Campbell Reproductive Biology Site
Endocrinology Lecture Illustrations

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2. Evolution of Basic Digestive Physiology and Endocrinology
3. Diabetes: Basics and Drugs
4. Introduction to Endocrinology
5. Overview of Male Endocrinology
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8. Opportunities and Active Research Areas in Endocrinology
9. Training in Endocrinology & Other Biomedical Sciences
10. Thyroid Physiology
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Endocrinology =
Intercellular Chemical Communication

**This is a course about communication systems and information transfer.
It covers the chemistry, biochemistry, molecular biology, genetics, cell biology, cell physiology, organismal physiology, whole animal biology, and behavior associated with chemical communication.**

Endocrine Functions

- **Maintain Internal Homeostasis**
- **Support Cell Growth**
- **Coordinate Development**
- **Coordinate Reproduction**
- **Facilitate Responses to External Stimuli**

Elements of a Communication System

- **Sender**
- **Signal**
- **Nondestructive Medium**
- **Selective Receiver**
- **Transducer**
- **Amplifier**
- **Effector**
- **Response (2ndary signal)**

Elements of an Endocrine System

- **Sender = *Sending Cell***
- **Signal = *Hormone***
- **Nondestructive Medium = *Serum & Hormone Binders***
- **Selective Receiver = *Receptor Protein***
- **Transducer = *Transducer Proteins & 2ndary Messengers***
- **Amplifier = *Transducer/Effector Enzymes***
- **Effector = *Effector Proteins***
- **Response (2ndary signal) = *Cellular Response (2ndary hormones)***

Known Hormonal Classes

1. **Proteins and peptides**
2. **Lipids *(steroids, eicosanoids)***
3. **Amino acid derivatives *(thyronines, neurotransmitters)***
4. **Gases *(NO, CO)***

Hormone

A molecule that functions as a message within an organism; its only function is to convey information.

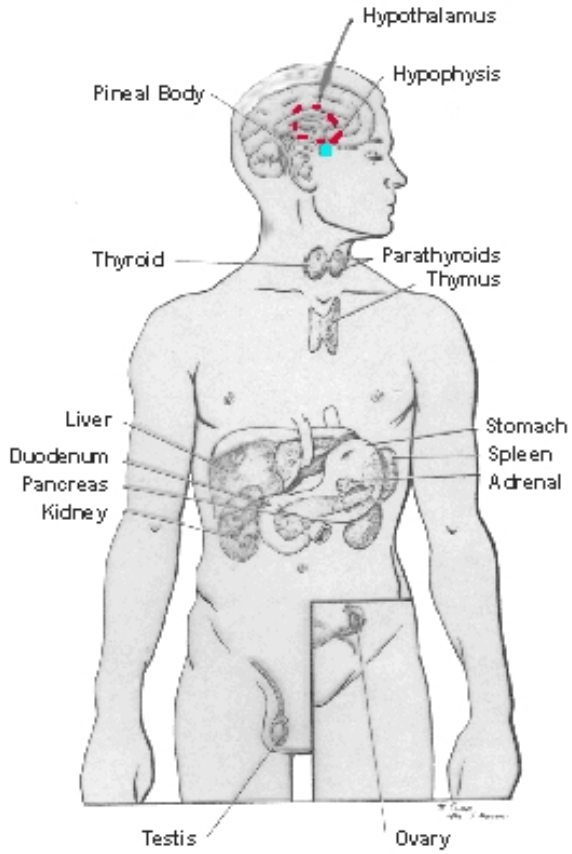
Because of this function, physical descriptions of a chemical suspected of being a hormone are inadequate to indicate the molecule's role in a biological system. A molecule is a hormone only when described in the context of its role in a biological communication system. Definition of a hormone requires testing of that molecule in a biological response system, running a bioassay.

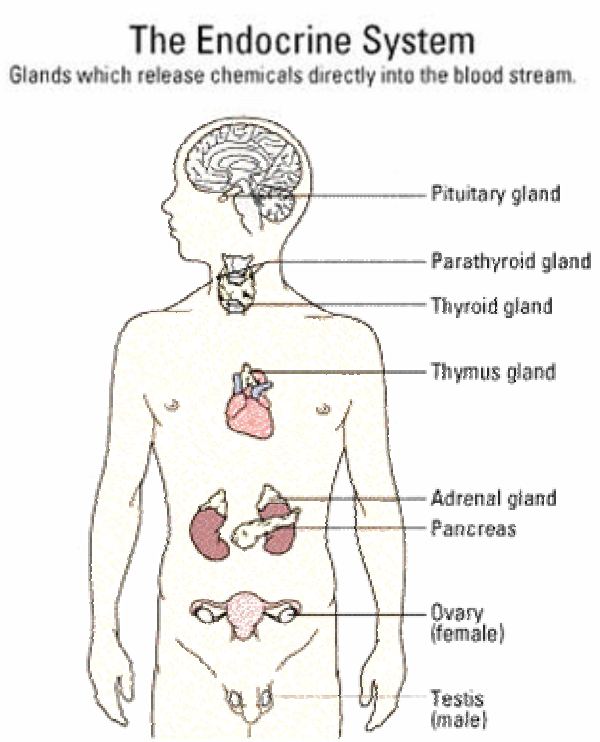
The existence of endocrinology is totally dependent on the existence and use of bioassays. (This is also true for pharmacology and toxicology.)

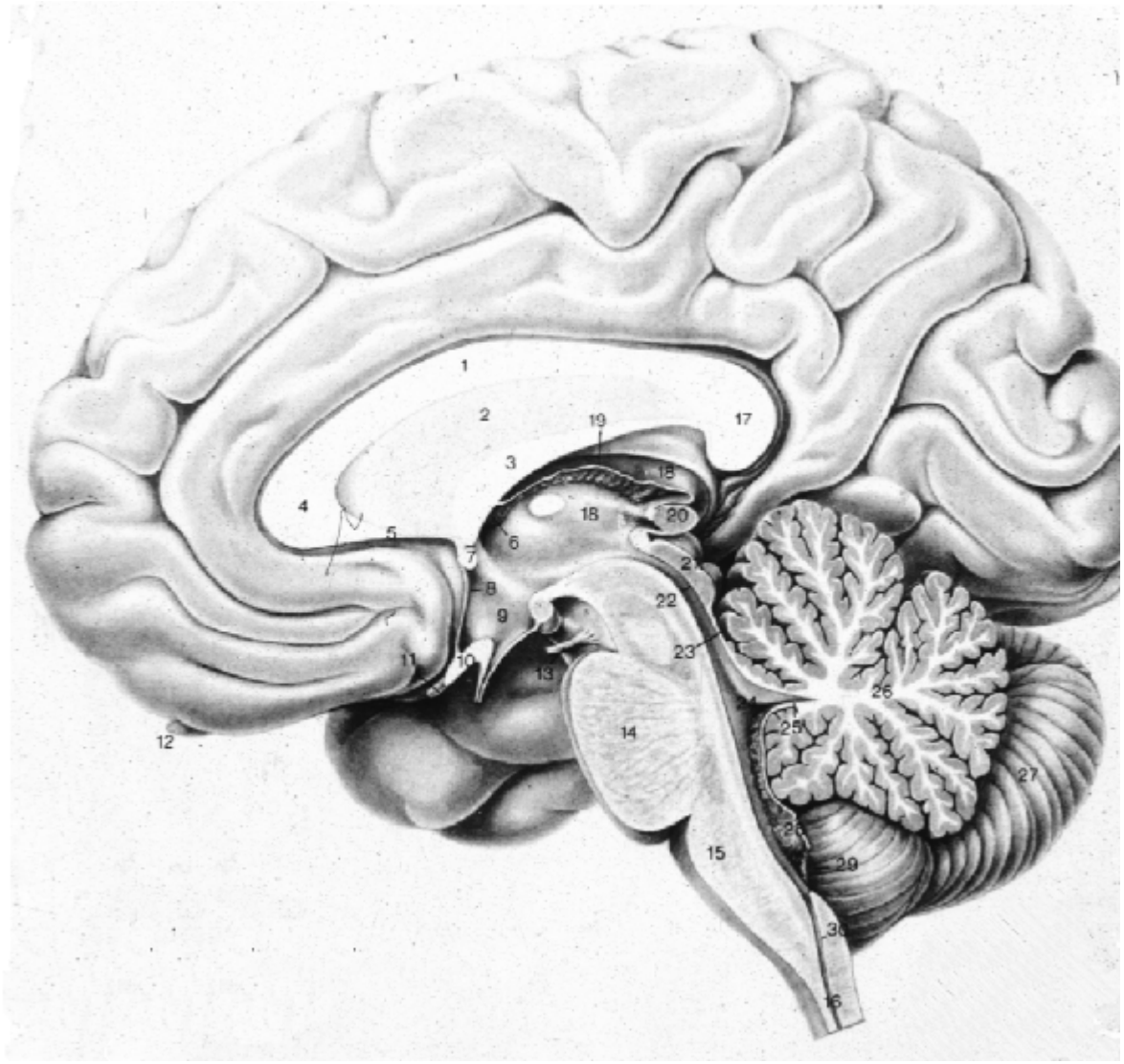
Forms of Intercellular Communication

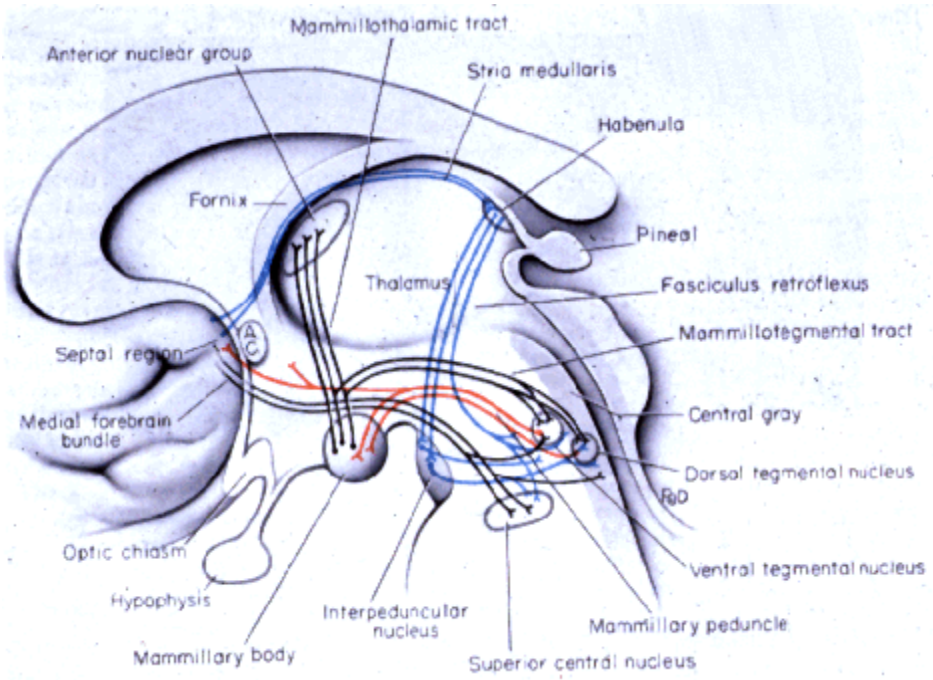
1. ***Endocrine*: secretion of a hormone by one cell with transmission via the blood, lymph, or intercellular fluid to a second, target, cell.**
2. ***Paracrine*: secretion of a hormone by one cell with transmission via intercellular fluid to a second, nearby cell.**
3. ***Autocrine*: secretion of a hormone by one cell with reception and response by the same cell.**
4. ***Pheromonal*: secretion by one organism and sensation and response by a second.**

