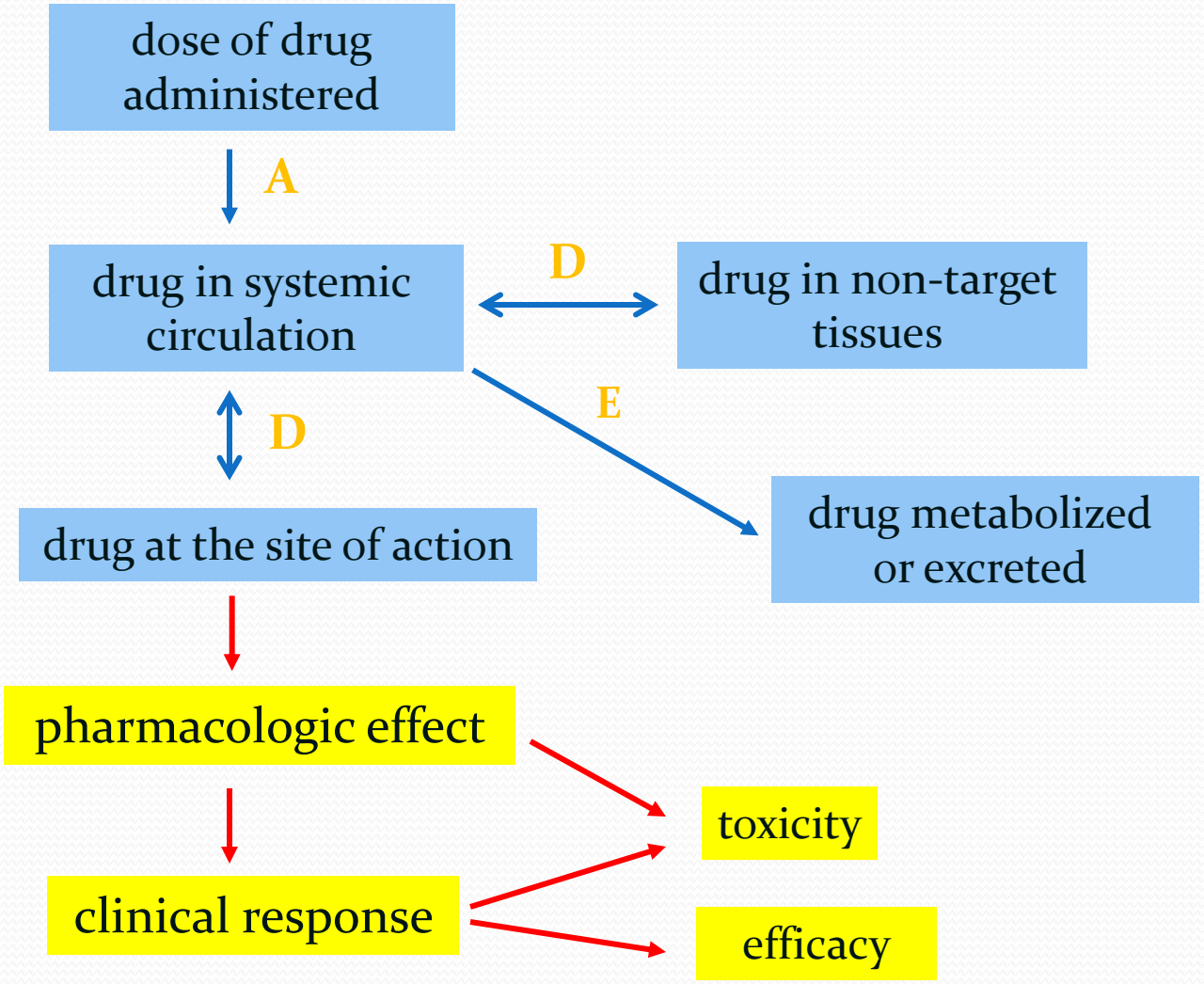
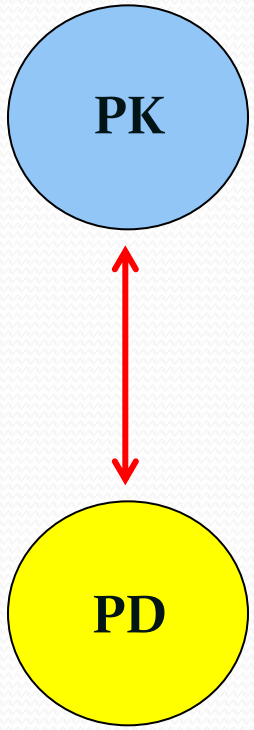
Topic # 3:

Drug receptors and pharmacodynamics

What Happens After Drug Administration?

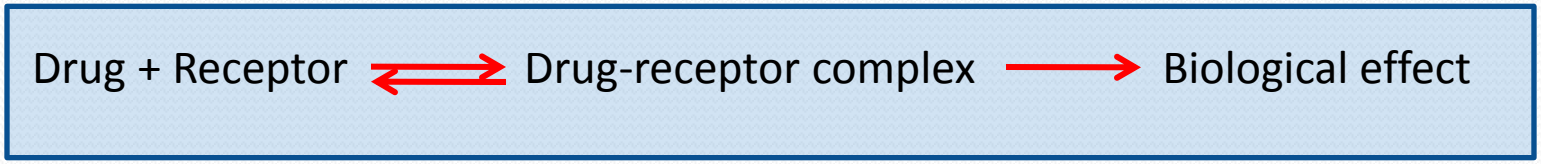




Receptors

- Both therapeutic and toxic effects of drugs result from their interactions with with macromolecular components of the organism (**receptors**)

- Drugs bind receptors (**Drug-receptor complex**) and initiate events leading to alterations in biochemical activity of a cell, and consequently, the function of an organ (**biological response**)



Receptor Theory

- Receptors largely determine the quantitative relations between dose or concentration of drug and pharmacologic effects
- Receptors are responsible for selectivity of drug action
- Receptors mediate the actions of pharmacologic agonists & antagonists

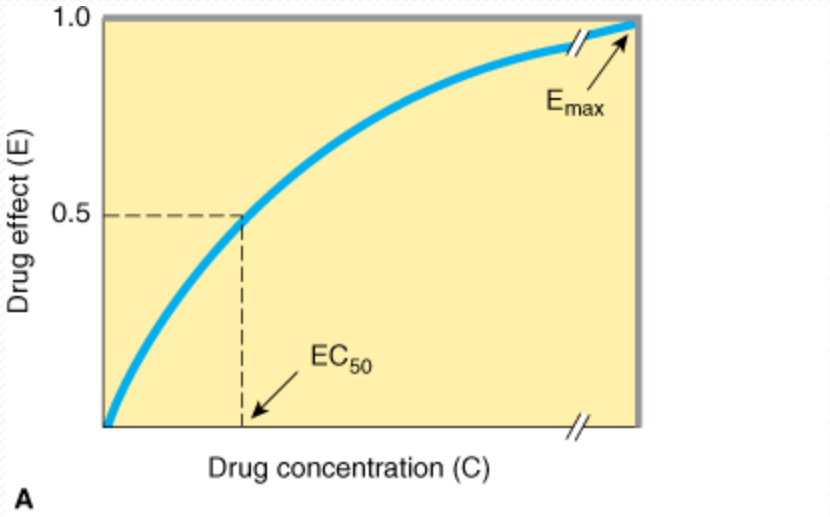
Concentration-Effect Curves & Receptor Binding of Agonists

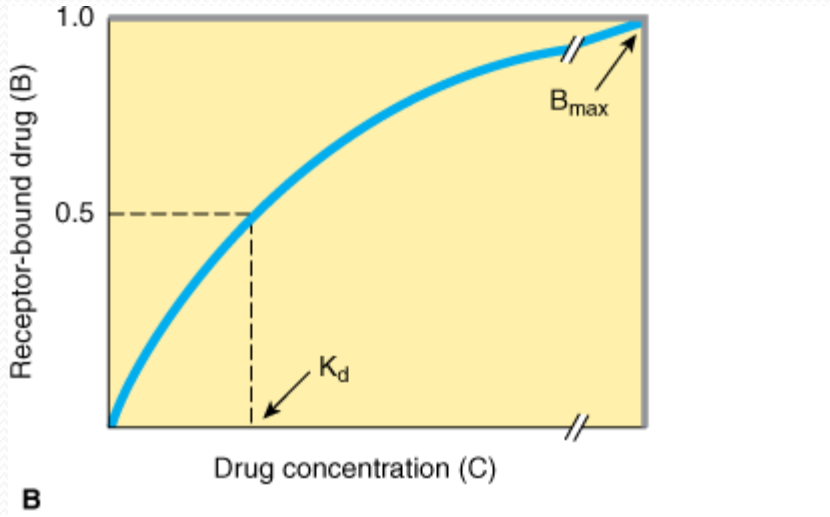
- **Responses** to low doses of a drug usually increase in **direct proportion to dose**

- As doses increase, however, the response increment diminishes

- Finally, doses may be reached at which no further increase in response can be achieved (E_{max})

Drug-receptor binding





Concentration-Effect Curves & Receptor Binding of Agonists

In *in vitro* systems, the relation between drug concentration and effect is described by a **hyperbolic curve**

$$E = \frac{E_{max} \times [C]}{[C] + EC_{50}}$$

Where [E] = is the effect observed at concentration [C];

E_{max} is the maximal response that can be produced by the drug;

and EC_{50} is the concentration of drug that produces 50% of maximal effect

Concentration-Effect Curves & Receptor Binding of Agonists

- The first step in drug action on specific receptors is the formation of a **drug-receptor complex**, the reactions being governed by the *Mass Action Law*
- The equation describes the relation between **drug bound to receptors (B)** and **the conc. of free drug (C)**

$$D + R \underset{}{\overset{k_1}{\rightleftharpoons}} D\text{-}R$$

$$B = \frac{B_{max} \times C}{C + K_d}$$

B_{max} indicates the **total *concentration*** of **receptor sites** (**sites bound** to the drug at **infinitely high concentrations** of free drug).

