

Lecture 28: Introduction to Endocrinology

Hormones

- Definition

- Classification

 - By origin

 - By site of action

 - By structure

- Mechanisms of action

 - Lipid soluble hormones (cytoplasmic receptors)

 - Lipid insoluble hormones (cell-surface receptors)

- Measurement

 - Immunoassay - RIA and ELISA

Endocrine systems

- Control of secretion

- Feedback

 - Long-loop, short-loop, short-short loop

Hormone-behavior relationships

- Organizational effects

- Activating effects

Hormone - a substance secreted by specialized cells, released in to the bloodstream, causing a response in target cells elsewhere in the body. Response is mediated by receptors that are specific to the hormone.

(Overhead: Figure 8-10 Eckert. Berthold's 1849 experiment with roosters, demonstrating distant effect of product from testes)

Classification

Hormones can be classified by several properties

1. Classification by site of action.

Secretions can be categorized by the site of action relative to the site of secretion.

(Overhead: Eckert Fig 8-1.)

Autocrine secretion - substance released by cell that affects the secreting cell itself (e.g. norepinephrine is released by a neurosecretory cell in the adrenal medulla, and norepinephrine itself inhibits further release by that cell - this is also an example of direct negative feedback)

Paracrine secretion - substance released by cell that affects neighboring cells. Not released into bloodstream
(e.g. histamine released at site of injury to constrict blood vessel walls and stop bleeding)

Endocrine secretion - substance released by cell into bloodstream that affects distant cells.
(e.g. testosterone is secreted by Leydig cells in testis, makes hair grow on your back)

Though hormones may also have autocrine or paracrine actions, we're mainly concerned with endocrine actions

Exocrine secretion - substance released by cell into a duct that leads to epithelial surface (onto skin or into gut). Action doesn't depend on receptors in target tissue.
(e.g. sweat, saliva, spider silk)

Endocrine and exocrine secretions are **glandular secretions**; they come from specialized secretory cells that are clumped together to form a **gland**. Endocrine glands are sometimes called ductless glands.

(Overhead: Eckert Fig 8.9)

Note that a secretion **may have several sites of action simultaneously**.
Example of norepinephrine above - **autocrine** action causes negative feedback on secretion. Simultaneously, **endocrine** action causes respiration rate to \uparrow , peripheral blood vessels to contract, etc.

2. Classification by origin. Another set of terms, related to those just discussed, is commonly used to classify secretions, based both on **origin** and site of action

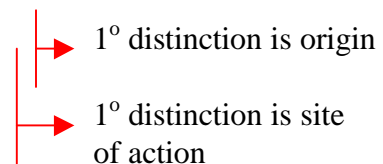
(Overhead: Table 9-1 Eckert)

Neurohormones - endocrine, source = nerve

Glandular hormones - endocrine, source = gland

Local Hormones - paracrine (source may not be gland)

Pheromones - exocrine



(Overhead: Fig 8.11: glandular hormone example - adrenal cortex)

(Overhead: Fig. 9.3, Eckert: neurohormone as trophic hormone, e.g. posterior pituitary)

(Overhead: Fig 8-15 Eckert: neurohormone example - adrenal medulla)

Our focus will be on glandular hormones with endocrine action.

3. *Classification by molecular structure.*

(Overhead: Fig 9.1 Eckert)

Amines- small molecules derived from amino acids. The **catecholamines** are epinephrine and norepinephrine, secreted by the adrenal medulla. Lipid insoluble

(Overhead: Fig 8-14 Eckert - synthetic pathway for catecholamines from AA phenylalanine)

Prostaglandins - fatty acids. Lipid soluble.

Steroids - cyclic hydrocarbons derived from cholesterol. Lipid soluble

(Overhead: Fig 9.23 Eckert - illustrates the small structural Δ between two androgens and between two estrogens. Remove the -OH group from C-17 on cortisol = corticosterone.)

Peptides and proteins - large, complex, structure can differ among species due to amino acid substitutions. Lipid insoluble.

(Overhead = Fig 8.10 Turner & Bagnara. Calcitonin from pig, human cow and salmon showing AA substitutions)

Focus in this class will be on peptides and steroids.