Classical Endocrine Tissues

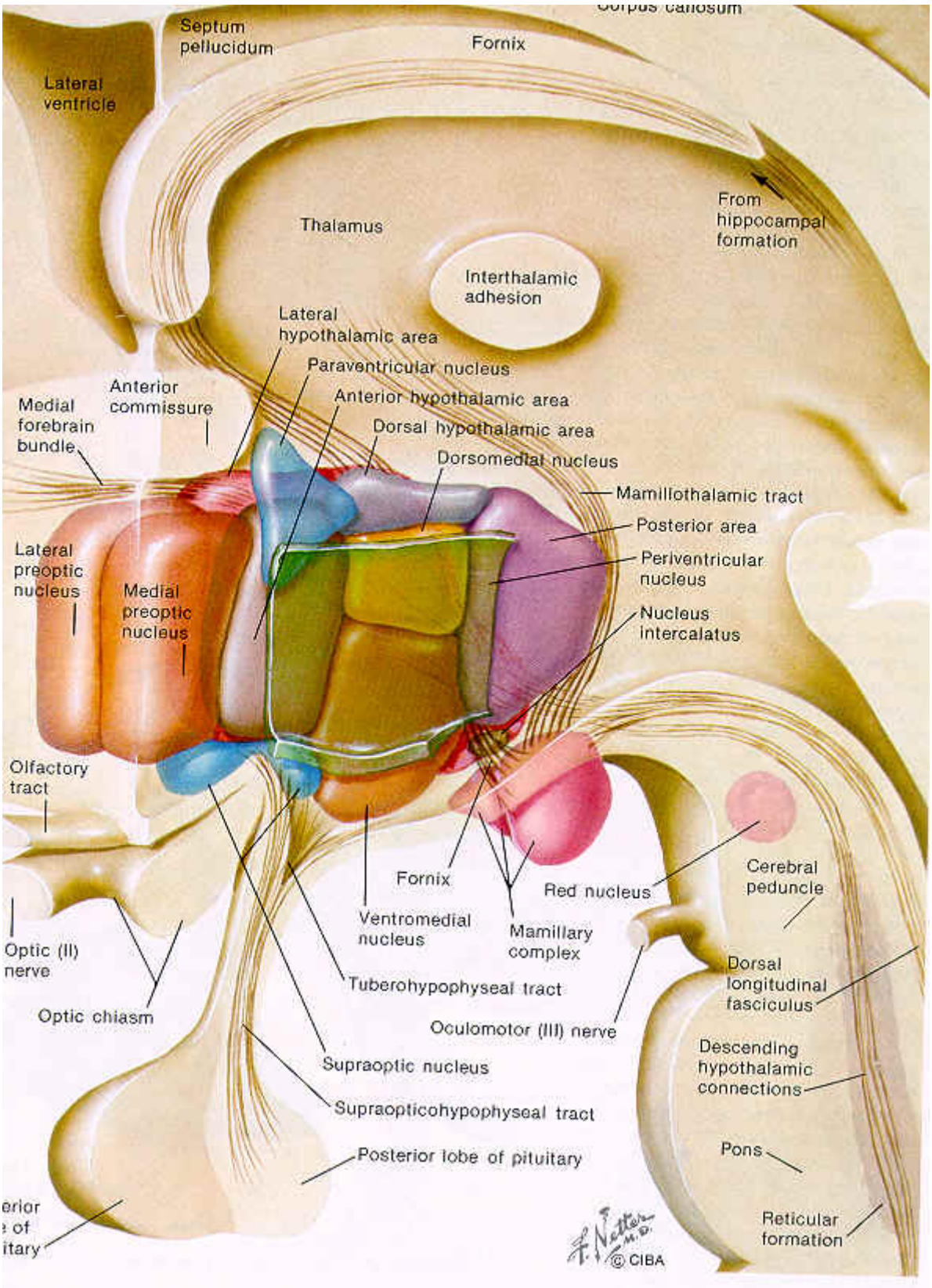


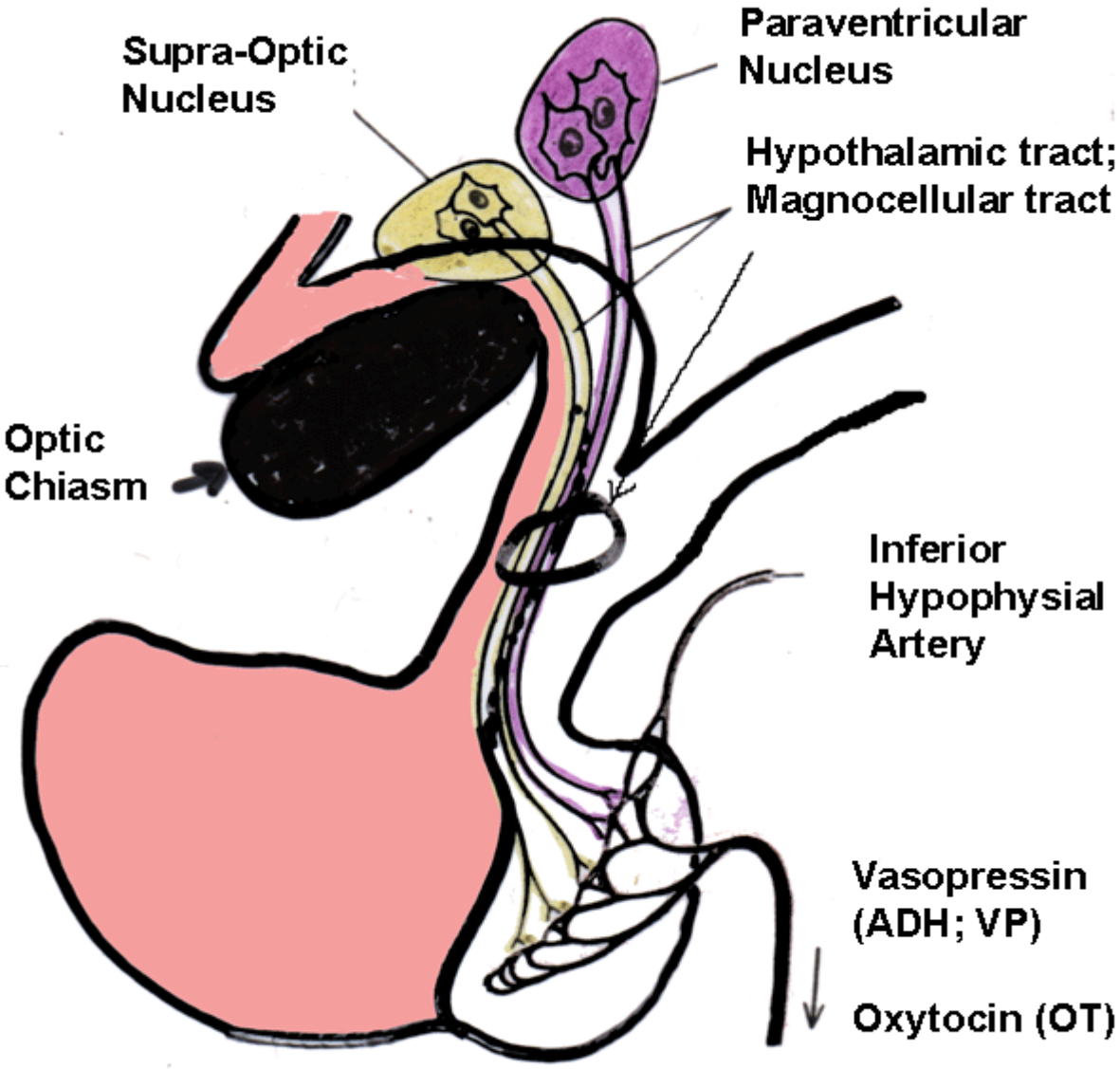






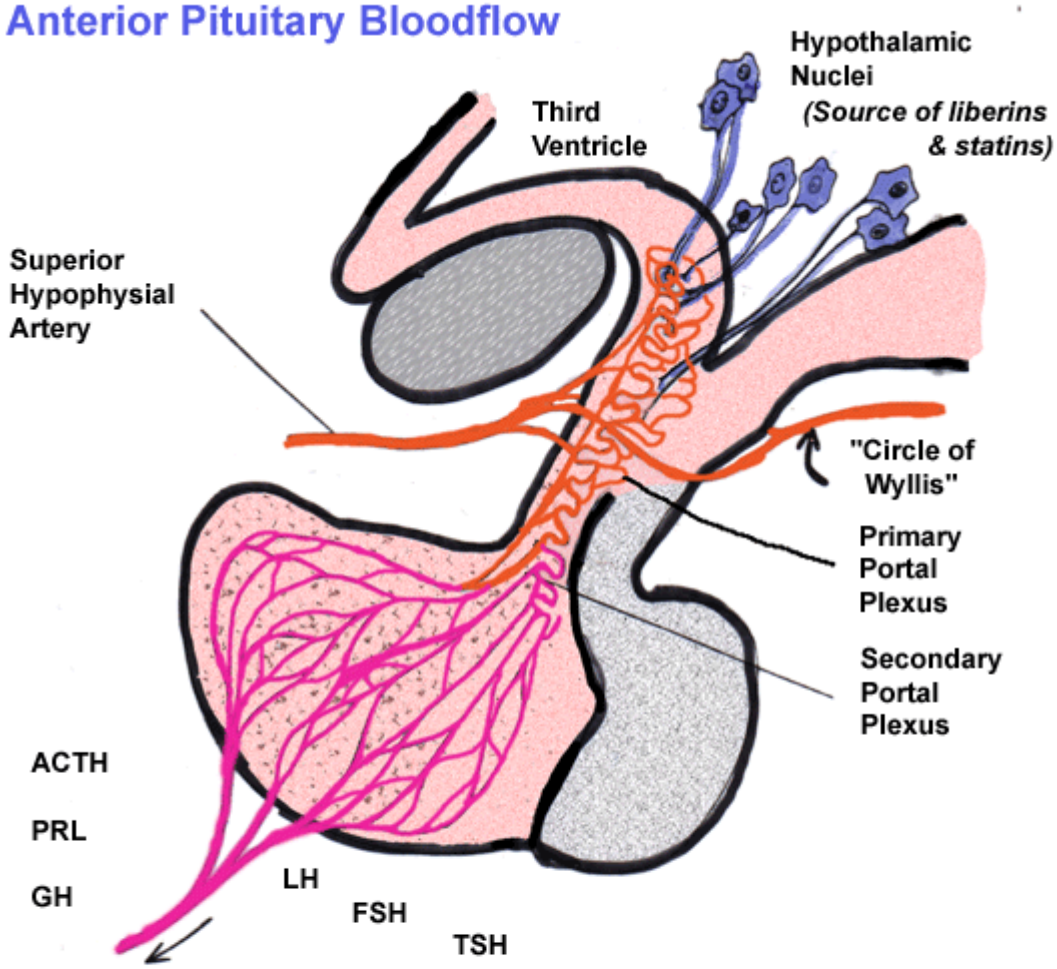
12) Hypothalamic Nuclei Function ~ http://cal.man.ac.uk/student_projects/2000/mnby6kas/function.htm
13) Hypothalamic Anatomy & Function ~ http://www.endotext.org/neuroendo/neuroendo3b/neuroendo3b.htm


Septum pellucidum
Fornix
Lateral ventricle
From hippocampal formation
Thalamus
Interthalamic adhesion
Lateral hypothalamic area
Paraventricular nucleus
Anterior commissure
Anterior hypothalamic area
Medial forebrain bundle
Dorsal hypothalamic area
Dorsomedial nucleus
Mamillothalamic tract
Posterior area
Lateral preoptic nucleus
Periventricular nucleus
Medial preoptic nucleus
Nucleus intercalatus
Olfactory tract
Fornix
Red nucleus
Cerebral peduncle
Ventromedial nucleus
Mamillary complex
Optic (II) nerve
Tuberohypophyseal tract
Dorsal longitudinal fasciculus
Optic chiasm
Oculomotor (III) nerve
Descending hypothalamic connections
Supraoptic nucleus
Supraopticohypophyseal tract
Pons
Posterior lobe of pituitary
Reticular formation
© CIBA