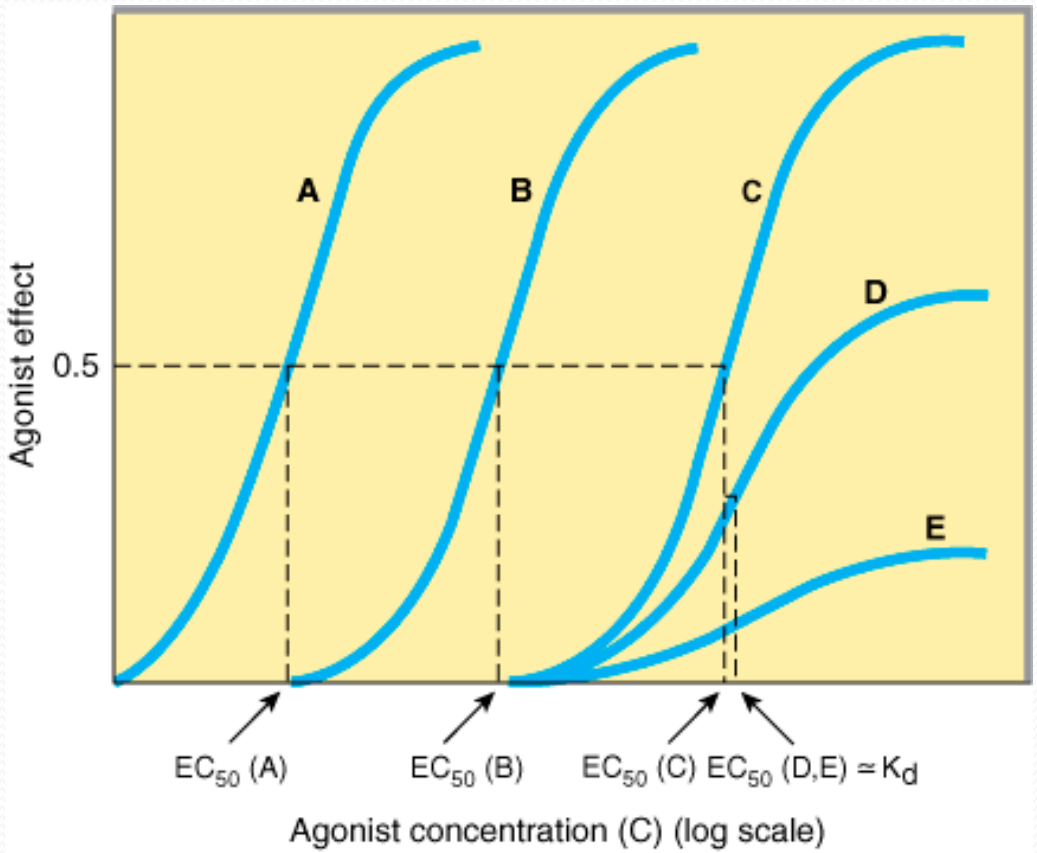
K_d (the equilibrium dissociation constant) is the concentration of drug required to bind 50% of the receptor sites (**reflects the drug affinity**)

Concentration-Effect Curves & Receptor Binding of Agonists

- Plotting the drug effect against the ***logarithm*** of the dose or concentration transforms the hyperbolic dose-response curve into a **sigmoid curve** with a linear midportion

- This curve has the expanded scale at low concentrations (where the effect is changing rapidly) and compresses at high concentration (where the effect is changing slowly)

Drug-receptor binding



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Drug receptor interaction

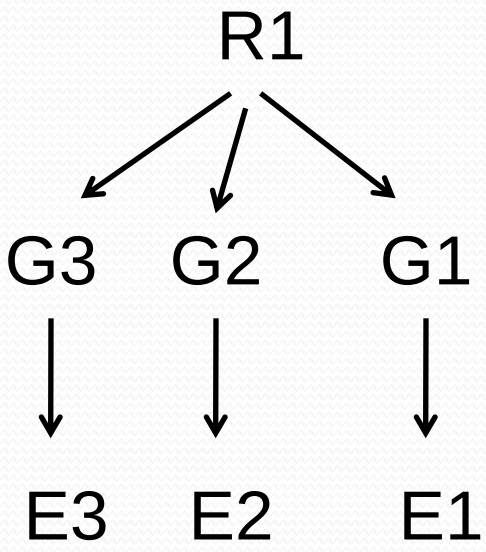
- In general, the drug-receptor interaction is characterized by:
  - **Binding of drug to receptor** which is governed by the chemical property of *affinity*, the **tendency** of a drug to **bind** the receptor. kd characterizes the receptor affinity for binding the drug in a reciprocal fashion i.e. A high affinity means a small kd
  - **Generation of a response in a biological system which** is governed by a property described as *efficacy (intrinsic activity)*; the **tendency** of a drug, **once bound**, to **activate** the receptor

Receptor-Effector Coupling

- The transduction process between occupancy of receptors and drug response is often termed **coupling**
- **Effectors:** molecules that translate the drug–receptor interaction into a change in cellular activity (adenylyl cyclase)
- The efficiency of occupancy-response coupling is determined by:
  - **Initial conformational change** (full agonist *vs.* partial agonist)
  - **Biochemical events** that transduce receptor occupancy into cellular response
    - Sometimes biological response is *linearly* related to the number of receptors bound /occupied (e.g. *drug-regulated ion channel*) OR increase *disproportionally* to the number of receptors bound

A single receptor interacts with one G-protein

A single receptor activates multi[le G-protein



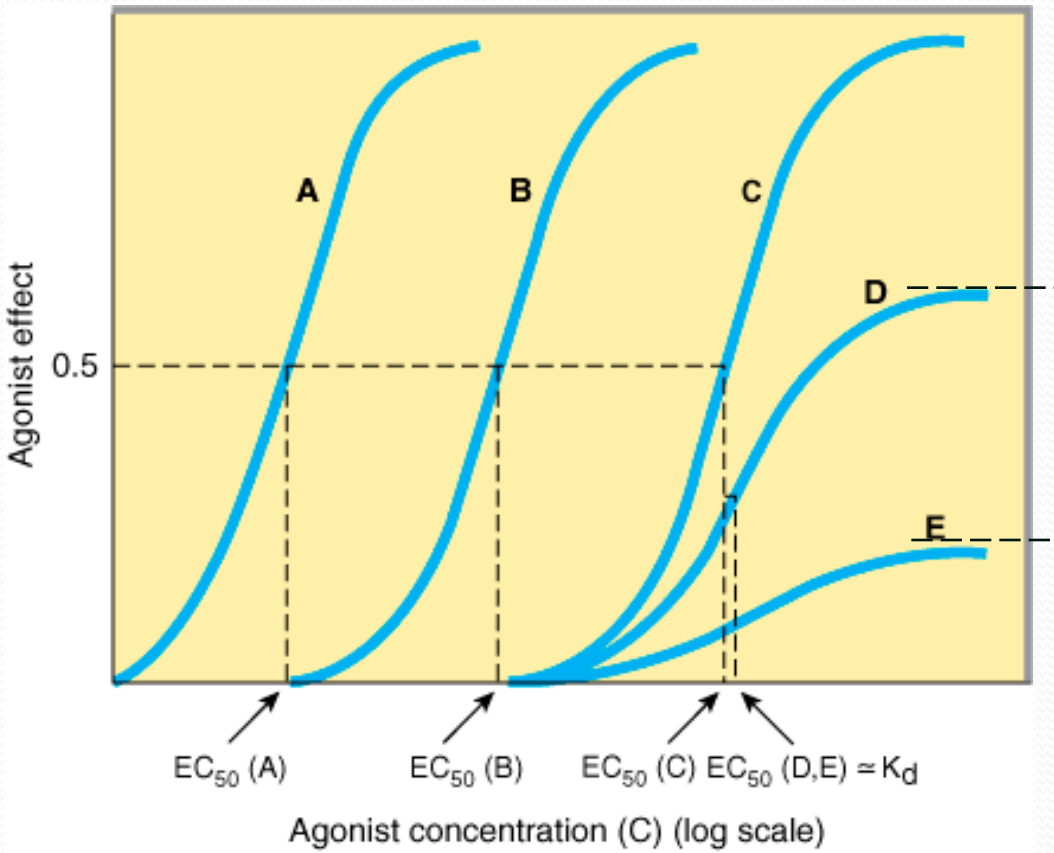
Spare Receptors

- Receptor are said to be "**spare**" for a given pharmacologic response when the maximal response can be elicited by an agonist at a concentration that does not result in occupancy of the full complement of available receptors
- *Spare receptors* are not qualitatively different from non-spare receptors
- When spare receptors are occupied, they can be coupled to response

Spare Receptors (Cont'd)

- **Experimentally,** spare receptors may be demonstrated by using irreversible antagonists to prevent binding of agonist to a **proportion** of available receptors and showing that high concentrations of agonist can still produce an undiminished maximal response

Spare receptors (Cont'd)



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How do we account for Spare Receptors?