Peptides from the hypothalamus and anterior pituitary

Steroids from 'somatic' glands - testes, ovaries, adrenal cortex.

Effects of hormones: mechanisms of hormone action on target tissue

What does a hormone do when it arrives somewhere via the blood?

1. First level controlling action: **target/non-target**.

Hormones have their effects only on **target tissues**. Cells in target tissue have receptors, molecules that bind specifically to that hormone. In non-target tissue, cells have no receptors and are not affected by the hormone no matter how much of it is present.

2. Second level controlling action: **receptor density**.

Target cells vary in the number of receptors they hold. A cell with many receptors is more strongly affected than one with few receptors, when exposed to the same amount of hormone.

Receptor density for a given tissue changes through time. **Down-regulation** is a decrease in receptor density. **Up-regulation** is an increase in receptor density.

Down and up-regulation commonly respond to the amount of hormone in circulation over the long-term, as a damping mechanism. If blood concentration of cortisol (a stress hormone from the cortex of the adrenal gland) is high for a long time, receptor densities will decrease to modulate the response.

3. Third level of control: *cellular mechanisms* of action.

Once they arrive at a target cell, peptide and steroid hormones have different mechanisms of action. The difference is based on fat-solubility, which determines whether or not the hormone can penetrate the target cell's plasma membrane (which is a lipid bilayer).

Peptides are not fat soluble, so they bind to receptors on the target cell's surface, and **act via a 'second messenger'** in the cell. Second messenger is cyclic adenosine monophosphate. CAMP activates an enzyme (a protein kinase), which activates other proteins that produce the final 'effect' once secreted from the cell. Peptides don't directly alter gene expression, so the effects are generally short-term.

Steroids are fat soluble, so they bind to receptors in the cytoplasm, either in the cytosol or directly in the nucleus. When the steroid binds to the receptor, **it disinhibits a DNA binding site** on the receptor. The steroid-receptor complex then moves into the nucleus and activates or suppresses specific genes. By altering gene expression, steroids produce long-lasting effects.

(Overhead: Fig. 9.8 Eckert general mechanism for fat soluble and insoluble)

(Overhead: Fig. 2.4 Turner & Bagnara- more detail, *especially for peptides*)

(Overhead: Fig. 9.9 Eckert - more detail, *for steroids*)

Δ 's in cellular mechanism of action cause other differences between peptides vs. steroids

(Overhead: Table 9.5 Eckert - binding proteins in blood, time course of effect)

Measuring hormone concentrations

How do you know what level of a given hormone is circulating in an individual's blood?

The old method was **bioassay**. Expose a target tissue to a fixed amount of the individual's blood and measure the response of target tissue. E.g. rat prostate glands exposed to testosterone grow larger. Bioassay for testosterone was to size of prostate in castrated rats injected with blood from different individuals. Problems with standardization, therefore imprecise.

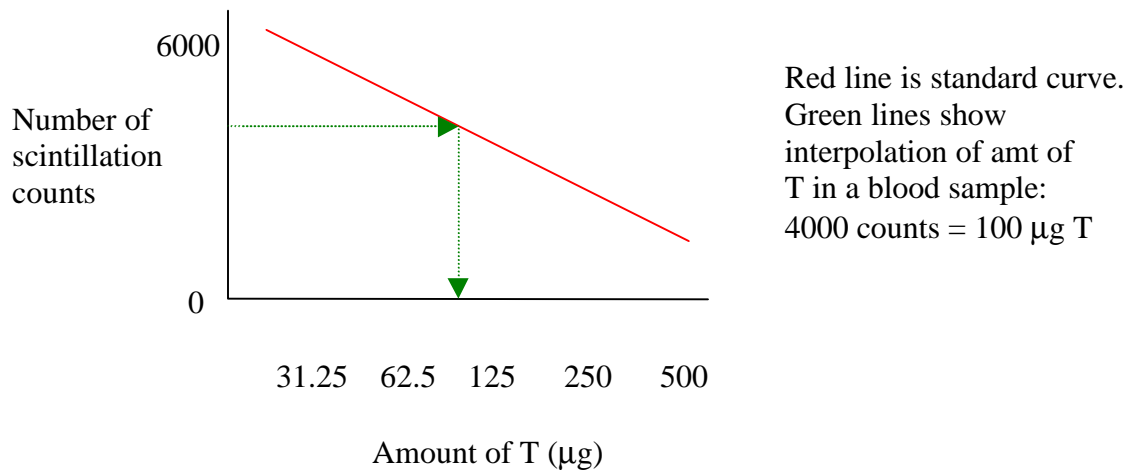
Now use immunoassay; either radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). The basic method is the same - illustrate with RIA, then explain Δ with ELISA. Rosalyn Yalow and Seymour Berson won a Nobel prize for the development of RIA).