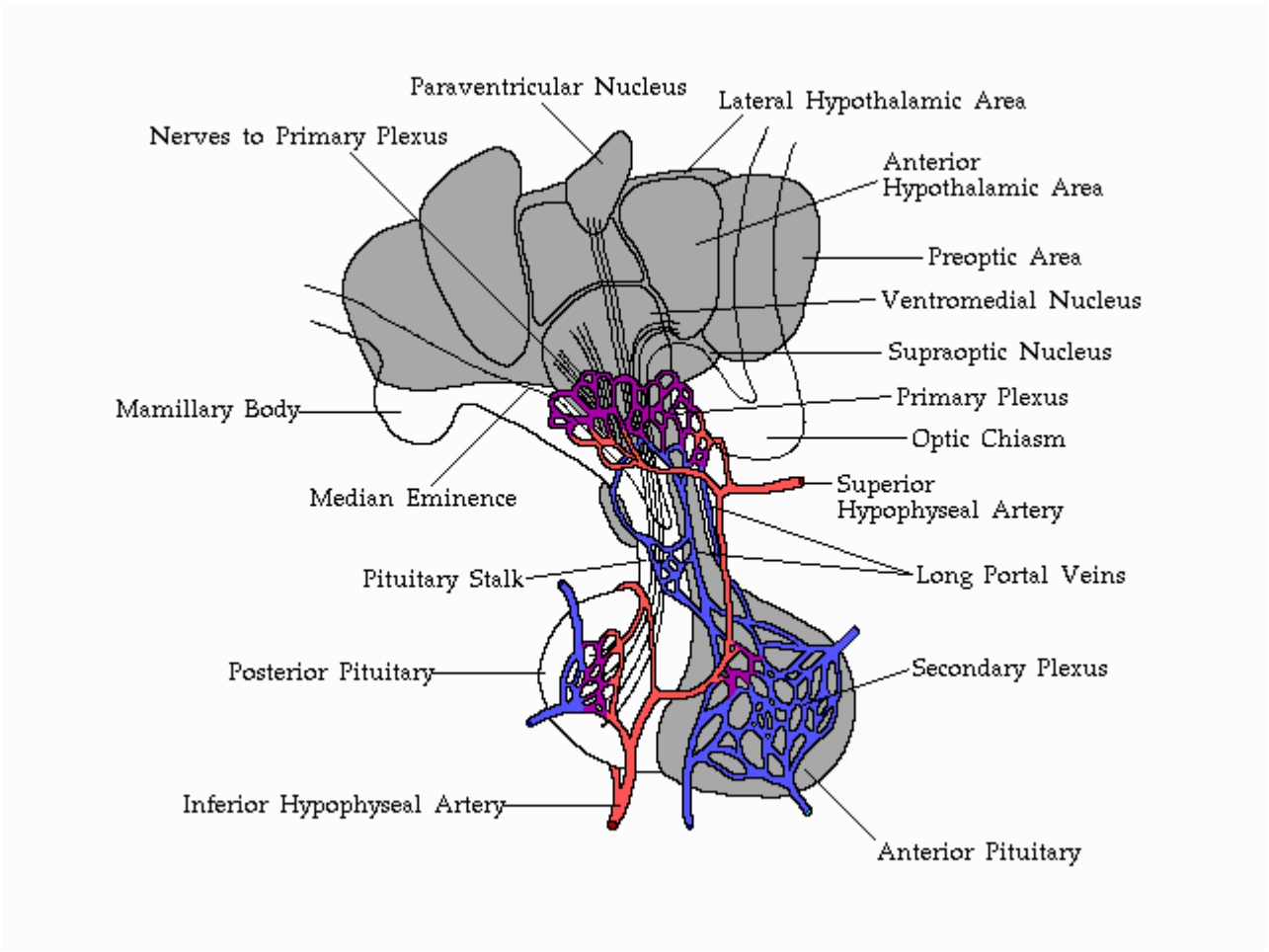


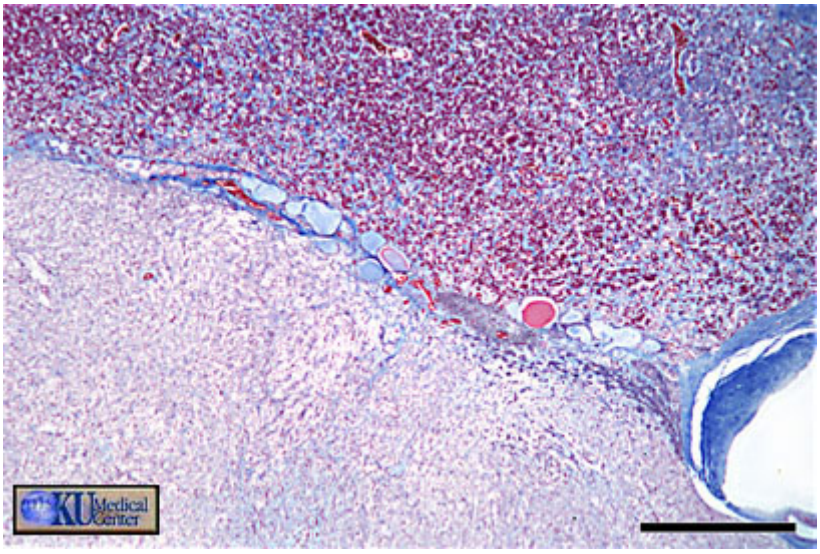
Neuroendocrine Products of the Neurohypophysis

Anterior Pituitary Bloodflow



The primary portal plexus, a "priviledged" or "leaky" portion of the brain vasculature, provides a port of entry for the neuroendocrine secretions of the cells of many hypothalamic nuclei. These are carried by pulsing capillary bloodflow to the cells of the adenohypophysis (anterior pituitary) where they bind and modulate the synthesis and secretion of the six anterior pituitary hormones. Note that the products of the anterior, posterior, and intermediate (where it exists) lobes may diffuse back to the hypothalamus during the nadir of capillary blood flow.


Paraventricular Nucleus
Lateral Hypothalamic Area
Nerves to Primary Plexus
Anterior Hypothalamic Area
Preoptic Area
Ventromedial Nucleus
Supraoptic Nucleus
Mamillary Body
Primary Plexus
Optic Chiasm
Median Eminence
Superior Hypophyseal Artery
Pituitary Stalk
Long Portal Veins
Posterior Pituitary
Secondary Plexus
Inferior Hypophyseal Artery
Anterior Pituitary


KU Medical Center

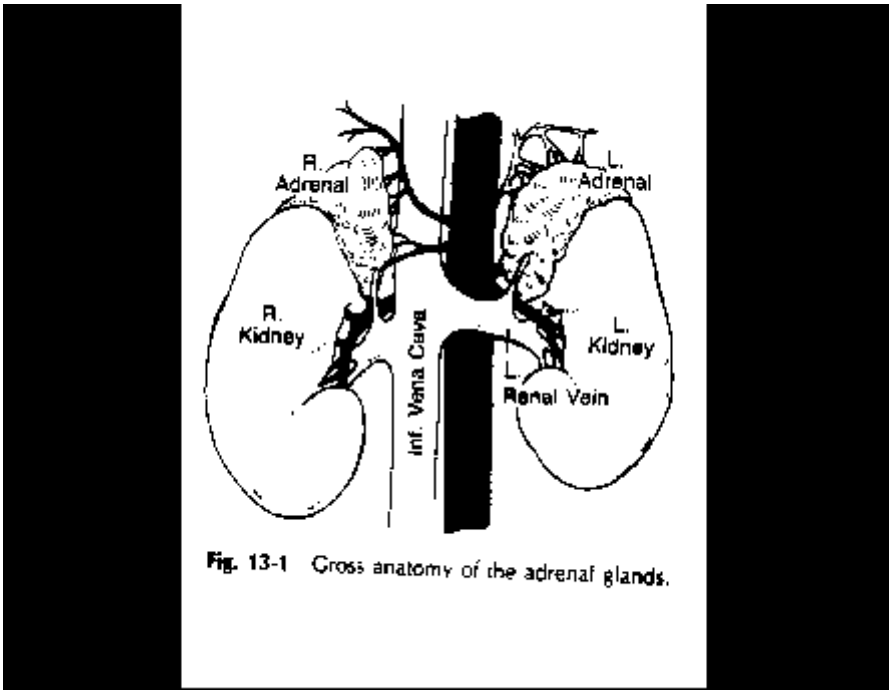
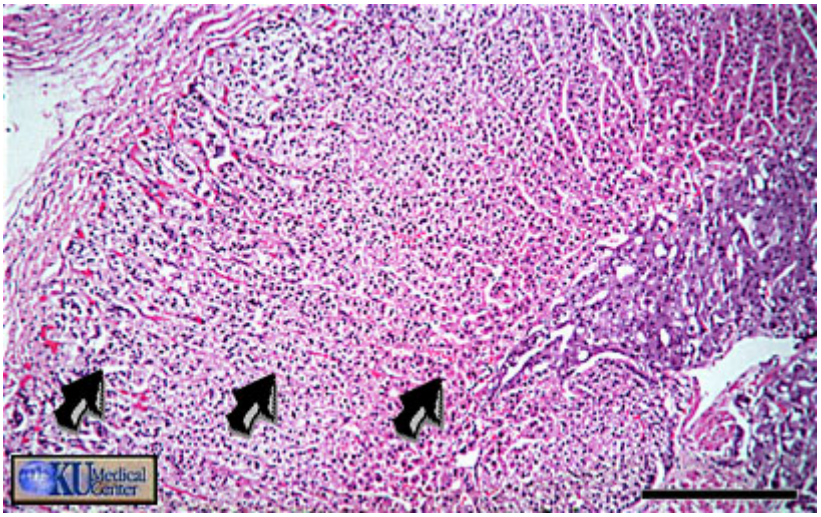


Fig. 13-1 Gross anatomy of the adrenal glands.



Information Content is Greatest When Levels of a Signal Fluctuate

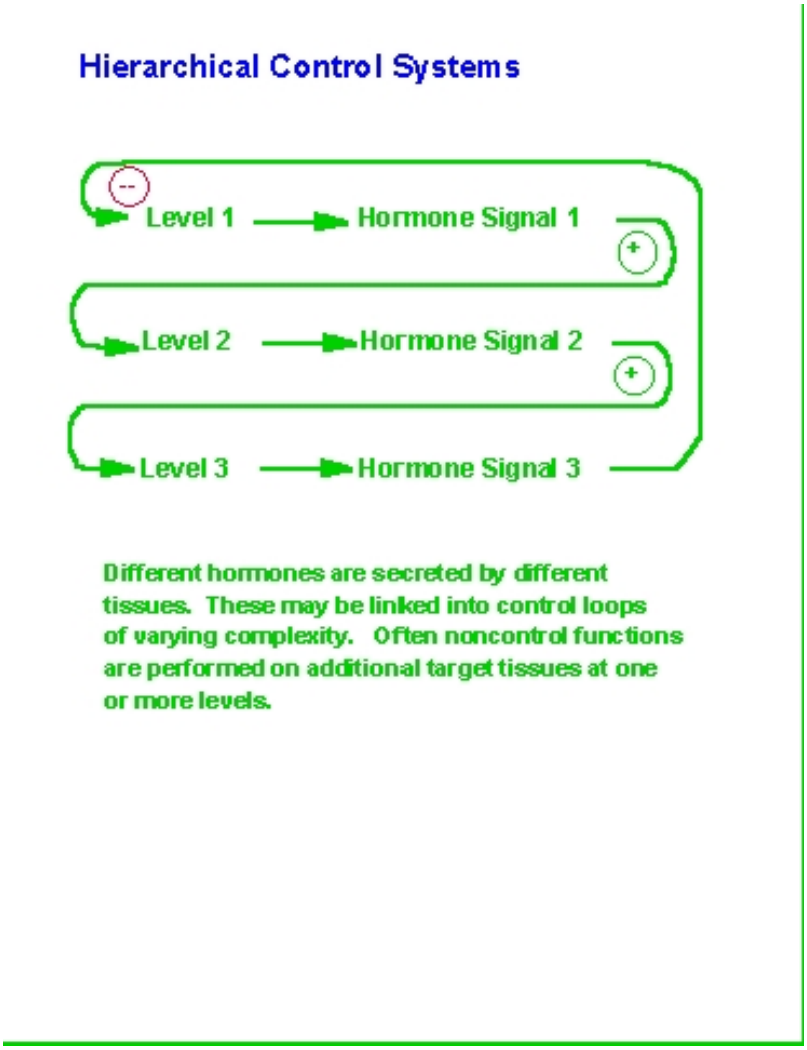
- **Fluctuations with distance provide cues to the position of the source, *e.g.*, developmental gradients.**
- **Fluctuations with time prevent saturation, *desensitization, down regulation*, habituation.**

Hormones Usually Released as Pulses

- **Pulses reflect the packaging of proteins, peptides, and neurotransmitters.**
- **Pulses reflect the coordinated actions of groups of releasing cells.**
- **Each pulse has an *amplitude* and *period.***
- **Groups of pulses have a *frequency*.**
- **Basal levels of hormone often reflect the spacing between pulses and its relation to hormonal clearance.**

