90% of secretion, **T3** for 10%. **Iodine deficiency** leads to decreased **T4** and **T3** synthesis, causing compensatory TSH increase in goiter formation, the most common cause of hypothyroidism worldwide. Iodized salt prevents this. Thyroid hormones are essential for normal brain development in utero and infancy.

Maternal hypothyroidism from **iodine deficiency** can cause cretinism, characterized by severe mental and growth retardation. **T3**, triiodothyronine, and **T4**, thyroxine, are both derived from tyrosine, with iodination of the phenolic ring. **T4** has 4 iodine atoms and is the **prohormone**, while **T3** has 3 iodine atoms and is the biologically active form.

T3 binds to the thyroid hormone receptor with approximately 10-fold higher affinity than **T4**. Conversion of **T4** to **T3** is catalyzed by diiodinase enzymes, D1 in the liver, kidney, and thyroid, D2 in the brain, pituitary, and brown adipose tissue, and D3 in the brain, placenta, and skin, which inactivates **T3** and **T4**.

D2 is particularly important for providing local **T3** in the brain and pituitary. D3 protects the fetus from excessive maternal thyroid hormone. The half-life of **T4** is about 7 days, while **T3** is about 1 day. This makes **T4** the preferred formulation for thyroid hormone replacement therapy, **levothyroxine**, as it provides stable levels and the body converts **T4** to **T3** as needed.

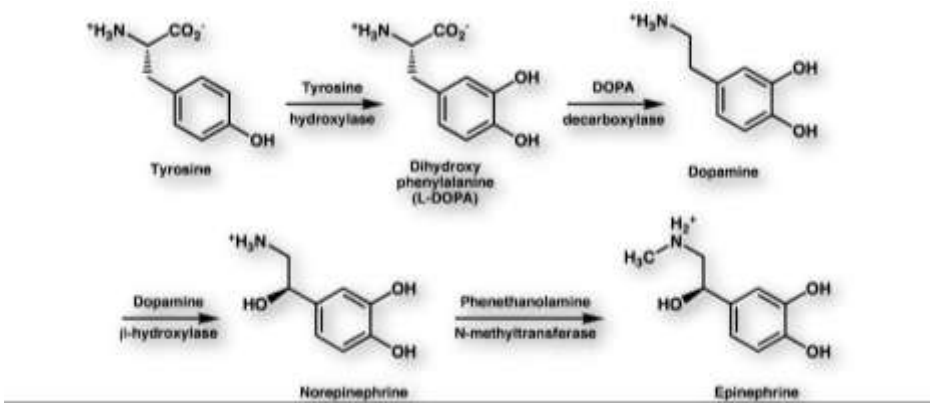
The primary route of thyroid hormone metabolism is diiodination, removing iodine atoms from the thyrinine ring. D1 and D2 convert **T4** to **T3**, activation, while D3 converts **T4** to reverse **T3**, R**T3**, inactive, and inactivates **T3**. In illness, sepsis, myocardial infarction, starvation, D1 and D2 activity is reduced while D3 activity is increased, leading to decreased **T3** and increased R**T3**, the euthyroid 6 syndrome.

Thyroid hormones can also undergo deamination, decarboxylation, and conjugation with glucuronic acid or sulfate in the liver, with conjugates excreted in bile and urine. Drug interactions are clinically important, propylthiouracil, **PTU**, inhibits **TPO** and D1, making it effective in hyperthyroidism. Glucocorticoids and beta-blockers, e.g., propranolol, inhibit D1, reducing **T4** to **T3** conversion, and are used as adjunctive therapy in hyperthyroidism.

Levothyroxine is preferred over lyothyronine, **T3**, for hypothyroidism because of its longer half-life and the body's ability to regulate conversion as needed.

Slides 13-16 | Catecholamine Synthesis and Breakdown

This slide serves as a transition to hormones that have plasma membrane receptors. Catecholamines are derived from tyrosine, from phenylalanine hydroxylation or dietary intake. Synthesis occurs in the adrenal medulla, **epinephrine** and **norepinephrine**, and sympathetic nerve terminals, **norepinephrine**. The first and **rate-limiting step** is conversion of tyrosine to **DOPA** by **tyrosine hydroxylase**, TH, which requires oxygen, iron, and tetrahydrobiopterin, BH4.