1. In some cases, the effect of the receptor activation may *greatly* **outlast the agonist-receptor interaction itself** (*spareness* is temporal)

eg, Activation of β-adrenoceptors promotes the binding of GTP to an intermediate that may greatly **outlast the agonist-receptor interaction**

→ Maximal response can be elicited by activation of relatively few receptors because the response initiated by an individual ligand-receptor binding event persists longer than the binding event itself

How do we account for Spare Receptors? (Cont'd)

2. The actual number of receptors may exceed the number of effector molecules available (**spare in number)**…(the sensitivity of a cell or tissue depends not only on the affinity but also on the degree of spareness)

Spare Receptors (cont'd)

- The presence of spare receptors increases sensitivity to the agonist……the likelihood of a D-R interaction increases in proportion to the number of receptors available;
- The sensitivity (EC_{50}) of a cell or tissue to a particular conc. of agonist depend on the affinity of the receptor for binding agonist (Kd) but also on the total no. of receptors present compared with the number actually needed to elicit a maximal (degree of spareness)

Spare Receptors (cont'd)

- If a large receptor reserve is present the EC_{50} will be lower than the Kd i.e. the concentration of drug required to give 50% of maximum response is lower than the concentration of drug required to occupy 50% of receptors

Spare Receptors (cont'd)

$$\frac{B}{B_{max}} = \frac{C}{C + K_D}$$

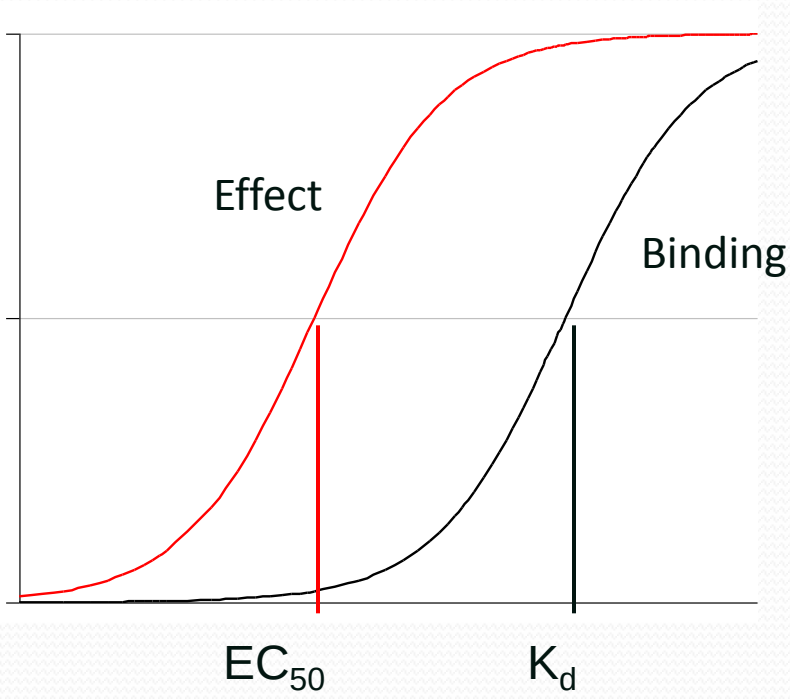
- If we have a cell with 4 receptors and 4 effectors, the number of effectors does not limit the maximal response, and the receptors are **not spare in number.**
- If the n. of receptors increases tenfolds to 40 receptors but the n. of effectors remains constant, most of receptors are now **spare in number**
- As a result, a much lower conc. of agonist suffices to occupy 2 of 40 receptors, and this same low conc. of agonist is able to elicit a half-max. response (2 of 4 effectors activated)

Spare receptors

$$[RL] = [R_{total}] \times \frac{[L]}{[L] + K_d}$$

$$E = E_{max} \times \frac{[D]}{[D] + EC_{50}}$$

Example:
Rat heart contractility
and b-adrenergic receptors
50% response at 1-3%
receptor occupancy



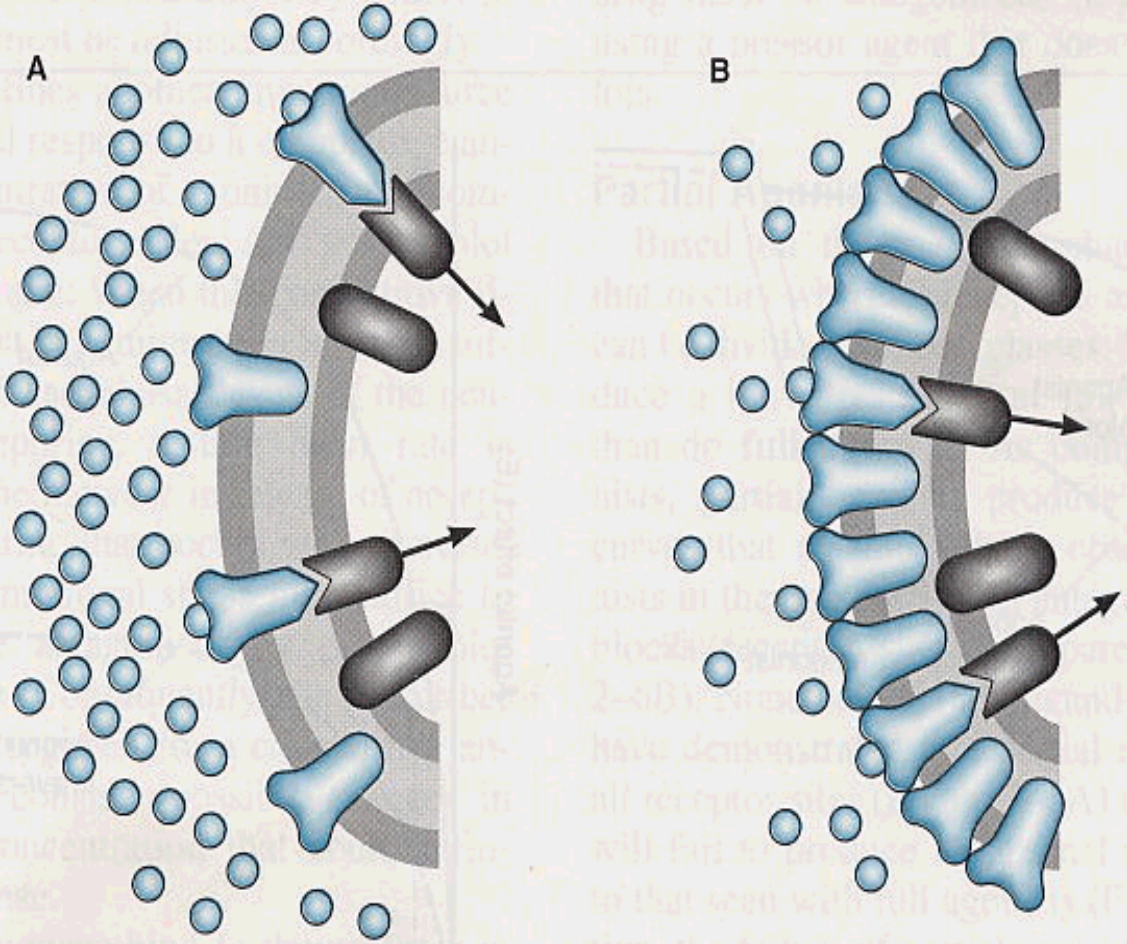
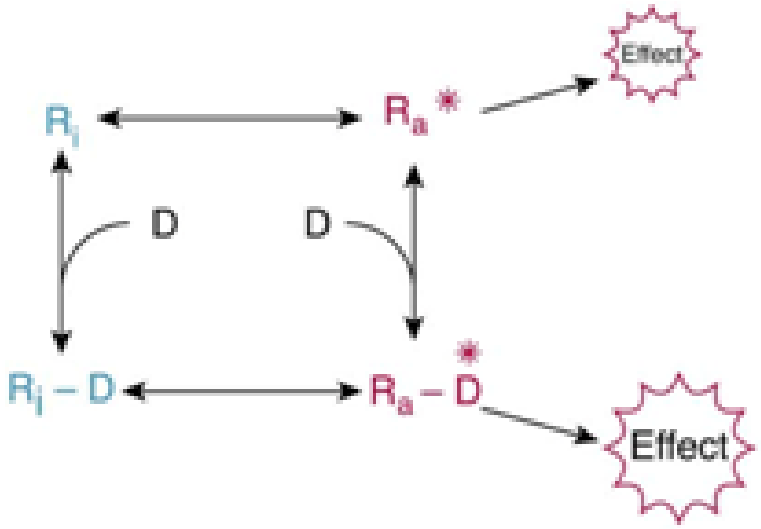
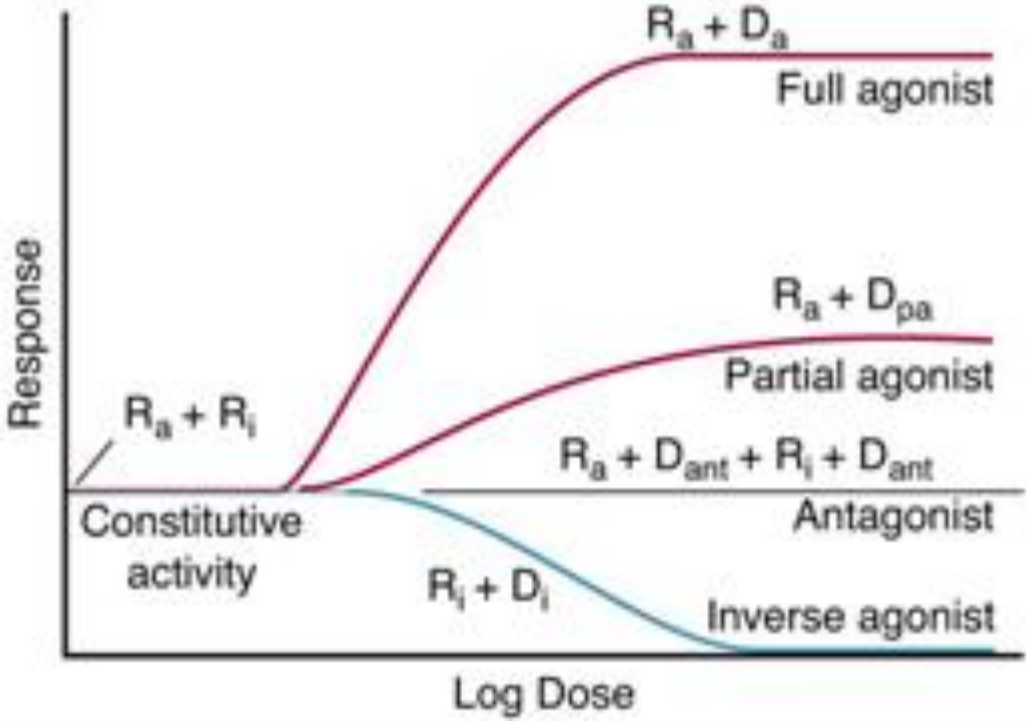