Biologically Available Hormone Levels Can Vary Due To:

- **Changes in synthesis**
- **Changes in secretion**
- **Changes in clearance**
- **Changes in degradation**
- **Changes in binding proteins**
- **Age**
- **Gender**
- **Developmental stage**
- **Reproductive status**
- **Stage of temporal rhythm**



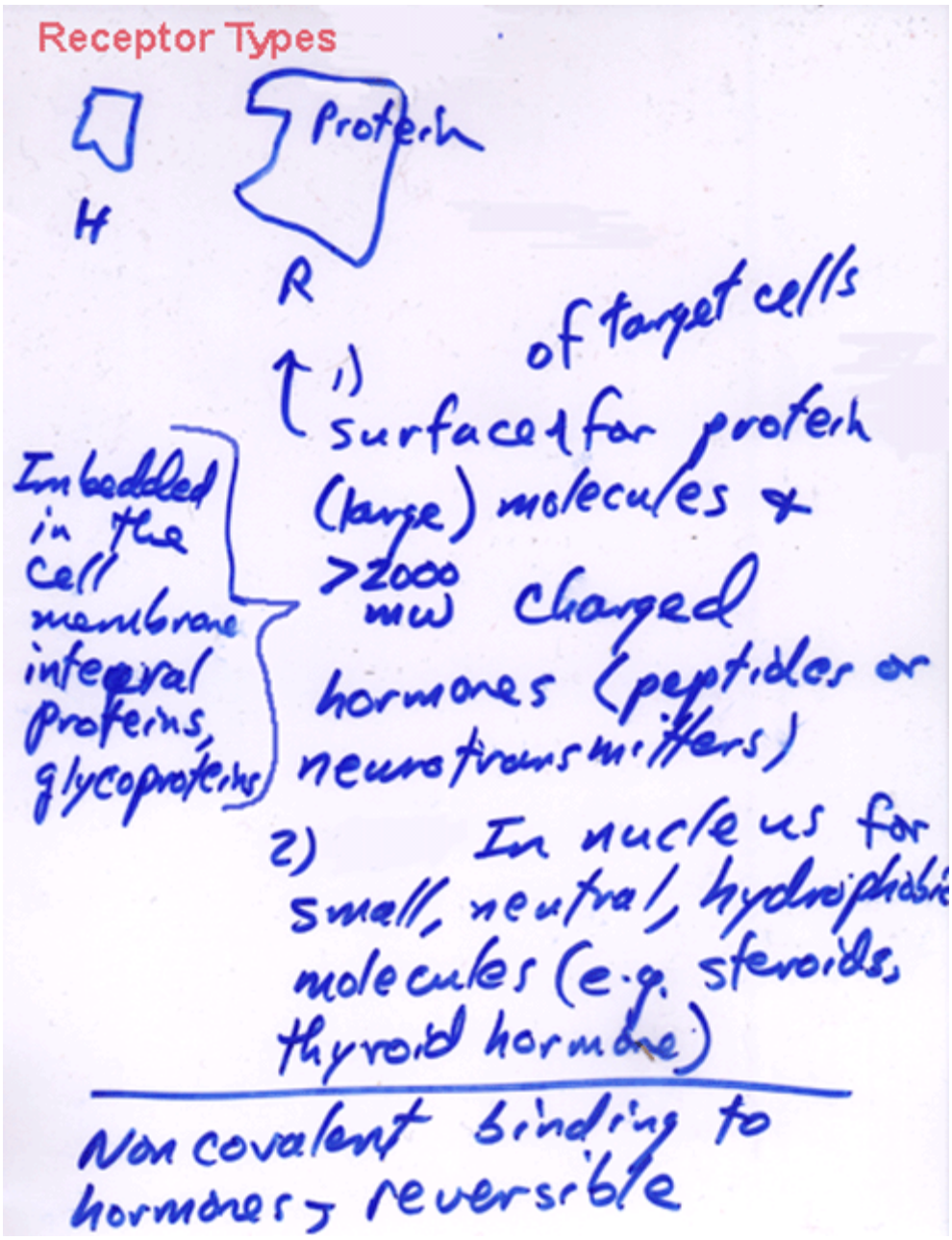
Control Loop Types

1. Negative

- **These maintain hormonal balance and are often linked to homeostatic processes.**
- **If the multiplicative effect of the several links in a control loop is negative, the entire control loop is negative.**

2. Positive

- **These cause physiologic changes in the system involved.**
- **If the multiplicative effect of the several links in a control loop is positive, the entire control loop is positive.**



Receptor Characteristics

- **Proteins**
- **Highly Specific for Hormone**
- **High Affinity for Binding (high K_a, low K_d)**
- **Saturable (low numbers per cell)**
- **Localized in responding tissues and necessary for specific cellular responses**

Hormone Receptors

Binding to hormones is noncovalent and reversible.

Membrane Receptors

- **Imbedded in the cell membrane, integral proteins, glycoproteins**
- **At the surface of target cells for protein molecules and charged hormones (peptides or neurotransmitters)**

Three major groups

- **Serpentine (7 transmembrane domains)**
- **Growth factor, cytokine receptors (1 transmembrane domain)**
- **Ion channels**

Nuclear Receptors

- **In the nucleus for small, neutral, hydrophobic molecules (steroids, thyroid hormones)**
- **Often involve cycling of R from cytoplasm where it resides as an inactive complex with heat shock proteins (HSPs) until binding of H and/or phosphorylation or dephosphorylation of the R or HSPs to the nucleus where the HR complex binds to specific Hormone Recognition Elements (HREs) or sequences on the DNA in the promoter regions of target genes. The HR often binds as a homodimer to palindromically oriented HREs and bends the DNA at the site of binding to allow transcriptional enzymes access to gene sequences. The HR may disaggregate as H levels fall or the complex may be phosphorylated or dephosphorylated so as to cause H loss and movement of the R back to the cytoplasm where HSPs can again bind.**

Transduction Systems:

- **Function to translate information contained in hormonal messages, when these are sensed by a receptor, into a language that can be interpreted and acted upon by target cells.**

- **For proteins, peptides, and hormones with a significant ionic charge at neutral pH, receptors are usually integral membrane proteins located at the cell surface. When hormones bind to the receptors, the receptors interact with membrane-bound or intracellular transducer proteins to begin the cascade of events leading to cellular response.**

- **Some membrane receptors, *e.g.*, the acetyl-choline receptor, act as ion channels that open or close in response to hormone binding and induce changes via changes of the intracellular ion/charge balance.**

- **For many lipophilic hormones, *e.g.*, steroids or thyronines, receptors are intracellular, usually intranuclear, proteins. When their specific *ligands* bind, the hormone-receptor complexes undergo conformational changes that allow them to interact with specific hormone recognition sites (HREs) in the DNA of the regulatory regions of certain genes.**

- **These transduction processes involve *allosteric* changes in receptor and/or transducer protein shape. The signaling cascades of membrane-bound receptors almost universally involve *protein phosphorylation* by kinases. These kinases may be activated initially via generation of secondary messengers produced by allosteric activation of enzymes like adenylyl or guanylyl cyclase or via unmasking of kinase activities that are part of the cytoplasmic portions of the receptor proteins themselves.**

- **Both allosteric changes and/or phosphorylation, which often changes protein charge, alter protein shape and/or intercellular location and protein *function*. These are exactly the kind of changes that would be needed to trigger a biochemical and cellular response. This may occur without the intervention of protein synthesis and therefore may be very rapid, milliseconds to minutes.**

- **Opening or closing of ion channels will also precipitate rapid responses either directly or via the intervention of phosphorylation cascades with their associated changes in protein functions.**

- **Intranuclear receptor transduction also involves allosteric and phosphorylation changes. It normally triggers changes in gene transcription and subsequent protein production and frequently modulates changes during a longer time course of minutes to days.**

Transduction Systems

2 Features of transduction that both alter protein shape and function:

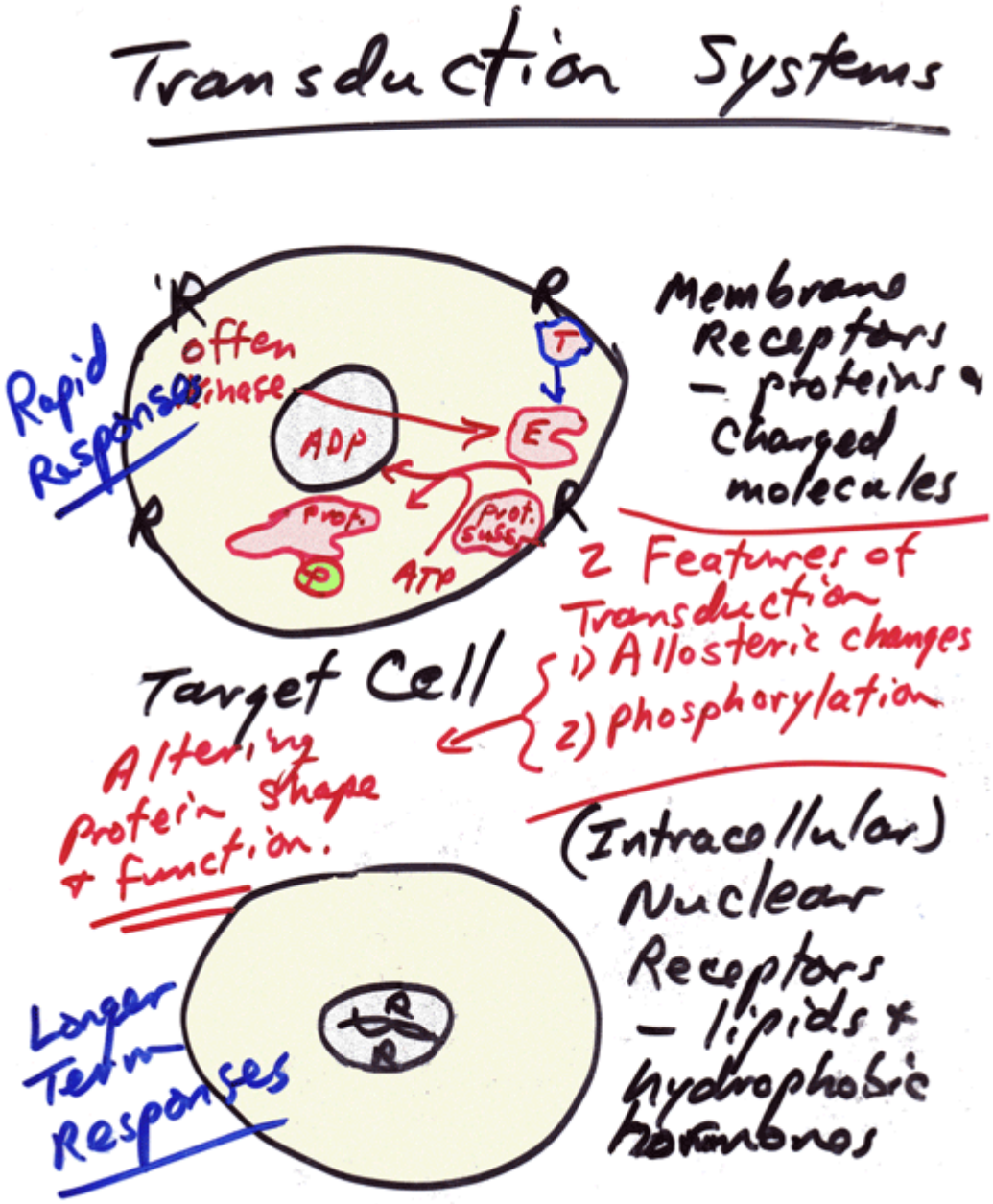
1. **Allosteric changes**
2. **Phosphorylation**

Membrane Receptors

- **usually for proteins and charged molecules**
- **rapid response systems, sec-min**