TH is inhibited by its end products, **norepinephrine** and **epinephrine**, via feedback inhibition. **DOPA** is decarboxylated to **dopamine** by **DOPA** decarboxylase. **Dopamine** is transported into secretory vesicles where **dopamine** beta-hydroxylase, DDH, converts it to **norepinephrine**. In the adrenal medulla, **norepinephrine** is converted to **epinephrine** by phenylethanolamine N-methyltransferase, **PNMT**, which requires **cortisol** as an inducer.

Clinically, alpha-methyltyrosine, metarosine, inhibits TH and is used to treat pheochromocytoma. Understanding this pathway is essential for diagnosing catecholamine -secreting tumors and interpreting measurements of catecholamines and their metabolites. A figure visually depicts the sequential steps of catecholamine synthesis, starting with tyrosine and showing conversion to DOPA via TH, dopamine, via DOPA decarboxylase, norepinephrine, via DBH, and epinephrine, via PNMT.

Tyrosine hydroxylase is the **rate-limiting step**, located in the cytoplasm, while **dopamine** beta-hydroxylase is located within secretory vesicles. The adrenal medulla is the primary site for **epinephrine** synthesis, while nerve terminals are the primary site for **norepinephrine** synthesis. Cofactors, BH4 for TH, vitamin C for DBH, are required for each step.

This pathway is clinically important for interpreting catecholamine assays, measurement of plasma **metanephrines** is the most sensitive test for diag**nos**ing pheochromocytoma. Understanding the pathway also aids in understanding the pharmacological effects of drugs that interfere with catecholamine synthesis, alpha-methyl tyrosine, or metabolism **MAO** inhibitors. Catecholamines have a very short half-life, minutes, because they are rapidly metabolized by two main enzyme classes, monoamine oxidase, **MAO**, and catecholomethyltransferase, **COMT**.

MAO is a mitochondrial enzyme that oxidatively deaminates catecholamines to alveohides, which are then oxidized to carboxylic acids or reduced to alcohols. **COMT** is a cytoplasmic enzyme that methylates the catechol group, inactivating the molecule. The combined action produces the major urinary metabolites, vanillylmandelic acid, **VMA**, and **metanephrines**, normetanephrine and metanephrine.

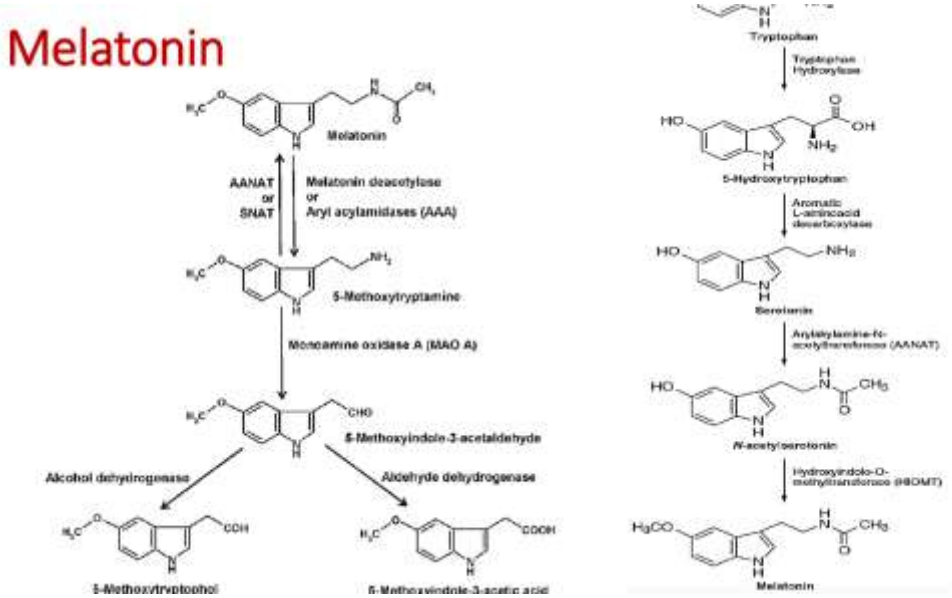
Plasma-free **metanephrines** are the preferred biomarkers for pheochromocytoma, with a sensitivity exceeding 95%, because they are continuously produced by intratumoral metabolism even when catecholamine secretion is **intermittent**. Understanding catecholamine metabolism is also important for the pharmacology of **MAO** inhibitors, used in depression, and **COMT** inhibitors, used in Parkinson's disease, which increase catecholamine levels by blocking degradation.