Figure 2–4. Spare receptors increase sensitivity to drug. In panel ***A,*** the free concentration of agonist is equal to the K_D concentration; this is sufficient to bind 50% of the four receptors present, resulting in the formation of two agonist-receptor complexes. (***Note:*** When the agonist concentration is equal to the K_D, half the receptors will be occupied. Remember that $B/B_{max} = C/[C + K_D]$.) Agonist occupancy of these two receptors changes their conformation so that they bind to and activate two effector molecules, resulting in a response. Because two of four effectors are stimulated by agonist-receptor complexes, the response is 50% of maximum. In panel ***B,*** the receptor concentration has been increased tenfold (not all receptors are shown), and the K_D for binding of agonist to receptors remains unchanged. Now a very much smaller concentration of free agonist ($= 0.05 \times K_D$) suffices to occupy two receptors and consequently to activate two effector molecules. Thus, the response is 50% of maximum (just as in panel A), even though the agonist concentration is very much lower than the K_D.

Drug-Receptor interactions

- The receptor can exist in the **inactive nonfunctional form (R_i)** and in the **activated form (R_a)**
- Even in the absence of any agonist, some of the receptor pool must exist in the R_a form some of the time and may produce the same physiologic effect as agonist-induced activity…….termed **constitutive activity**
- **Agonists** ⟶ have much higher affinity for the R_a configuration and stabilize it….
- ……so large % of the total pool resides in the R_a–D fraction and large effect is produced


Effect
R_i
R_a *
D
D
R_i – D
R_a – D *
Effect


$R_a + D_a$
Full agonist
$R_a + D_{pa}$
Partial agonist
$R_a + D_{ant} + R_i + D_{ant}$
Antagonist
Response
$R_a + R_i$
Constitutive activity
$R_i + D_i$
Inverse agonist
Log Dose

Drug-Receptor interaction

- **Full agonists** → drugs that activate the R_a receptor configuration to the maximum extent
- **Partial agonists**?? → stabilize both the R_a and R_i configuration…….**low intrinsic efficacy** (pindolol)
  - Partial agonists do not stabilize the Ra configuration as fully as full agonists, so that a significant fraction of receptors exists in the R i –D pool
- *Intrinsic efficacy is independent of affinity for the receptor*

Drug-Receptor interaction

- **Antagonist: as in the absence of any drug** fixes the fractions of drug-bound R_i and R_a in the same relative amounts (same as constitutive effect)
- No change will be observed, so the drug will appear to be without effect
- *Block access of agonists* to the receptor and prevent the usual agonist effect → neutral antagonism
- What will happen if a drug has a stronger affinity for the R_i than for the R_a state (stabilizes a large fraction in the R_i–D pool?)
  - Would reduce any constitutive activity resulting in the opposite effects produced by agonists → **Inverse agonists**

Inverse agonist

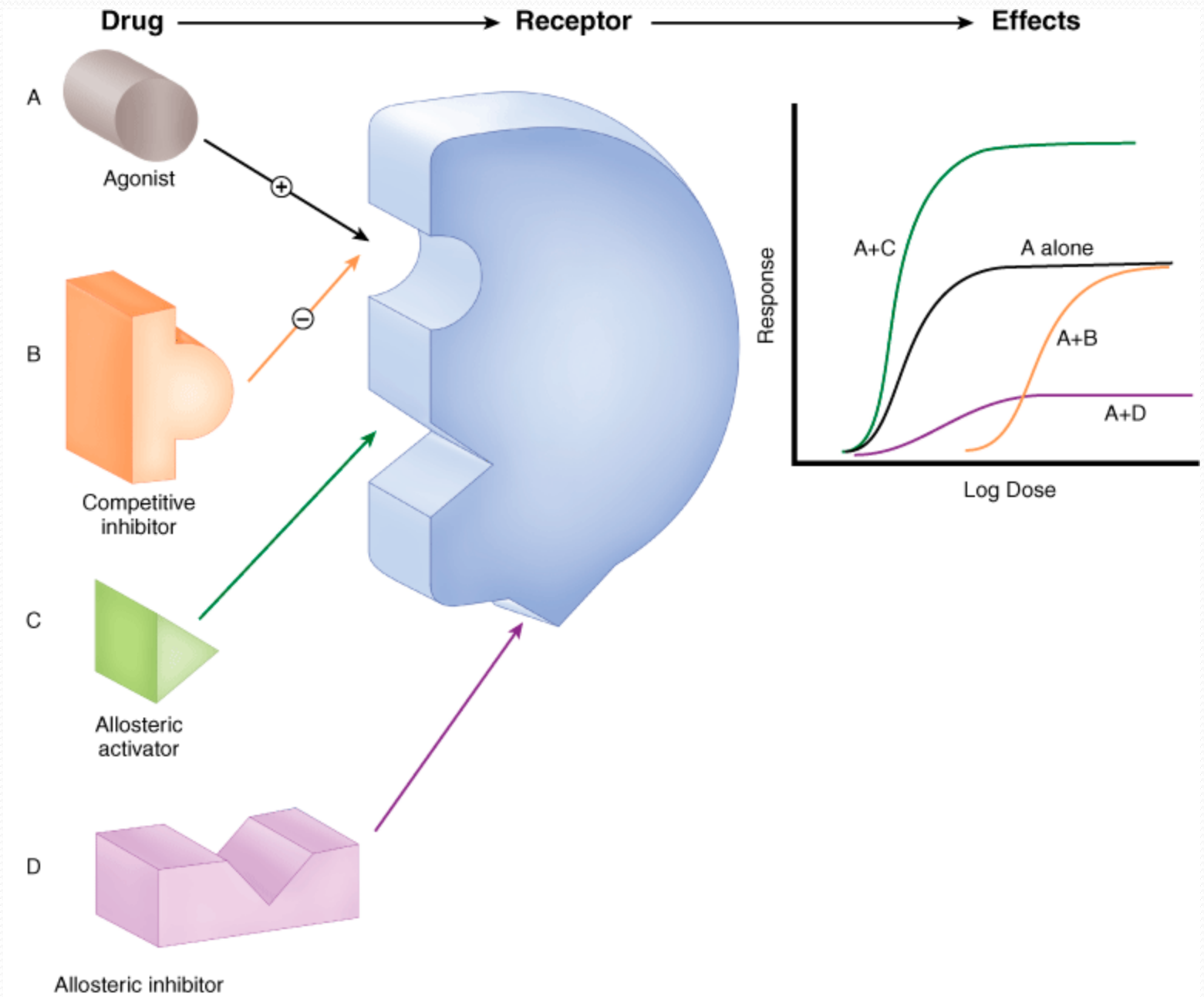
- Drugs with negative efficacy
- **More apparent** in **systems** that express **relatively high receptor levels** and **consequently** have **higher basal activity (constitutive activity)**
- Treatment with an inverse agonist may be appropriate if:
  - receptor is over-expressed (mutation);
  - higher level of basal activity i.e. High Ra conformation
- Examples: famotidine, losartan, and metoprolol

Antagonism

- Antagonists are drugs that decrease the actions of another drug or endogenous ligand to a specific receptor

- Reversible? Irreversible? Compete or not?
  - **Competitive Antagonist (reversible, same binding site)**
  - **Non-competitive antagonism (allosteric [diff. binding site, or irreversible [same binding site])**
    - **Allosteric can be reversibly or irreversibly bound to the allosteric site, based on the type of binding.**