Intranuclear Receptors

- **lipids and hydrophobic hormones**
- **longer term responses, min-days**

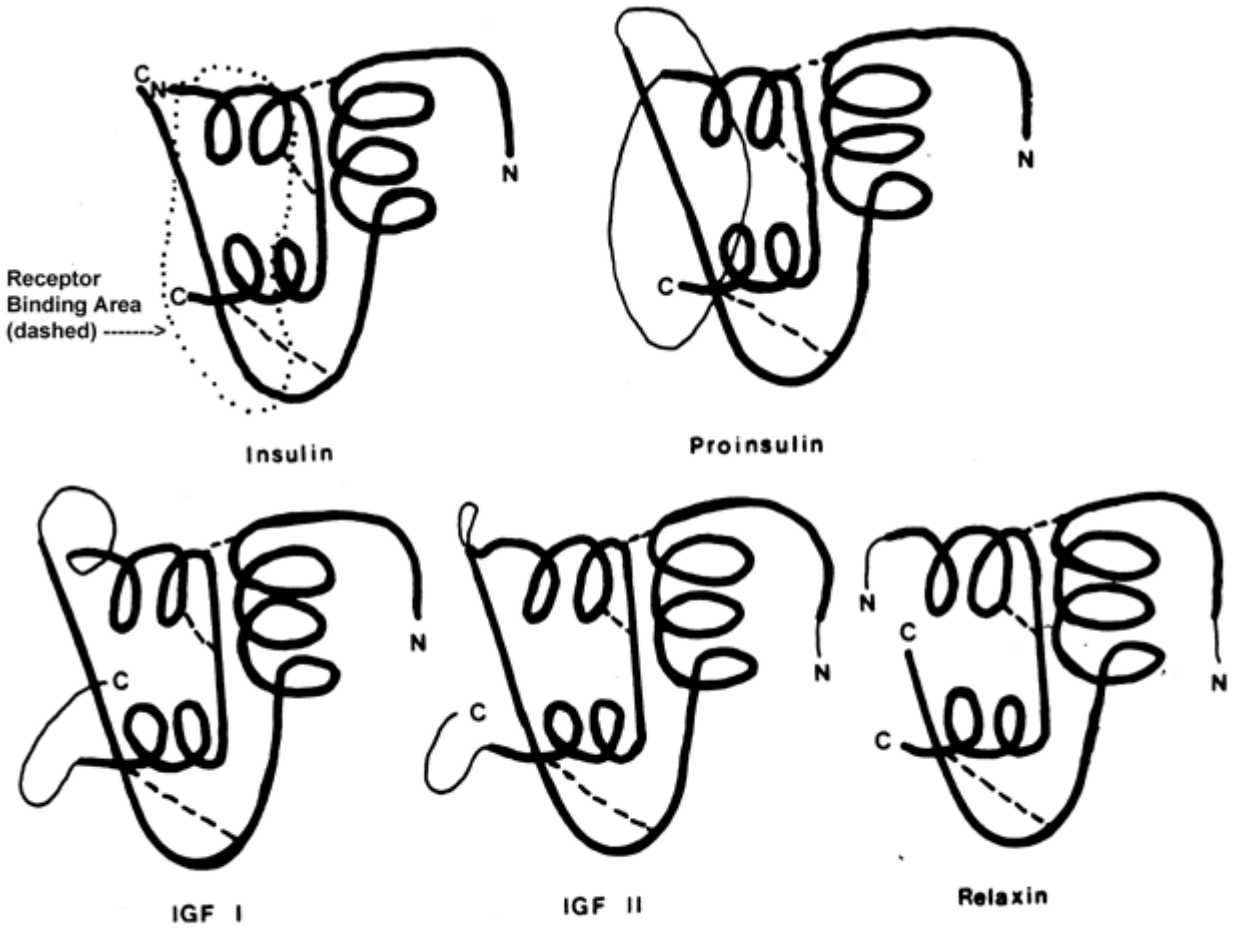


Hormones are often evolutionarily and genetically closely related to other hormones.

Receptors are often related to one another in similar ways.

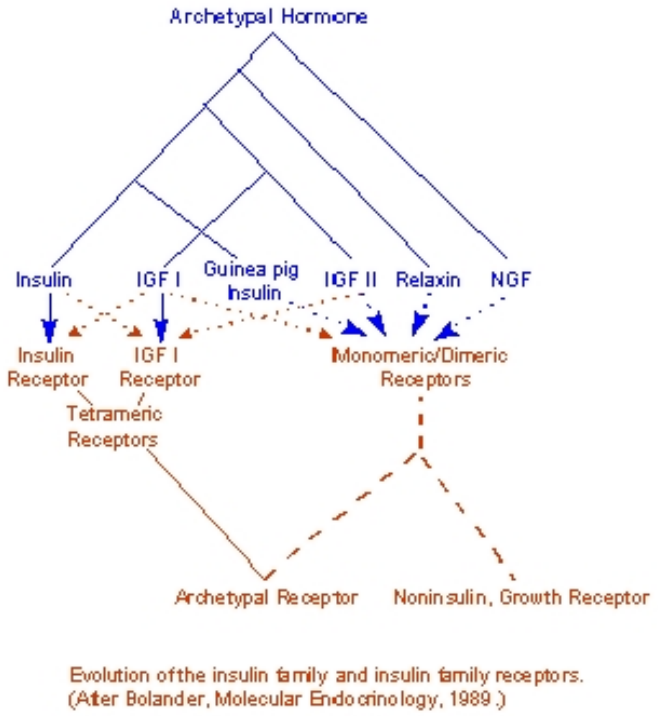
Genetic relatedness among hormones or receptors leads to *promiscuity* among hormone/receptor systems.

- **GH, PRL**
- **Insulin, IGF-I, IGF-II**
- **hCG, TSH**



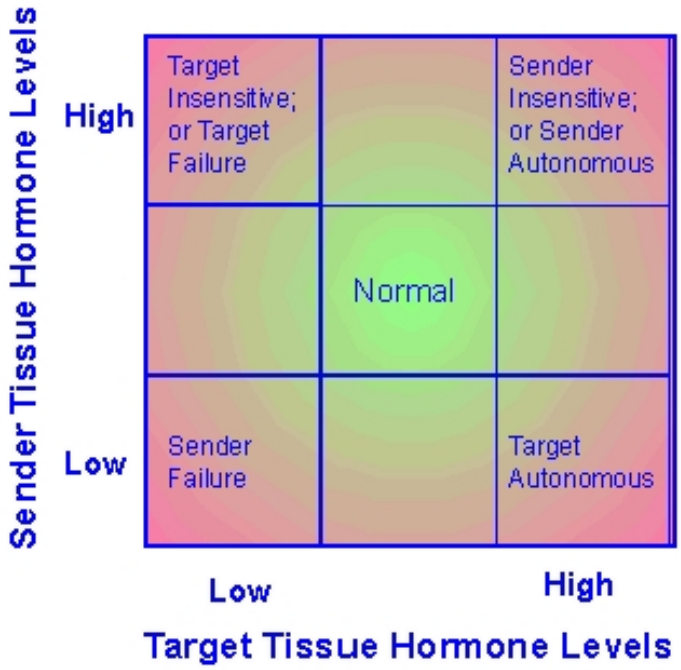
Insulin family structural homologies. A and B chains are heavy lines. C-peptides or N- and C- terminal extensions are thin lines. S-S bonds are dashed lines. (Modified from Bolander, *Molecular Endocrinology,* Academic Press:San Diego, CA, 1989.)

35) Insulin Molecular Structure ~ http://c4.cabrillo.cc.ca.us/projects/insulin_tutorial/index.html



Evolution of the insulin family and insulin family receptors.
(After Bolander, Molecular Endocrinology, 1989.)

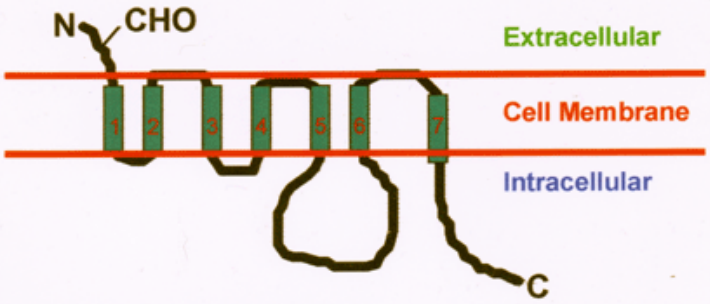
Diagnostic Relationships in Negative or Homeostatic Feedback Loops



Relative Affinities for Receptors of the Insulin Family to Family Members

Receptor	Relative Affinities
Insulin	Insulin >Proinsulin (10%) >IGF II >IGF I >>Relaxin(~0)
IGF I	IGF I > IGF II >Insulin ~ Proinsulin
IGF II	IGF II = IGF I >>Insulin ~ Proinsulin
Relaxin	Relaxin >NGF >Proinsulin > IGF >>Insulin (~0)
NGF	NGF (only)

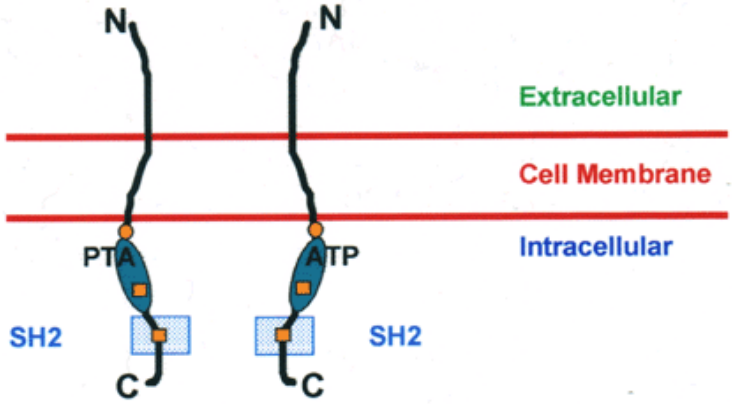
Serpentine Receptors



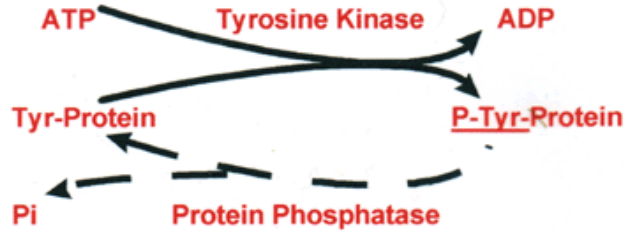
- 7 Transmembrane helices
- Coupled to "G" Proteins
- Interactions (allosteric) with intracellular proteins on intracellular loops, especially the third, and on the C-terminal tail
- Often phosphorylated on the third intracellular loop
- Additional phosphorylations can occur on ser/thr on the intracellular tail

- Examples:
  - β Adrenergic receptors
  - LH receptor
  - ACTH receptor
  - VP receptor
  - Glucagon receptor
  - .
  - .
  - .