Suppose you want to measure testosterone (T) concentration in blood. Requires three things.

1. synthetic or purified testosterone = 'top standard'
2. synthetic or purified testosterone with radioactive label = 'tracer' or 'hot-T'
3. antibody that binds to testosterone and nothing else (Antibodies are proteins produced by the immune system that bind to and eliminate foreign ['non-self'] molecules that have invaded the body)

Steps in RIA

1. Centrifuge the blood and collect the plasma. If you are assaying hormone levels in urine or feces, additional extraction steps to remove stuff that interferes with binding of hormone to antibody.
2. Put a fixed amount of antibody into each tube. Each tube now has the same number of binding sites for testosterone molecules.
3. Put a fixed amount of hot-T into each tube. If there was no other testosterone in the tube, then all of the binding sites will be occupied by hot-T.
4. Make a standard curve.
 - Suppose the top standard is a solution of 500 μg of T /ml of solution.
 - Make a dilution series so you have solutions that are 500 $\mu\text{g}/\text{ml}$, 250 $\mu\text{g}/\text{ml}$, 125 $\mu\text{g}/\text{ml}$, 62.5 $\mu\text{g}/\text{ml}$, 31.25 $\mu\text{g}/\text{ml}$ (a series of 2x dilutions).
 - Add 1 ml of the top standard to one tube, 1 ml of standard 2 to the next tube, etc.
 - Wait a while, so that the hot-T and unlabelled T can compete for binding sites and reach equilibrium.
 - In the tube with top standard, there are 500 μg of unlabelled T competing with the hot-T for binding sites. In the tube with standard 2, there are 250 μg of unlabelled T competing with the hot-T for binding sites. The greater the amount of unlabelled testosterone, the less hot-T will be bound to antibody.
 - Remove all of the T (hot and unlabelled) that is not bound to antibody binding sites. This is done by adding dextran coated charcoal (which grabs the 'free' hormone but leaves 'bound' hormone behind), centrifuging (to pellet the charcoal), and pouring off the liquid (which holds the antibodies and bound T).
 - Add scintillation fluid to the liquid. Scintillation fluid emits a photon when struck by a beta or gamma particle from radioactive decay. A scintillation counter just counts the number of photons emitted from a sample in a fixed period of time.
 - High scintillation count = lots of hot-T in tube = little unlabelled T in tube.
Low scintillation count = little hot-T in tube = lots of unlabelled T in tube.
The amount of unlabelled T in each standard tube is known
Plot scintillation counts vs. amount of T standard. This is the standard curve.
5. Compare blood samples to standard curve.
 - Can use the standard curve to interpolate how much T is in 1 ml of plasma. To tubes with antibody and tracer, add 1 ml of *plasma* instead of 1 ml of T-standard solution. Equilibrate, strip the free hormone, scintillation count. Plot the counts on the Y-axis of the standard curve, and determine amount of T on X-axis.



(Overhead: Fig 6.3 Nalbandov)

The difference between RIA and ELISA: RIA measures scintillation due to decay of radioactive tracer. ELISA measures a color change due to an enzyme tracer. The tracer antigen (hormone) is labeled with an enzyme that catalyzes a color change reaction. Instead of scintillation fluid, substrate for the enzyme is added, and a spectrophotometer is used to read absorbance of light at specific wavelength to quantify how much of substrate has been converted to new compound. More tracer → bigger shift in absorbance. Use standard curve same way as in RIA.