Slide 17 | Melatonin

Melatonin is synthesized from tryptophan, tryptophan 5-hydroxytryptophan, tryptophan hydroxylase, serotonin, aromatic L-amino acid decarboxylase, N-acetylserotinin, serotonin N-acetyltransferase, and N-melatonin, hydroxyindolomethyltransferase, HIEMT. The rate-limiting enzyme is NAT, activated by **norepinephrine** from sympathetic nerves innervating the pineal gland. Synthesis is regulated by the circadian clock and the suprachiasmatic nucleus, SCN. Light exposure inhibits the SCN signal to the pineal gland, reducing melatonin production.

At night, the SCN signals the pineal gland, increasing NAT activity and melatonin production. Thus, melatonin levels are low during the day and peak at night, 2-4 a.m. Melatonin acts on MT1 and MT2 receptors to regulate circadian rhythms, sleep, and seasonal reproduction. Clinically, melatonin is used as a supplement for jet lag, shift work sleep disorder, and insomnia, and is being investigated for neuroprotective and antioxidant effects.

Understanding melatonin physiology is important for counseling patients about sleep hygiene and the effects of shift work on circadian rhythms.



Slides 18-20 | Protein and Peptide Hormones & Precursor Polypeptides

This slide serves as a transition to hormones that have plasma membrane receptors. Protein and peptide hormones constitute the largest and most diverse class of hormones, ranging from small neuropeptides, TRH, a tripeptide, to large glycoproteins, TSH, a 200-plus amino acid heterodimer, and growth factors, IGF-1. Examples include CNS mediators, hypothalamic-releasing hormones, pituitary peptides, insulin, and glucagon, and growth factors.

To this list, we must add the incretin hormones, particularly glucagon-like peptide 1, **GLP-1**, and glucose-dependent insulinotropic polypeptide, GIP, produced by intestinal L-cells and K-cells. **GLP-1** is a 30 or 31 amino acid peptide secreted in response to food intake, enhancing glucose-dependent insulin secretion, suppressing glucagon, delaying gastric emptying, and promoting satiety.

GLP-1 acts through a G-protein-coupled receptor, **GLP-1**R, coupling to G-alpha-tis, activating adenylyl cyclase and increasing cAMP. Synthetic **GLP-1** receptor agonists, semaglutide, liraglutide, have become blockbuster drugs for diabetes and obesity, with substantial cardiovascular and renal protection. Peptide hormones are synthesized as larger precursors, pre -**prohormone**s, undergoing extensive post-translational processing.

Clinically, many peptide hormones are used as drugs, including insulin, EPO, GH, gonadotropins, and GLP-1 receptor agonists. Because peptide hormones are water-soluble, they are administered by injection or infusion, although oral semaglutide, rebelsis, is now available. The synthesis of peptide hormones begins with transcription of the gene encoding the hormone, producing a large precursor called a pre-pro-protein.

The pre-portion is a signal peptide, 15 -30 amino acids, at the interminus that directs the growing polypeptide to the endoplasmic reticulum, ER, recognized by the signal recognition particle, SRP. The signal peptide is cleaved by signal peptidase, producing the pro-protein, **prohormone**. The **prohormone** is transported to the Golgi, where it undergoes proteolytic cleavage by **prohormone** convertases to remove the propeptide and generate the mature hormone.

The mature hormone is stored in secretory vesicles until released by exocytosis in response to a stimulus. Classic examples include **proinsulin** to insulin and **POMC** to **ACTH** and **MSH**.