Cytokine/Growth Factor Receptors



- 1 Transmembrane domain
- Often folded extracellularly
- Often form dimers upon hormone binding
- Have multiple phosphorylation sites on intracellular C-terminus at ser/thr (●) and tyr (■) residues
- Have an ATP binding site and a tyrosine kinase activity that is activated by hormone binding
- Phosphorylated receptors participate in multiple intracellular signalling pathways via SH2 and SH3 domains



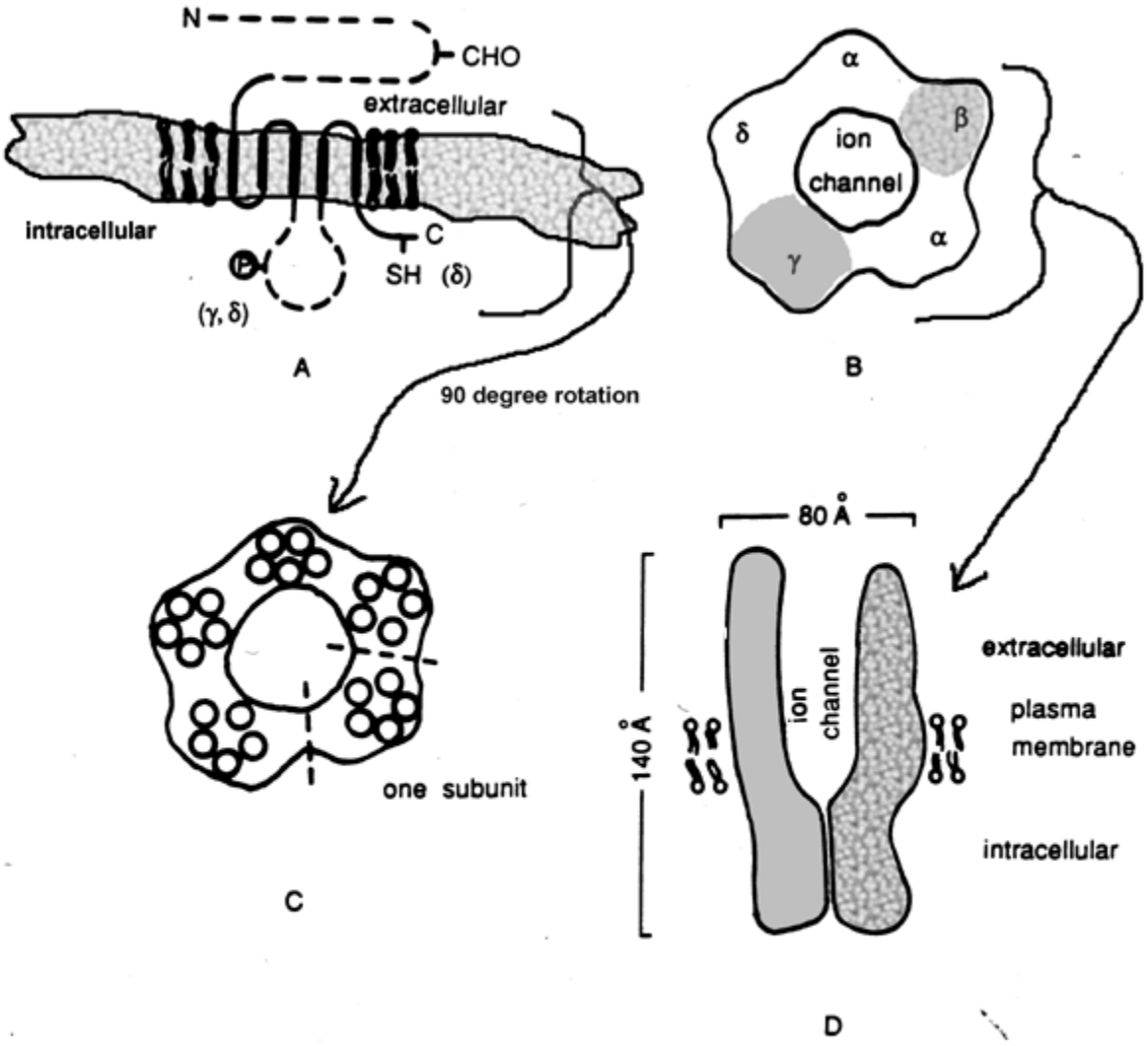
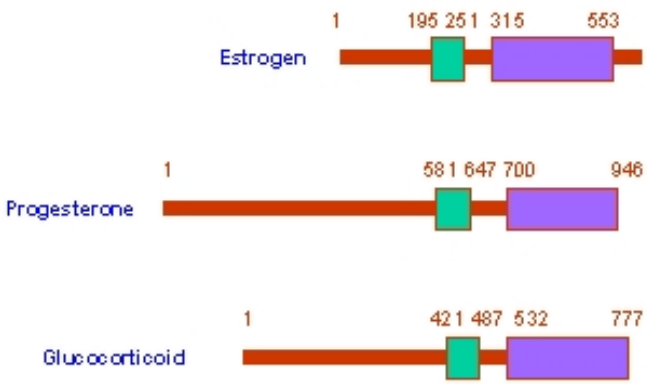
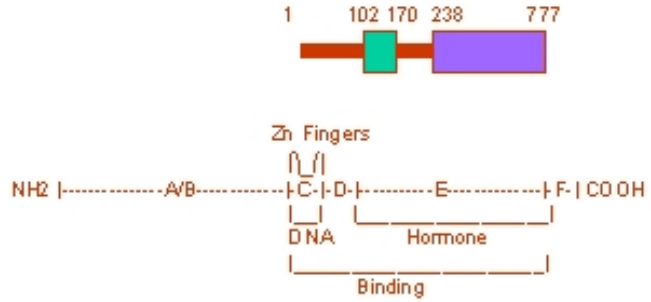


Fig. 5-6. The nicotinic ACh receptor. (A) Membrane orientation of a typical subunit; (B) transverse section showing the arrangement of the five subunits as viewed from the extracellular side; (C) transverse section showing the hypothetical arrangement of the five transmembrane helices within each subunit; (D) longitudinal section of the receptor *in situ*. The figure in (D) is adapted and **modified** from *Nature (London)* **315**(6019), 474–477. Copyright © 1985 MacMillan Magazines Limited.

Steroid Receptors



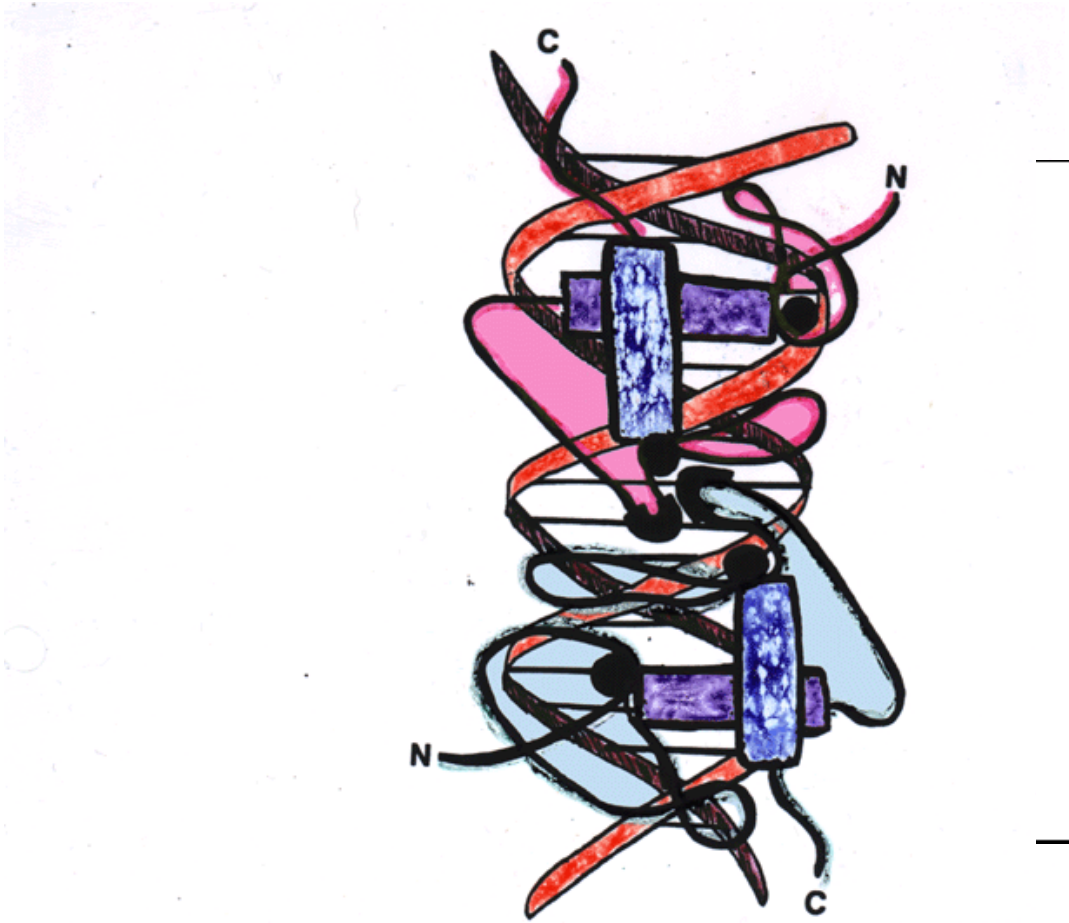
Thyroid Hormone Receptor



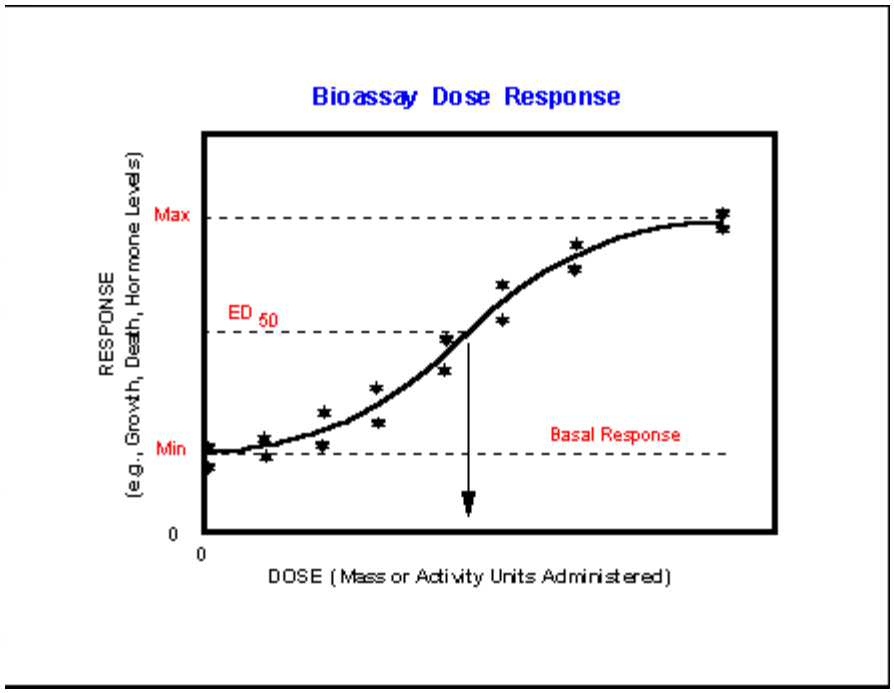
Nuclear Receptor Response Elements

Estradiol	GGTCANNNTGACC CCAGTNNNACTGG
Androgen	AGAACAgcaAGTGCT T CTT GTcgtT CACGA
T3	AGGTAAGatcAGGGACG T CCATT CtagTCCCT GC

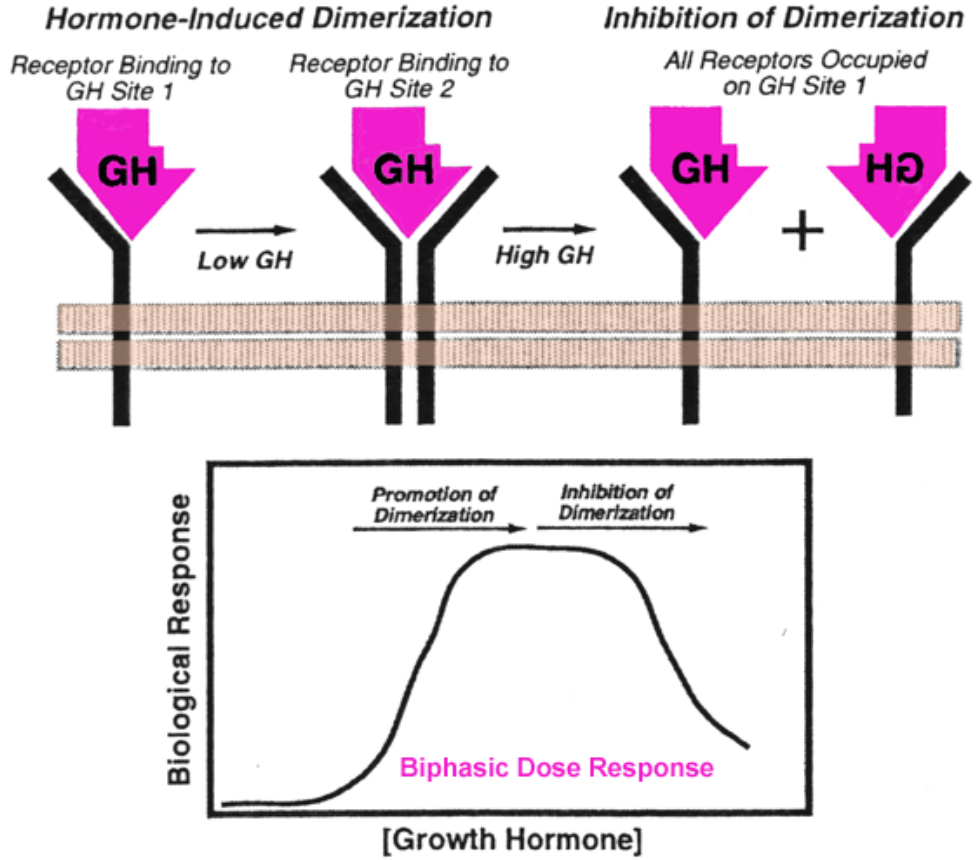
- Hormone-Receptor complexes bind to RE's in DNA, often as homodimers that wrap around the DNA or as heterodimers with other non-identical H-R complexes.
- The dimers interact with other transcription factor proteins and often alter transcription, in part, by bending the DNA molecule.