Specificity of immunoassays - monoclonal antibodies

Antibodies for assays used to be raised by injecting a rabbit with the hormone (antigen), collecting blood, isolating antibodies from the blood. Problem with this is that it yields **polyclonal antibodies** - a mixture of antibodies that recognize parts of same antigen molecule. Different antibodies within polyclonal mixture will cross-react (bind) to molecules other than the original antigen, with variable affinity, reducing the **specificity** of the assay.

(Recall that structures of different hormones can be very similar, e.g. cortisol and corticosterone differ only by one hydroxyl group - an OH rather than an H on one carbon)

(Overhead: Eckert Fig 2.3)

Identical or **monoclonal antibodies** are desirable, to reduce cross-reactivity with molecules other than the one you're interested in measuring. Not practical to sort out monoclonal antibodies from the polyclonal mix in rabbit blood - too few of each type. Kohler & Milstein won Nobel prize for discovery that a β lymphocyte (which produces

antibodies) can be fused with a cancerous myeloma cell (which is self-perpetuating in culture) to form a hybridoma. The hybridoma is self-perpetuating, and a culture of hybridomas produces large amounts of monoclonal antibody.

Endocrine systems.

Up to now have just described hormones themselves, nothing about the ways they are integrated into systems that regulate physiology and behavior.

Initiation of an endocrine response.

Hormones generally secreted at some (non-zero) resting rate or ***baseline***. Secretion regulated up or down by some signal.

(Overhead: Fig. 9.3 Eckert)

A chain of endocrine responses is usually initiated by ***neurohormone***. Nerve cells are stimulated by neural activity, release a neurohormone that then alters secretion of second hormone. Neurohormones ***transduce a neural signal into an endocrine signal***.

Modulation of an endocrine response.

Sets of endocrine glands are usually organized into hierarchical loops that allow ***feedback*** to regulate responses.

(Overhead: Fig. 9.2 Eckert)

1. ***Feedforward*** circuit or open loop.
2. ***Feedback*** or closed loops

Short loop - hormone A affects secretion of hormone B, and hormone B affects secretion of A. No intervening steps.

Long loop - hormone A affects secretion of B, hormone B affects secretion of C, and hormone C affects secretion of A. Intermediate steps.

Feedback is usually ***negative***, so that endocrine response is self-limiting; secretion modulates itself and does not 'run away'.

Feedback is sometimes ***positive***, when a quick, large response is necessary. When a system shows positive feedback, it will run away (like a microphone held near an amplifier) unless something changes to stop the positive feedback.

(Overhead: Fig. 1.9 Becker et al.)

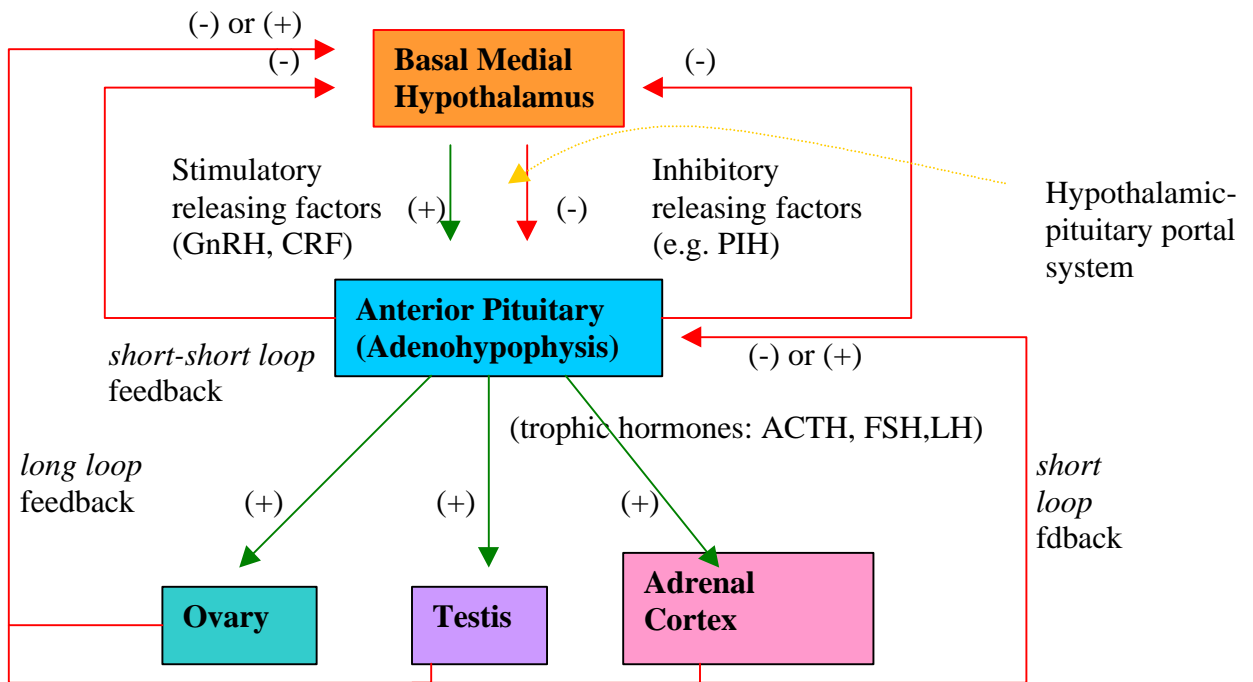
(Overhead: Fig. 2.1 Turner & Bagnara)