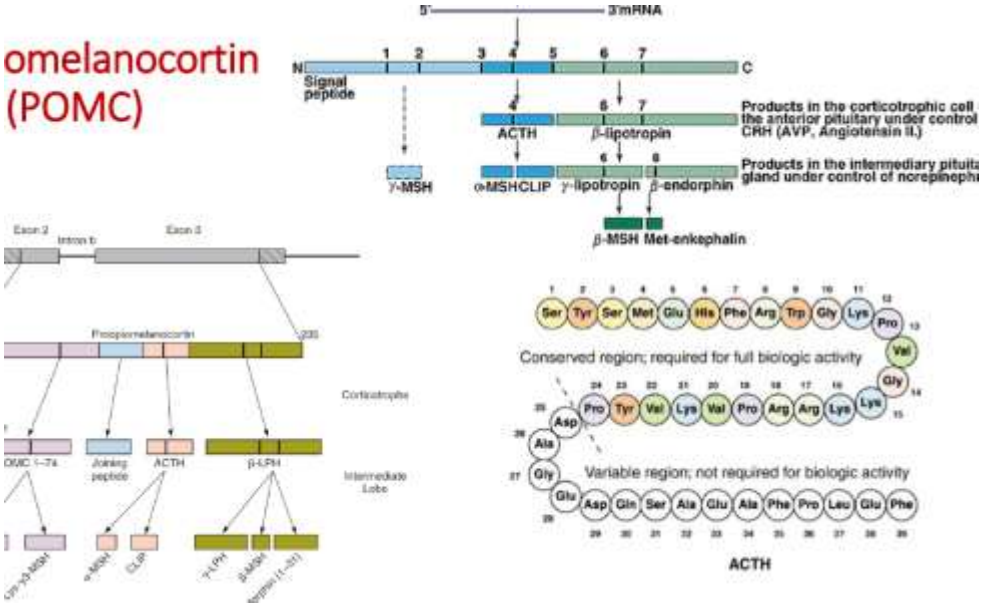
Slide 21 | Proopiomelanocortin (POMC)

Tissue-specific processing, as seen with **POMC** where different **prohormone** convertases are expressed in different tissues, leads to different hormone products. Mutations in **prohormone** convertases can cause endocrine disorders, and the secretion of precursors can serve as biomarkers, e.g., **proinsulin** and **C-peptide**. The **POMC** genome chromosome 2 contains three exons and is expressed primarily in the anterior pituitary, intermediate lobe, vestigial in humans, hypothalamus, and other tissues.

The **POMC** precursor protein is 241 amino acids and contains sequences for **ACTH**, β-lipotropin, γ -lipotropin, α-**MSH**, β-**MSH**, and β-endorphin. Processing is carried out by **prohormone** convertases PC1 -3 and PC2. In the anterior pituitary, PC1-3 cleaves **POMC** to **ACTH**, 1-39, and β-lipotropin, 1 -91. In the hypothalamus, PC2 further cleaves **ACTH** to α-**MSH** and CLIP, and β-lipotropin to β-endorphin and γ-lipotropin.

This differential processing results in different biological effects in different tissues. **POMC** is involved in the stress response, **ACTH**, pigmentation, and appetite regulation, α-**MSH**, and pain modulation, β-endorphin. Defects in **POMC** processing or signaling cause adrenal insufficiency, obesity, due to loss of anorexigenic α -**MSH** signaling, and hypopigmentation.

POMC has provided fundamental insights into **prohormone** processing and tissue-specific gene expression.