Model of the binding of two nuclear receptors as a dimer with the partners facing in opposite directions. Note this fails to convey the tendency such dimers have to bend the DNA, thereby opening it up to interactions with transcription factors. The D-boxes of the two molecules are shown in blue and purple. (After Bolander, *Molecular Endocrinology, 2nd Ed*, Academic Press:San Diego, CA, 1994.)



Down Regulation or Desensitization; Loss of Unoccupied Receptors or Transduction?



Modified from Mayo, Receptors: Molecular mediators of hormone action, In Conn & Melmed, ed., *Endocrinology: Basic and Clinical Principles,* Humana Press: Totowa, NJ, 1997.

Bioassays – useful but slow & relatively imprecise, consume animals & lots of reagents; expensive to run

---

Other assays to allow running many samples quickly & accurately, cheaply.

Antibodies allow production of highly specific reagents in reasonable volumes which can be used in chemical-type assay.
– This is an improvement over strictly physical-chemical assays, e.g., UV-absorption, Gas Chromatographic behavior, IR-absorption, Mass-spectra.

Chemical Assays Include Those Using Biochemical Reagents Like Receptors or Antibodies

Competitive Binding Assays

1. **Isolate/prepare a receptor suspension, or a serum binding protein, or an antibody**
2. **Isolate/prepare a small amount of purified hormone**
    1. **Need to label part of it so it can be *visualized***
    2. **Need pure hormone for standards or references, analytical references.**
3. **In each of a series of tubes or wells place the same small amount of label and binder - *the binder will be the limiting reagent in this assay***
4. **In part of this series of tubes add increasing amounts of unlabeled hormone, one amount in each tube or well starting at zero additional.**
5. **In the rest of this series of tubes add a measured volume of the unknowns, one unknown per tube.**
6. **Incubate for a period of hours**
7. **Separate the hormone bound to the binder from the remaining free hormone by some simple method.**
8. **Quantify the amount of label that is bound to the binder; it will be inversely proportional to the amount of unlabeled hormone present since the binder is the limiting reagent - this is a molecular game of musical chairs.**

Analytical Assay Performance Parameters

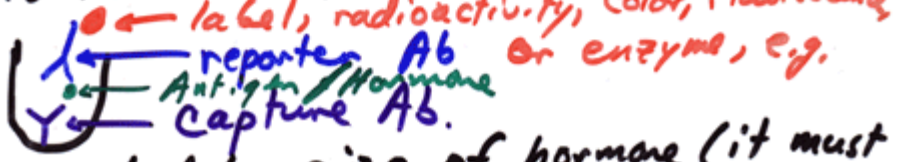
- Specificity: uniqueness of the detected species, lack of cross-reactivities, parallelism of diluted samples
- Precision: reproducibility of measurements
- Accuracy: ability to estimate the "correct," known, amount of analyte contained in standards or reference samples; lack of Bias: comparability of results with other assays for the same analyte
- Sensitivity: (analytically) slope of the assay response curve, height of the response/amount of analyte yielding the response; also, (diagnostically) limit of detection or the lowest measurable, nonzero, amount of analyte

Antibody-based assays — Immunoassays

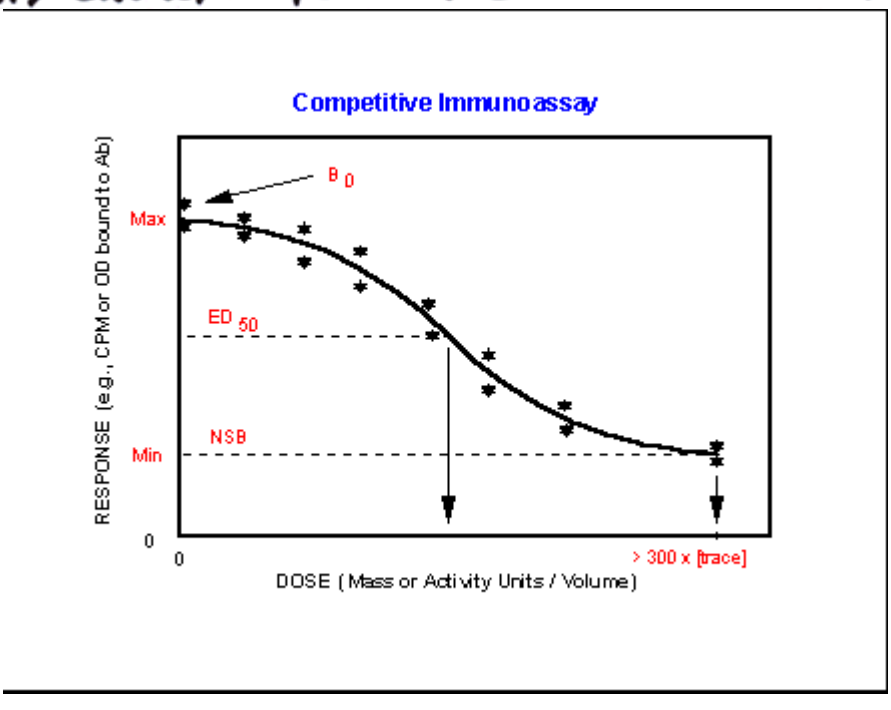
1. Competitive assay –
2. Noncompetitive assay –

Competitive –
Antibody is the limiting reagent
"Molecular game of musical chairs"
– work regardless of the size of antigen (hormone) molecules used.

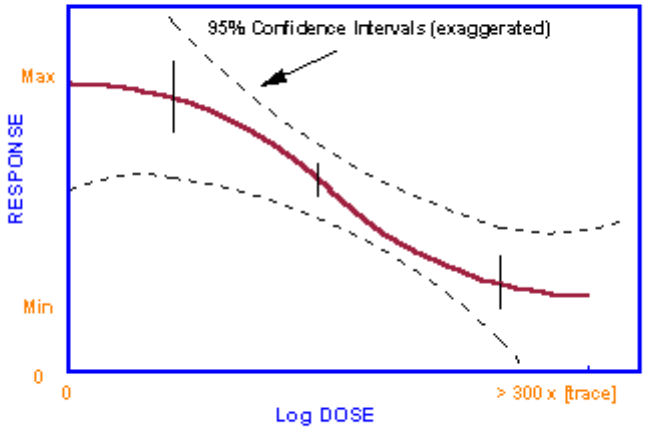
Noncompetitive –
2 complementary antibodies used, 1 is labeled (think "sandwich")



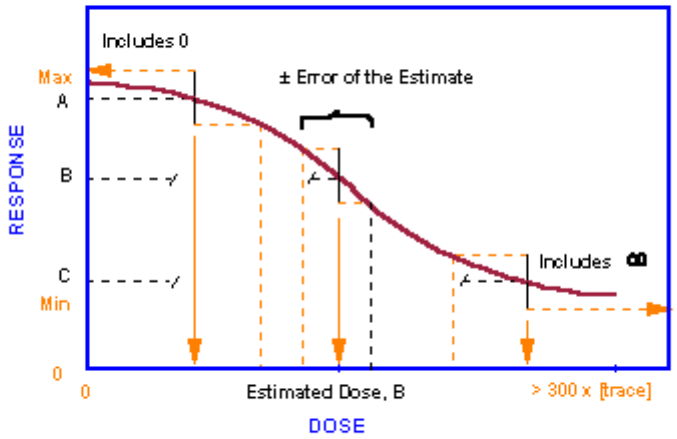
– Limited by size of hormone (it must be big enough to bind 2 Ab's at same time)



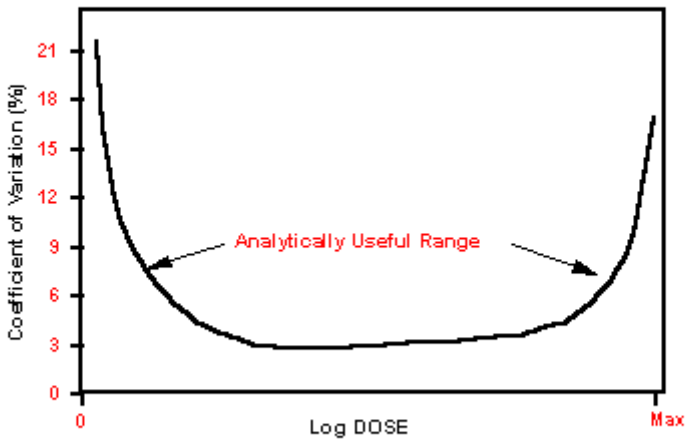
Error Structure, Competitive Immunoassay



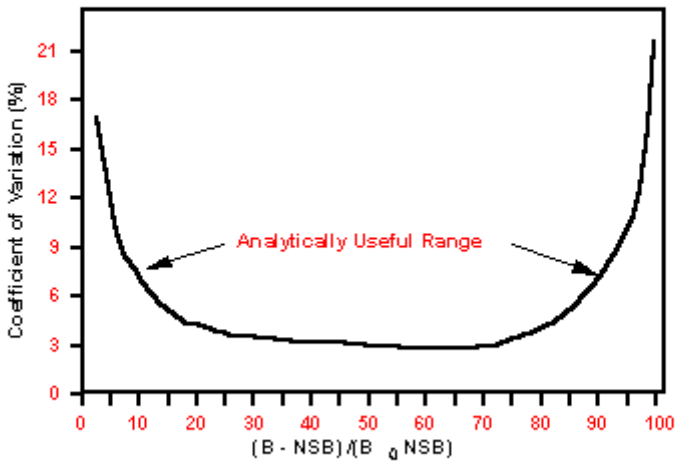
Estimation Errors, Competitive Immunoassay

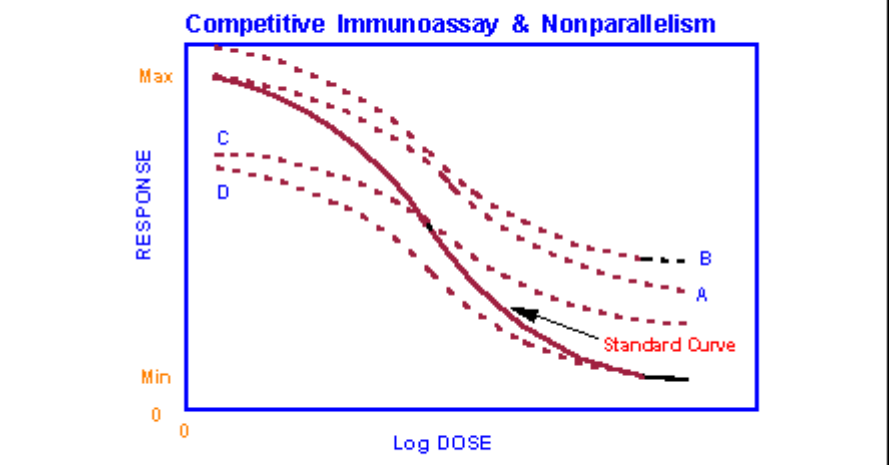


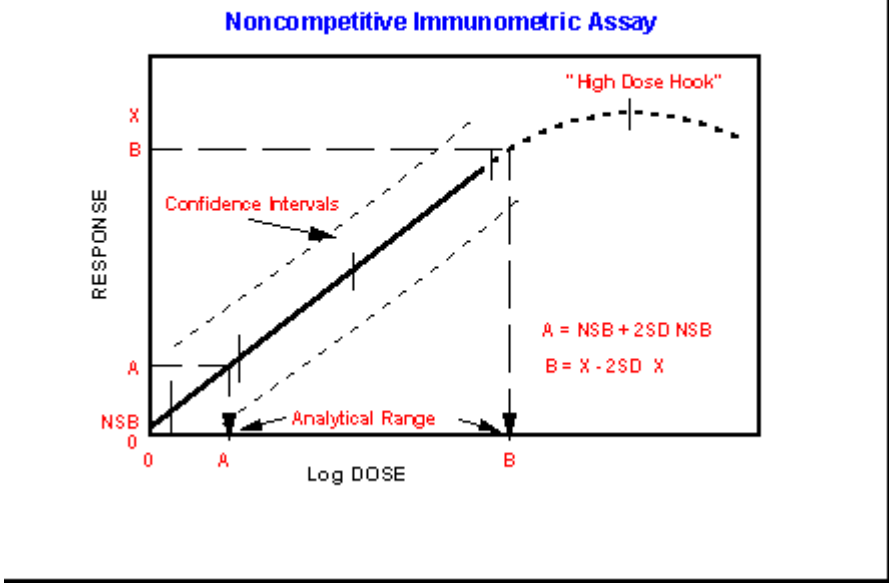
Competitive Assay Precision Profile



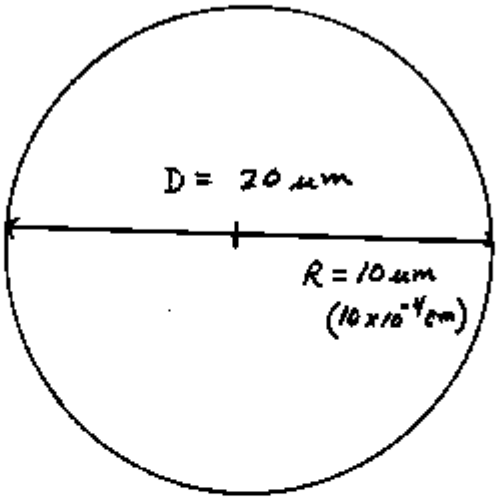
Competitive Assay Precision Profile




Competitive Immunoassay & Nonparallelism
Max
RESPONSE
C
D
B
A
Standard Curve
Min
0
0
Log DOSE