  - **Physiological/ functional**
  - **Chemical antagonism**



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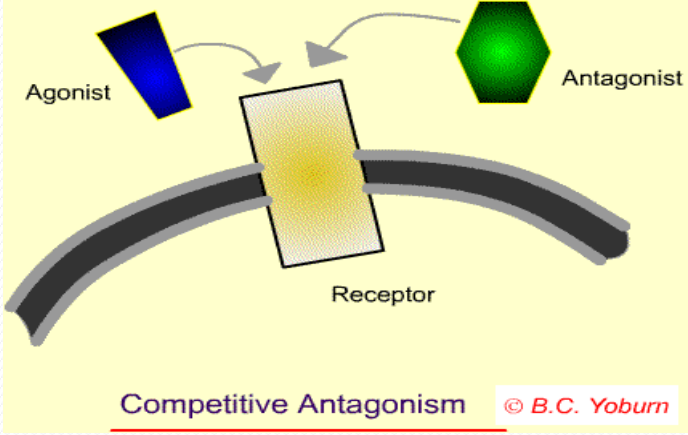
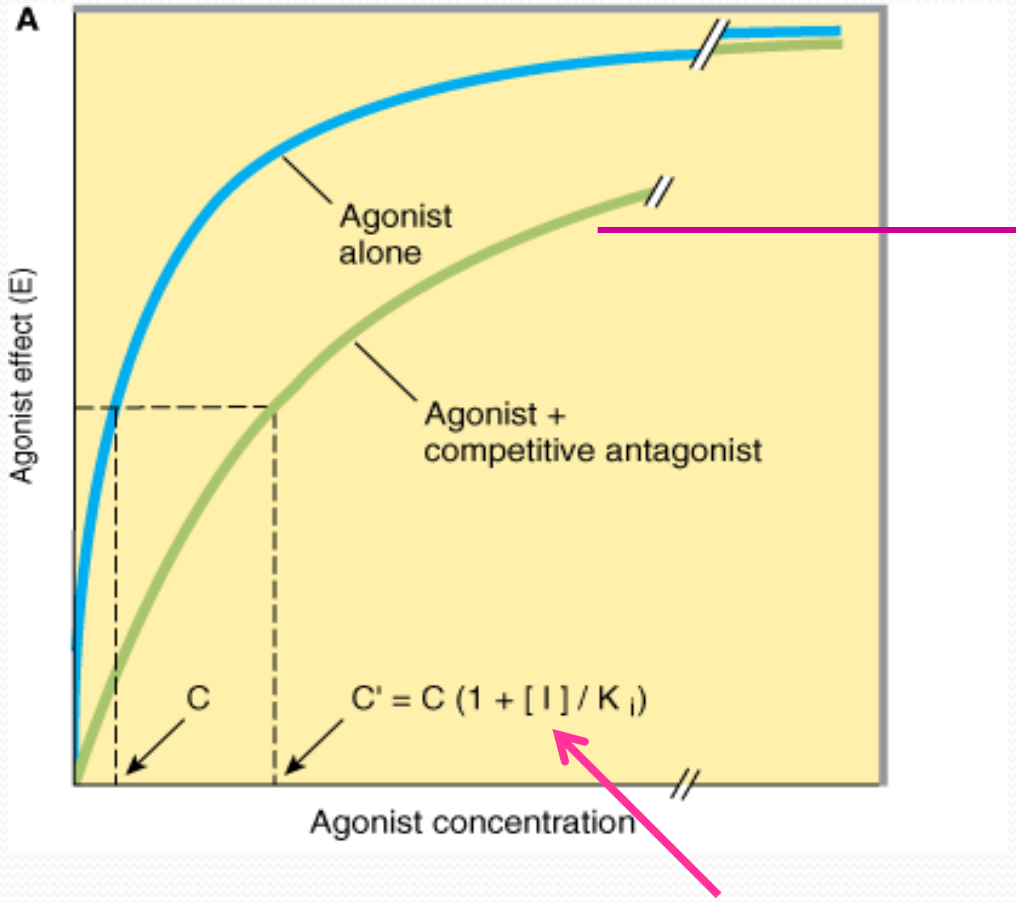
Competitive "reversible" antagonists



- In the presence of a fixed concentration of agonist,
  - increasing concentrations of a reversible **competitive antagonist** progressively inhibit the agonist response;
  - **high antagonist concentrations prevent response completely**

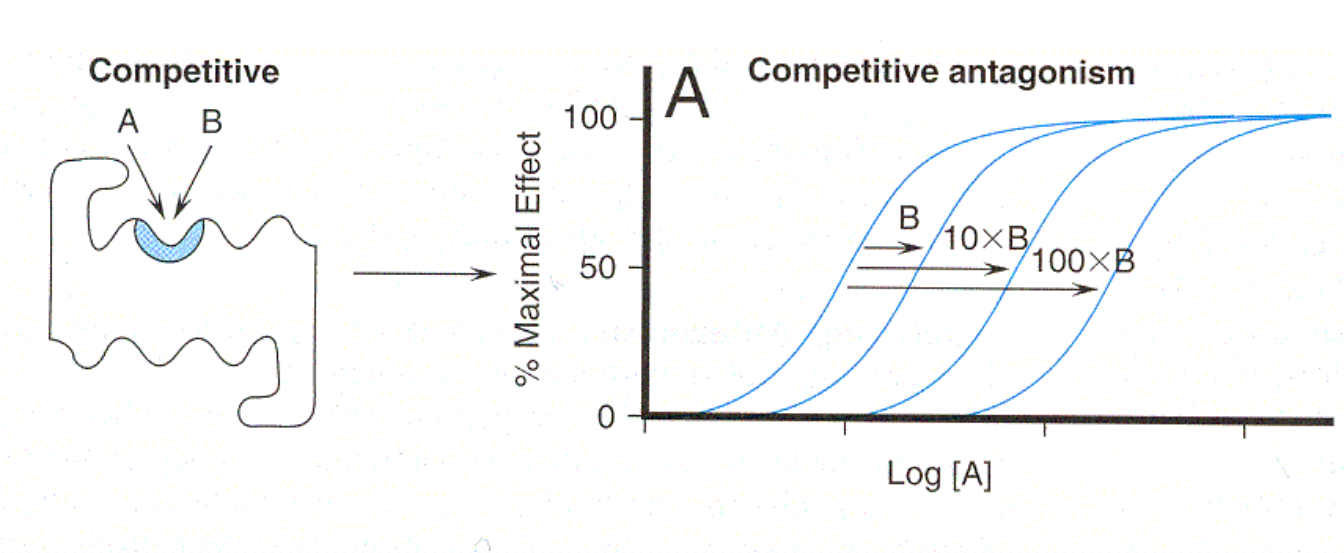
- **Conversely,** sufficiently high concentrations of agonist can surmount the effect of a **given concentration** of the **antagonist;**
-
- That is, the $\mathbf{E_{max}}$ **f**or the **agonis**t remains **the same** for **any fixed concentration of antagonist** (Figure 2–3A). (need higher conc of agonist)

Competitive “reversible” antagonists



Partial Agonist

Competitive “reversible” antagonists



Competitive "reversible" antagonists

- The concentration (C') of an agonist, required to produce a given effect in the presence of a fixed concentration of competitive antagonist ([I]), is **greater than** the agonist concentration (C) required to produce the same effect in the absence of the antagonist
- The ratio of these two agonist concentrations (dose ratio) is related to the dissociation constant (K_i) of the antagonist by the **Schild equation**:

$$\frac{C'}{C} = 1 + \frac{[I]}{Ki}$$

Competitive "reversible" antagonists

- **Two important therapeutic implication of Schild equation:**

1. The degree of inhibition produced by a competitive antagonist depends on the **concentration of antagonist**??

- ……the extent & duration of action of such a drug will depend upon its concentration in plasma and will be influenced by its rate of elimination

  Ex. Beta-blockers conc. may vary between individuals (due to differences in clearance) and must be therefore adjusted

Competitive “reversible” antagonists

- **Two important therapeutic implication of Schild equation:**

2. The concentration of agonist also determines the degree of response to competitive antagonist

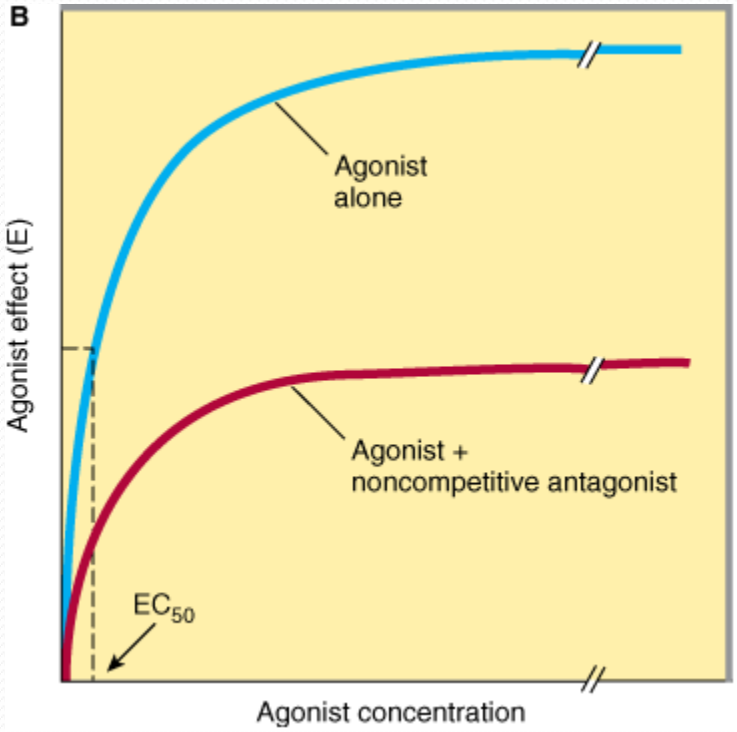
- Ex. action of **propranolol on heart rate** in a **resting heart** but……
- **in** exercise may be overcomed by increase catecholamines…..accordingly, the same dose of propranolol may have little effect under these conditions

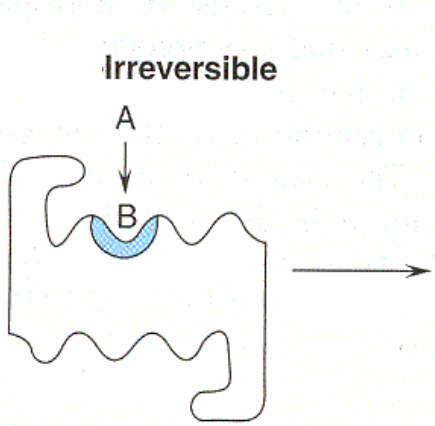
Irreversible antagonists

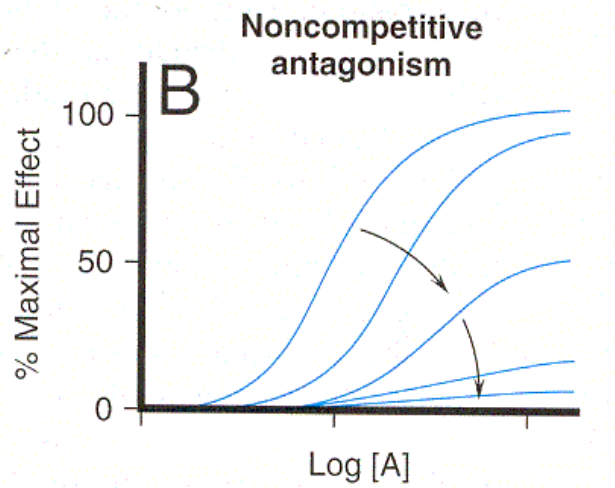
- If the antagonist's affinity for the receptor is so high (bind so tightly or covalent bonds, i.e: antagonist dissociate very slowly) that the receptor is unavailable for binding of agonist
- ….the **number** of **remaining unoccupied** receptors may be **too low** for the **agonist** (**even at high concentrations**) to **elici**t a r**esponse** comparable to the previous maximal response (fig. 2-3B)

- **If spare receptors are present**???
- …..**low dose** of an irreversible antagonist may leave enough receptors unoccupied to allow achievement of maximum response to agonist, (with higher agonist conc.)