(Overhead: Fig. 2.2 Turner & Bagnara)

This class will focus on:

1. stress-response by adrenal cortex (adrenal corticosteroids = glucocorticoids)
2. female reproduction and ovarian hormones (estrogens and progesterone)
3. male reproduction and testicular hormones (androgens)

Overheads illustrate the glands (and their locations) in the feedback loops that control these three systems.



Organizational and activating effects

Organizational effects are permanent changes due to hormones that occur during development.

(Overhead: Breedlove, Fig 2.1)

Example of organizational effect - sexual differentiation of reproductive organs.

Initially, reproductive tract is composed of 'indifferent gonads' connected to body wall by Mullerian and Wolffian ducts.

A gene on the Y chromosome produces TDF - testis determining factor.

TDF present → indifferent gonad becomes testis

TDF absent → indifferent gonad becomes ovary

If gonad become testis, it secretes testosterone and Mullerian regression factor which in turn cause the ducts and external genitalia to develop male. Absence of T and MRF → female ducts and genitalia.

Organizational effects can be *behavioral* as well as physical.

Example. In guinea pigs, early exposure to T causes a female to exhibit little or no lordosis as an adult. (Lordosis is a reflexive, arched-back posture that female rodents show during mating.)

Absence of T → female brain

Presence of T → male brain

(Overhead: Fig. 2.3 Breedlove)

This is true of any sexually dimorphic behavior, not just mating behavior. Good example is song-circuit of song-bird brains.

Paradoxical effect of estrogens:

(Overhead: Breedlove Fig. 2.4 - rat SDN-POA experiments)

Experiments show that injecting *either testosterone or estrogen* has masculinizing effect on brain. It is the ***absence of androgens, not the presence of estrogen***, that produces female development of brain and body.

(Overhead: Breedlove Fig 2.2)

Estrogen (estradiol 17 β) and testosterone have very similar molecular structure. Convert T to estrogen by aromatization (converts the A ring to a benzene-like structure that is literally aromatic).

The masculinizing effects of circulating testosterone are, in fact, produced by estradiol. T is converted within the cell from T to estradiol 17 β , and the E₂-17 β causes the masculinization.

1. Absence or presence of circulating T determines female or male organizational effects.
2. Immature ovary produces very little estrogen, so it does not have masculinizing effect.
3. But all fetuses are exposed to estrogens from mother - so how do female fetuses avoid masculinization? In rodents, at least α -fetoprotein is produced by female fetuses, and this simply binds to the mother's estrogen and keeps it from affecting the fetus.
4. Injecting estrogen overwhelms the α -fetoprotein, and leads to masculinization.
5. Testosterone produced by male fetuses is not bound by α -fetoprotein.

Activating effects are short-term, reversible changes produced by hormones. Can be physiological (e.g. secretion of LH causes ovulation) or behavioral (e.g. secretion of testosterone increases aggression in male birds).