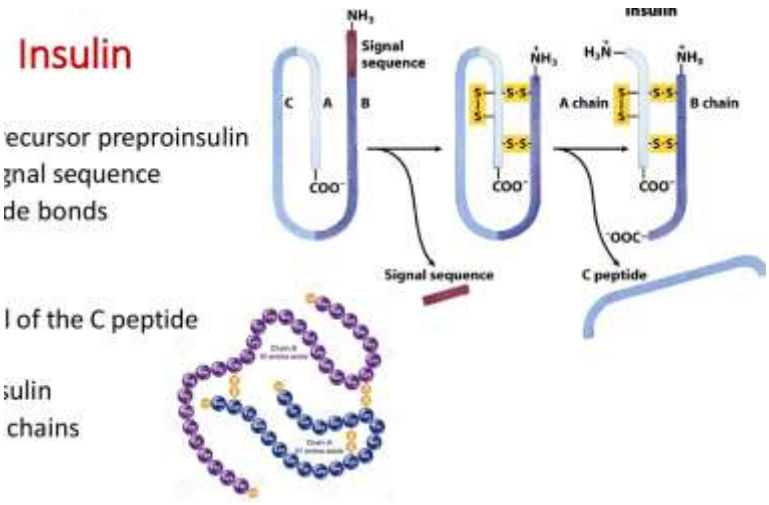


Slide 22 | Insulin Synthesis

The insulin gene on chromosome 11 encodes a 110-aminoacid precursor called **preproinsulin**, containing a 23 -aminoacid signal peptide, followed by the B-chain, 30-aminoacids, **C-peptide**, 35-aminoacids, and A -chain, 21-aminoacids. The signal peptide directs the nascent protein into the ER, where it is cleaved to **proinsulin**, 86-aminoacids. **Proinsulin** contains the B-chain, **C-peptide**, and A-chain with three disulfide bonds, two connecting the A and B-chains, a 7b7, a 20b19, and one intramolecular within the A-chain, a 6a11.

In the Golgi, **proinsulin** is transported to secretory granules, where **prohormone** convertases, PC1-3 and PC2, cleaved to remove **C-peptide**, and carboxypeptidase removes basic amino acids, yielding **mature insulin**, A-chain and B-chain linked by two disulfide bonds. **C-peptide** is co-secreted equimolarly. Clinically, **C-peptide** measurement assesses endogenous insulin secretion, very low or undetectable levels indicate beta-cell failure, type 1 diabetes, while normal or elevated levels indicate insulin resistance, type 2 diabetes.

C-peptide itself has biological activity and is being studied as a therapeutic agent for diabetic complications.



Slide 23 | Degradation of Peptide Hormones

Peptide hormones have short half-lives, minutes to hours, and are degraded by three primary mechanisms. First, lysosomal degradation after endocytosis of the hormone receptor complex, the receptor ligand complex is internalized and degraded in lysosomes, allowing receptor recycling or degradation, important for down-regulation and signal termination. Second, chemical modification in the liver, involving proteolytic cleavage, disulfide bond rearrangement, and other modifications that inactivate the hormone.

The liver is the primary site of degradation for most peptide hormones, insulin, glucagon, GH. Third, renal excretion, particularly for small peptides filtered by the glomerulus and degraded in the proximal tubule. In chronic kidney disease, peptide hormone clearance is impaired, leading to accumulation and prolonged effects, e .g., insulin accumulation and renal failure.

The short half-life explains why peptide hormones require continuous infusion or multiple daily injections, and why continuous glucose monitoring has transformed diabetes management.

Slides 24-25 | Target Cell Interactive Effects

Two types of hormone interactions are **permissive** and **integrative**. A **permissive** effect occurs when one hormone enhances the response to another without directly producing a response itself. Estrogen induces **progesterone** receptor synthesis in the uterus, making **progesterone**'s effects possible essential for the menstrual cycle. Thyroid hormone increases beta-adrenergic receptor number and activity in adipose tissue, smooth muscle, and heart.

Hypothyroid patients have reduced catecholamine responsiveness, while hyperthyroid patients have increased sympathetic symptoms, tachycardia, tremor, anxiety. An **integrative** effect occurs when two hormones produce complementary effects on different tissues for a common goal. PTH and calcitriol both increase extracellular calcium, PTH acts on bone and kidney, while calcitriol acts on the intestine, providing redundancy and robustness.

Clinically, treating one hormonal deficiency may require correcting the permissive hormone, thyroid hormone replacement may be necessary for epinephrine to be effective in treating anaphylaxis in hypothyroid patients. Synergistic effects occur when two hormones together produce a greater effect than the sum of their individual effects. FSH and estrogen are both required for oocyte development FSH stimulates granulosa cell proliferation, while estrogen promotes follicular maturation.

FSH and testosterone act together to promote spermatogenesis FSH stimulates Sertoli cells, while testosterone supports germ cell and Sertoli cell function. **Antagonistic** effects occur when hormones oppose each other's actions. The classic example is **insulin and glucagon**, insulin lowers blood glucose while glucagon raises it. Other examples include PTH and calcitonin, opposing calcium effects, and estrogen and testosterone, opposing effects on sexual differentiation.

Understanding synergy and antagonism is clinically important, in type 2 diabetes, insulin resistance, reduced insulin effect, combined with elevated glucagon leads to hyperglycemia. Therapeutic strategies aim to restore the balance, such as using GLP-1 receptor agonists to enhance insulin secretion and suppress glucagon secretion.

End of Lecture 3