Irreversible antagonists







Antagonists

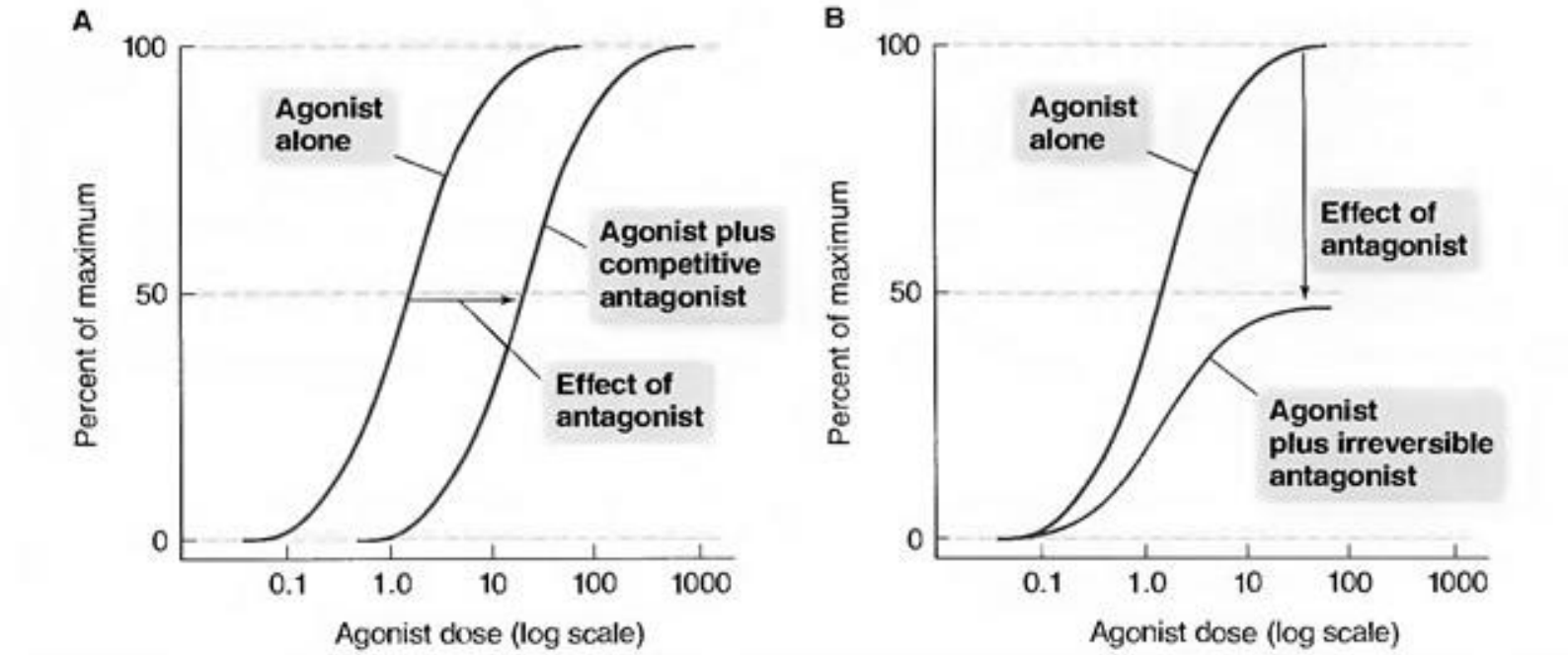


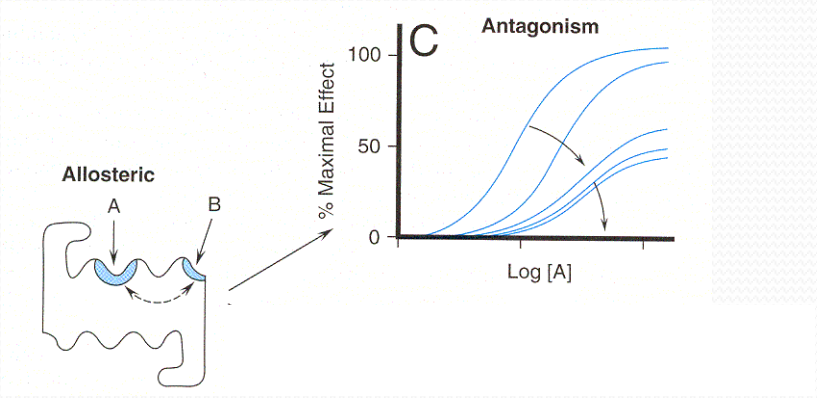
Figure 2–5. Agonist dose-response curves in the presence of competitive and irreversible antagonists. Note the use of a logarithmic scale for drug concentration. ***A.*** A competitive antagonist has an effect illustrated by the shift of the agonist curve to the right. ***B.*** A noncompetitive antagonist shifts the agonist curve downward.

Therapeutically, irreversible antagonists present distinctive advantages and disadvantages

Irreversible antagonists

- **Advantage:** the duration of action of irreversible antagonists is independent of their rate of elimination but on the rate of turnover of receptor molecules
- Phenoxybenzamine (irreversible α-adrenoceptor antagonist) & pheochromocytoma: able to prevent response to high and varying concentrations of agonist;
- **Disadvantage:** difficulty to treat the overdose!
- **Solution:** the excess effects of the drug must be antagonized "*physiologically*", ie by using an agent **that binds to a different receptor and cause the opposing effect**

Non-competitive "allosteric" antagonism



- Allosteric antagonist binds to a site other than where the agonist binds
- **Prevent receptor activation without blocking agonist binding**
- Their actions may be reversible or not if they bind covalently or not
- **Allosteric modulators:** alter the function of the receptor without inactivating the receptor
- Ex. benzodiazepines…..

Physiological antagonism

- Describes interaction between two drugs acting on endogenous regulatory pathways mediated by different receptors….does not involve single type of receptor
- In general, produces **effects that are less specific and less easy to control** than are the effect for receptor-specific antagonist
- Ex: insulin opposes hyperglycemic effect of glucocorticoids through different receptors
- Ex. bradycardia caused by Ach may be managed by atropine OR the physician could use isoproterenol

Chemical antagonism

- Some types of antagonism **does not involve interaction of drug with a receptor**
- Example: **protamine (+)** can be used clinically to **counteract the effects of heparin (-)**
- In this case, one drug acts as a **chemical antagonist** of the other simply by **ionic binding** that makes the other drug unavailable for interactions with proteins involved in blood clotting

Agonism

- **Agonist….**bind to a receptor and produces a biologic response **that mimic directly or indirectly the response to the endogenous ligand**

- In general……strong affinity to receptor and good efficacy

- Because they amplify the effects of physiologically released agonist ligands, their effects are sometimes more selective and less toxic than those of exogenous agonists

Full & Partial Agonist

- Based on the Emax that occurs when all receptors are occupied, agonists can be divided into two classes:
  - **Full agonist:** is capable of producing a maximal response
  - **Partial agonist:** produces a lower response at full receptor occupancy than do **full agonists**
  - **Produce concentration-effect curves that resemble those observed with full agonists in the presence of irreversible antagonist**
  - Many drugs used clinically as antagonist are actually weak partial agonist….(less ADE)
  - Decreased affinity for binding to receptors??
  - *Self-study: Figure 2-4*