Noncompetitive Immunometric Assay
"High Dose Hook"
X
B
Confidence Intervals
RESPONSE
A = NSB + 2SD NSB
B = X - 2SD X
A
NSB
0
Analytical Range
0
A
B
Log DOSE

OBW.B membrane ~ 10 nm thick, protein (large) ~ 10-20 nm across ⇒ "area" of a protein ~ 314 - 1257 nm^2
⇒ 397 could fit in the area available for each molecule @ a density of 3000/cell.



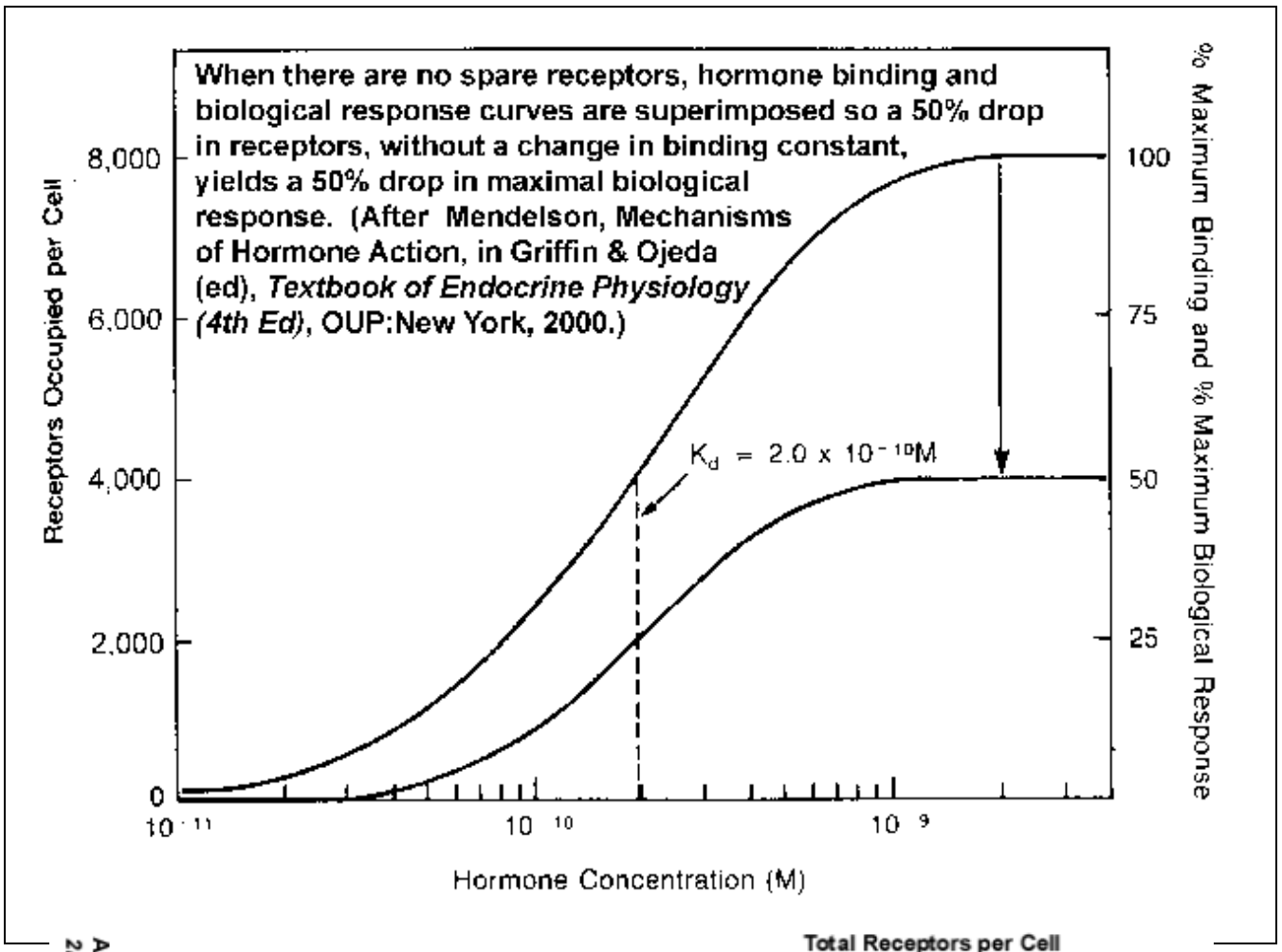
$V = \frac{4}{3}\pi r^3 = 4.19 \times 10^{-9}\ cm^3 = 4.19 \times 10^{-9}\ ml = 4.19\ pL$

$A = 4\pi r^2 = 12.57 \times 10^{-6}\ cm^2 = 1.257 \times 10^{-3}\ mm^2$
$= 1257\ \mu m^2 = 1.257 \times 10^{9}\ nm^2$

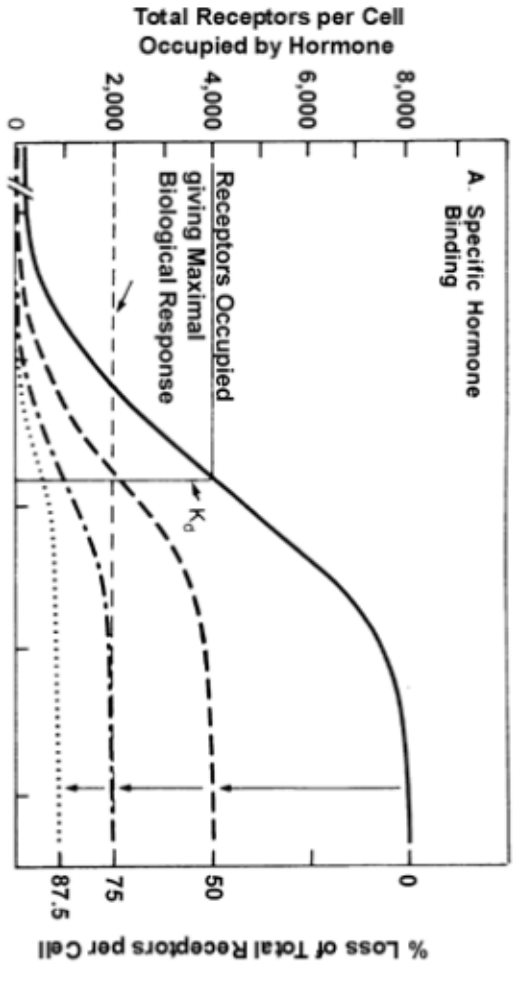
@ 1 μMolar concentration & 4.19 pL:
1×10^{-6} Moles/L x 6.023×10^{23} molecules/mole x 4.19×10^{-12} L =
2,523,637 molecules/cell

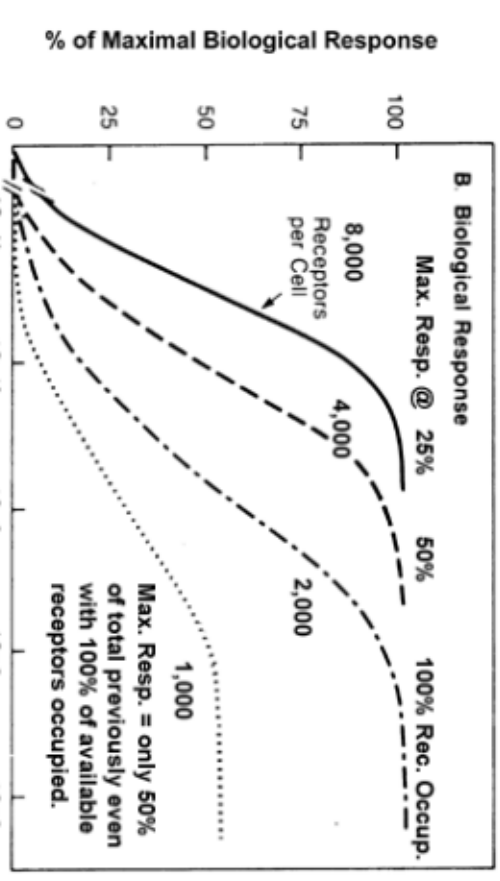
@ 1 nMolar → 2524 molecules/cell

@ 3000 receptors/cell (surface) = 3000/1257 μm^2 = 2.39 $\frac{\text{receptors}}{\mu m^2}$
or $\frac{1257\ \mu m^2}{3000\ \text{receptors}}$ = 0.419 μm^2/receptor = 419,000 nm^2/receptor



Impact of Receptor Number on Biological Response





After Mendelsohn, Mechanism of Hormone Action, in Griffin & Ojeda, *Endocrinology, 2nd ed.*, Oxford University Press:New York, 1994.

Adjusting Cellular Sensitivity to Hormone

1. **Number of Receptors**

   Spare Receptors - Multiple Hypotheses

   1. **Cell has 2 pools, 1 "true" or active, 1 "spare" or inactive**
   2. **"Spare" R only reflect those not coupled to the response being monitored**
   3. **Spare R [R] [H] + [R] [HR], favors [HR] and responses to HR, allows regulation without altering K_a**

2. **Location of Receptors**

   Expression on part of the cell surface regulates exposure to hormone,
   may allow directional or polar responses to hormone

3. **Biochemical Status of Receptors**
   1. **Receptor aggregation alters surface location and may trigger cellular internalization of HR with proteolysis of H and/or R (*receptor - mediated endocytosis*; associated with at least temporary R inactivation and inaccessibility to H)**
   2. **Receptor aggregation may trigger tyrosine kinase activities that alter receptor association with hormone (K_a), other receptors or with transducers**
   3. **Transducer/Effector molecules may phosphorylate receptors and alter receptor associations as in 3b.**

4. **Number of Transducers**

   Interactions with multiple transducer systems may deplete available molecules.

5. **Location of Transducers**

   Intracellular localization of transducers in membranes, near receptors make them more available than if they must diffuse along cytoskeletal elements in the cytoplasm; recruitment to one end of the cell by a highly localized receptor may make them unavailable for coupling to receptors at the other end of the cell.

6. **Biochemical Status of Transducers**
   1. **Allosteric interactions mediated by binding to GTP/GDP, ATP, IP_3,DAG, or other small molecules may activate or inactivate transducer elements.**
   2. **Phosphorylation, or other covalent biochemical modifications may activate or inactivate transducer elements.**

7. **Number of Effectors**

   As for transducers.

8. **Location of Effectors**

   Proximity to receptor/transducer elements helps speed modulations of activity.

9. **Biochemical Status of Effectors**

   As for transducers, except that availability of suitable substrates may also alter activity levels and these may be affected by activities of transducers.