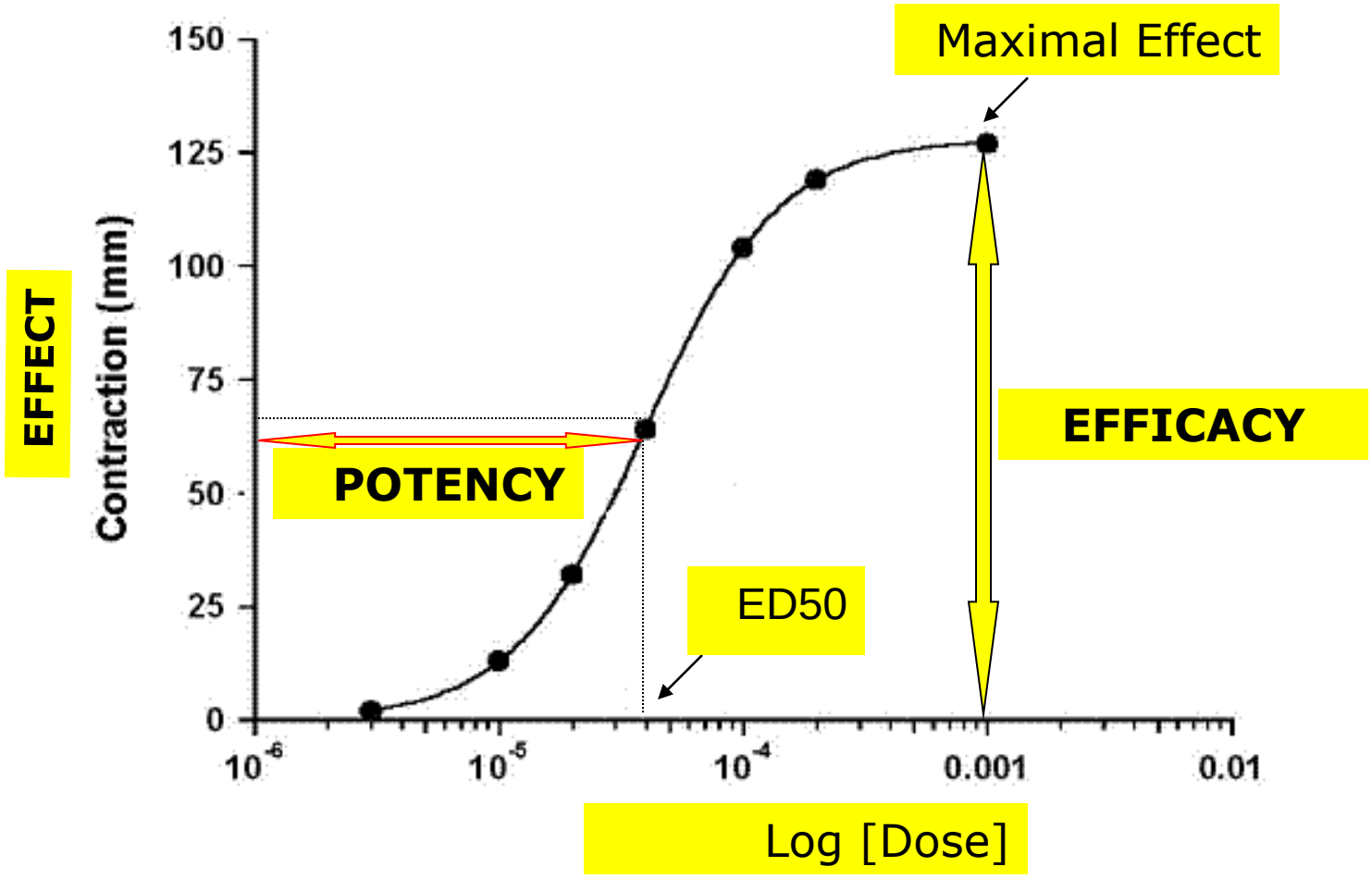
RELATION BETWEEN DRUG DOSE & CLINICAL RESPONSE

Dose & Response in Patients

A. Graded dose-response relation

To choose among drugs and to determine appropriate doses of a drug, the clinician must know the relative **pharmacologic potency** and **maximal efficacy** of the drugs in relation to the desired therapeutic effect

→ **Graded dose-response** curves **relate do**ses of **drugs** to a **particular therapeutic effect** (decrease BP, increase urinary sodium excretion)


Maximal Effect
EFFECT
Contraction (mm)
150
125
100
75
50
25
0
POTENCY
EFFICACY
ED50
10^{-6}
10^{-5}
10^{-4}
0.001
0.01
Log [Dose]

A. Graded dose-response relations

- **Potency** refers to the concentration (EC50) or dose (ED50) of a drug required to produce 50% of the drug's maximal effect (Emax)
- **Potency** of **a drug depends** in part on the **affinity (Kd)** of receptors for binding the drug **and** in part on the efficiency with which drug-receptor interaction is coupled to response (**efficacy)**
- It is necessary to distinguish between a drug's potency and its efficacy

A. Graded dose-response relations

- The **clinical effectiveness** of a drug depends not on its potency but:
  I. Maximal efficacy
  II. Ability to reach the relevant receptors 
- In choosing a drug, clinicians must consider **relative effectiveness** than potency
- Pharmacologic potency can largely determine the administered dose of the chosen drug
- Potency is expressed in dosage units, in terms of a particular end point (50mg for mild sedation)
- Relative potency???

A. Graded dose-response relations

- **Maximal efficacy** is determined by:
  - Drug's **mode of interactions with receptors** (partial agonists!)
  - Characteristics of **the receptor-effector system** involved (e.g. diuretics)
- The practical efficacy of a drug may be limited by the drug's propensity to cause a toxic effect (eg, cardiac arrhythmia with a positive inotropic drug
- **Maximal efficacy….Therapeutic efficacy**….

A. Graded Dose-response Relations (cont'd)

- **Limitations of the graded dose-response curve**:
  I. Graded dose-response curves may be impossible to construct if the pharmacologic response is an either-or (quantal) event, such as **prevention of convulsions, arrhythmia, or death**

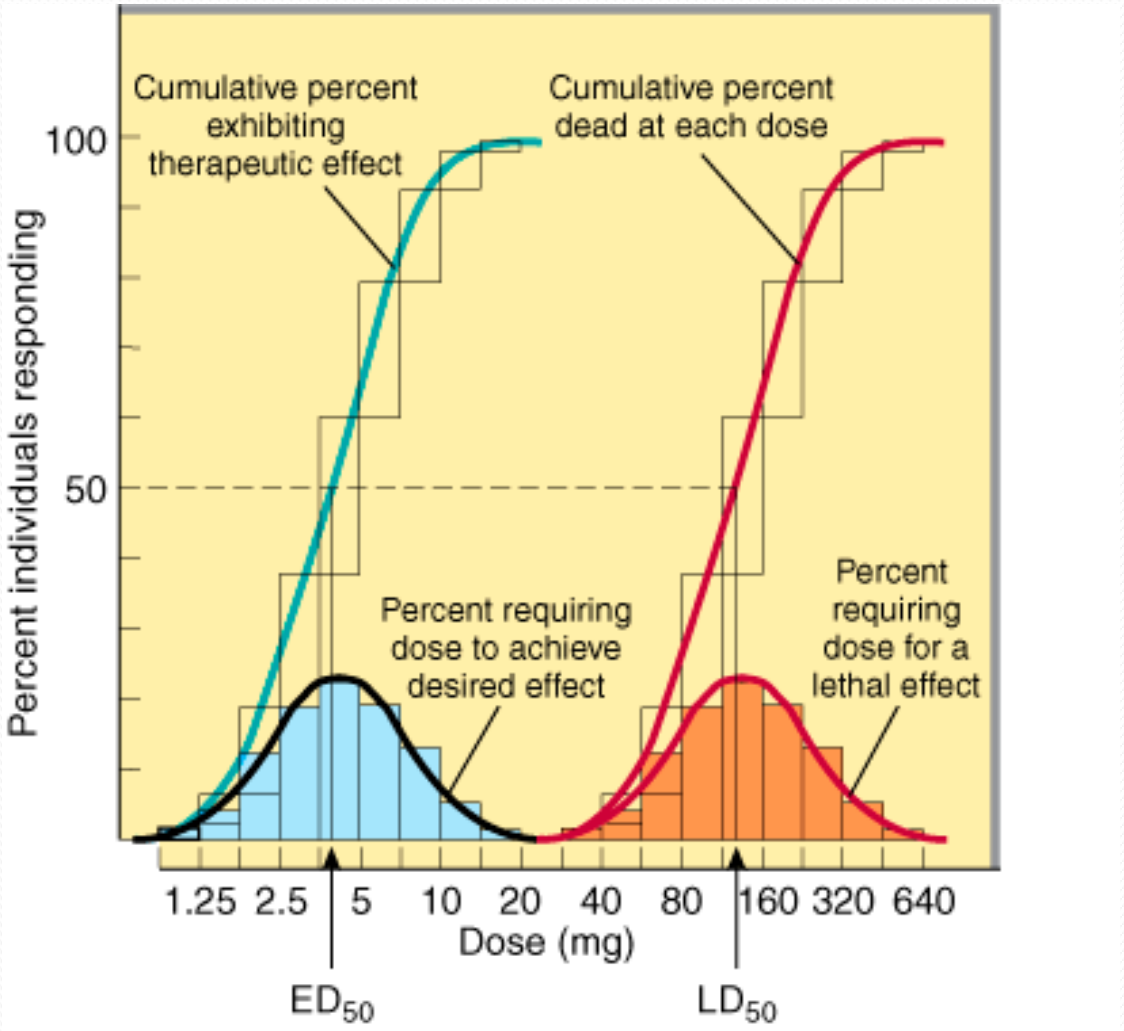
  II. **Graded dose-response curves** obtained **in a single** patient may be **limited in application** to **other patient** (variability among patients in severity of disease and responsiveness to drugs)

B. Quantal dose-response relation

- Determines the **dose of a drug** required to produce **a specific** magnitude of effect in a **large number of individuals** (or **experimental animals**)
- Determines the **cumulative frequency** distribution of responders **versus** the **log dose**
- For most drugs, the **doses** required **to produce** a **specified quantal effect** in individuals are l**ognormally** distributed that is, a frequency distribution of such responses plotted against the log of the dose produces a (gaussian normal curve of variation)

B. Quantal dose-response relation

- When these responses are **summated,** the resulting cumulative frequency distribution constitutes **a quantal dose-effect curve** (or **dose-percent curve**) of **the percentage** of **individuals who exhibit the effect** plotted as a **function of log dose**



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B. Quantal dose-response relation (Cont'd)

The **quantal dose-effect curve** is often **characterized** by:

- **Median effective dose (ED_{50})**: **the dose** at which **50%** of the **individuals/population exhibit** the **specified** **desirable/therapeutic effect**

- **Median toxic dose (TD_{50}):** the **dose** at which **50%** of individuals/population exhibit **a particular toxic effect**
- If the toxic effect is death of the animal, a **median lethal dose (LD_{50})** may be experimentally defined

B. Quantal Dose-Response Curves

These values allow us to:

a. **Compare potencies** of drugs

b. **Obtain index of selectivity** of **drug action** (e.g. **suppression of cough** against **analgesia** for opioids drugs)

c. Estimating **the margin of safety (i.e. therapeutic index**)

THERAPEUTIC INDEX = TD_{50}/ED_{50}

* **Relates the dose of a drug required to produce a desired effect to that which produces an undesired effect**

Therapeutic index

- In humans, the therapeutic index of a drug is almost never known with real precision;
  - is determined using drug trials and accumulated clinical experience. These usually reveal a range of effective doses and a different (**sometimes overlapping** e.g. warfarin) range of toxic doses

- The clinically acceptable risk of toxicity depends critically on the severity of the disease

Therapeutic drug monitoring (TDM)

- Given the multiple factors that alter drug disposition, measurement of the concentration in body fluids can assist in individualizing therapy with selected drugs
- Determination of the concentration of a drug in blood, serum, or plasma is particularly **useful when** well-defined criteria are fulfilled:
  1. A demonstrated relationship exists between the concentration of drug in plasma and the desired therapeutic effect or the toxic effect to be avoided (i.e. therapeutic window)
  2. There is **sufficient variability in plasma level** that **the level cannot** be predicted from **the dose alone**

Therapeutic drug monitoring (TDM) (Cont'd)

3. The drug produces effects, intended or unwanted, that are difficult to monitor
4. **The concentration required to produce the therapeutic effect is close to the level that causes toxicity**

- The use of the population therapeutic window to adjust dosage of a drug should be complemented by monitoring appropriate clinical and surrogate markers for drug effect

Therapeutic drug monitoring (TDM) (Cont'd)

DRUG CONCENTRATION (KINETIC-DYNAMIC)

1. Choose the target concentration, TC
2. Predict volume of distribution (Vd) and clearance (CL) based on standard population values (eg, Table 3–1) with adjustments for factors such as weight and renal function
3. Give a loading dose or maintenance dose calculated from TC, Vd, and CL
4. Measure the patient's response and drug concentration
5. Revise Vd and/or CL based on the measured concentration
6. Repeat steps 3–5, adjusting the predicted dose to achieve TC

Variation in drug responsiveness (Cont'd)

- Four general mechanism may contribute to variation in drug responsiveness:

1. Alteration in concentration of drug that reaches the receptor (i.e. pharmacokinetic factors, P-gp)
2. Variation in concentration of an endogenous receptor ligand (antagonist; partial agonist)
3. Alteration in number or function of receptors (efficiency of coupling) *ligand induce increase or decrease in n. of receptors….(*up* or *down-regulation, desensitization….overshot phenomenon)/*genetic variations
4. Changes in components of response distal to the receptor; biochemical processes (largest and most imp. mech of variation)

Variation in drug responsiveness

- A **single** individual may respond differently to the same drug at different times during the course of treatment
- Occasionally, individuals exhibit an unusual or **idiosyncratic** drug response, (infrequently observed in most patients)
- The idiosyncratic responses are usually caused by **genetic differences in metabolism of the drug or by immunologic mechanisms**
- Quantitative variations in drug response are more common and more clinically important: **hyporeactive** or **hyperreactive** (no to be confused with hypersensitivity)

Variation in drug responsiveness

- Often, the effect of a drug gradually diminishes when it is given continuously or repeatedly
- **Tolerance** is a decrease in the intensity of response to a drug as a result of **continued drug** administration
- **Tachyphylaxis,** when responsiveness diminishes **rapidly** after administration of a drug
- The clinician must be prepared to change either the dose of a drug or the drug; taking into consideration that some drug produce tolerance or tachyphylaxis more than other
- Other factors are **age**, **sex**, **body size**, **disease state**, **genetic factors**, and **concurrent drug administration**
- ***Drug resistance??***

Clinical Selectivity: Beneficial vs. Toxic Effects of Drugs

- Although we classify drugs according to their principal actions, it is clear that ***no drug causes only a single, specific effect***
- Drugs are only *selective*—rather than specific—in their actions, because they bind to one or a few types of receptor more tightly than to others and because these receptors control discrete processes that result in distinct effects
- *Any drug*, no matter how trivial its therapeutic actions, *has the potential to do harm*
- Selectivity can be measured by comparing binding affinities of a different receptors **or** by comparing **ED_{50}s** for **different effect**s of **a drug** in vivo (*Therapeutic vs. Toxic effect*)

Clinical Selectivity: Beneficial vs. Toxic Effects of Drugs

"Side effects"…

- **Beneficial & toxic** effects mediated by the same **receptor-effector mechanism** (i.e. **extension of the therapeutic effect**) *eg. **bleeding** caused by **anticoagulant therapy; hypoglycemic coma due to insulin….!(synergic effect)***

- **Beneficial & toxic effects** mediated by identical receptors but in different tissues or by different effector pathways*? lower doses, synergism, local effect (eg. aerosol, glucocorticoid)*

- Beneficial and Toxic Effects Mediated by **Different Types of Receptors** *(H1 and H2 antihistamines, nicotinic and muscarinic blocking agents…)…SAR!*

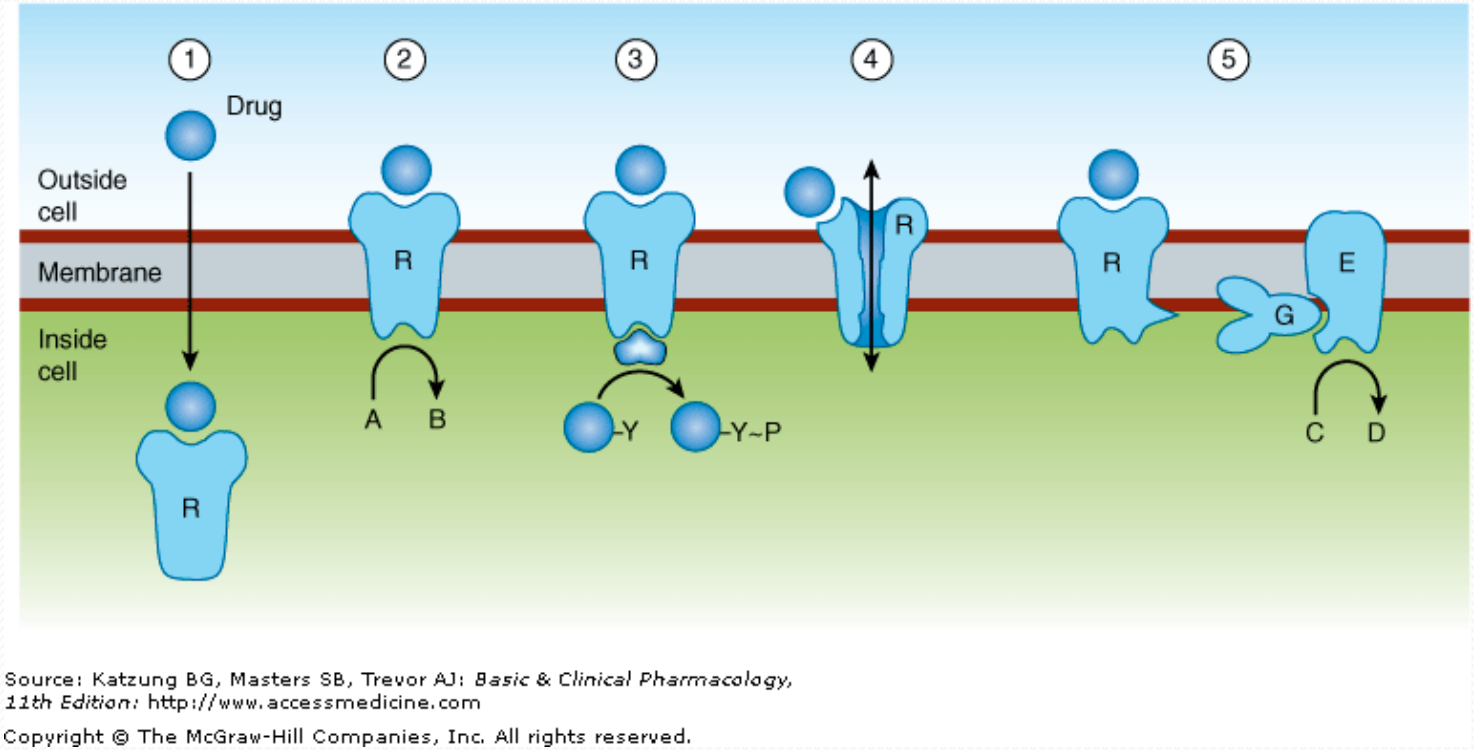
Drug, Receptor, Effector

- Drug (D) + receptor-effector (R) → drug-receptor-effector complex → effect
- D + R → drug-receptor complex → effector molecule → effect
- D + R → D-R complex → activation of coupling molecule → effector molecule → effect
- Inhibition of metabolism of endogenous activator → increased activator → increased effect

Overview

- Receptors elicit many different types of cellular effect. Some of them are of very rapid , intermediate, or long timescales
- Therapeutically exploitable pharmacologic receptors are responsible for transducing extracellular signal into intracellular response (the transduction mechanism)
- The transmembrane signalling is accomplished by a small number of different mechanisms. Each type of mechanism has been adapted to transduce many different signal i.e. Linkage between receptor occupation and response

Five Known Transmembrane Signaling Mechanism



I. Intracellular Receptors for Lipid-Soluble Agents
II. Ligand-Regulated Transmembrane Enzymes Including Receptor Tyrosine Kinases
III. Cytokine Receptors
IV. Ligand- and Voltage-Gated Channels (*ionotropic*)
V. G Proteins & Second Messengers (*metabotrobic*)

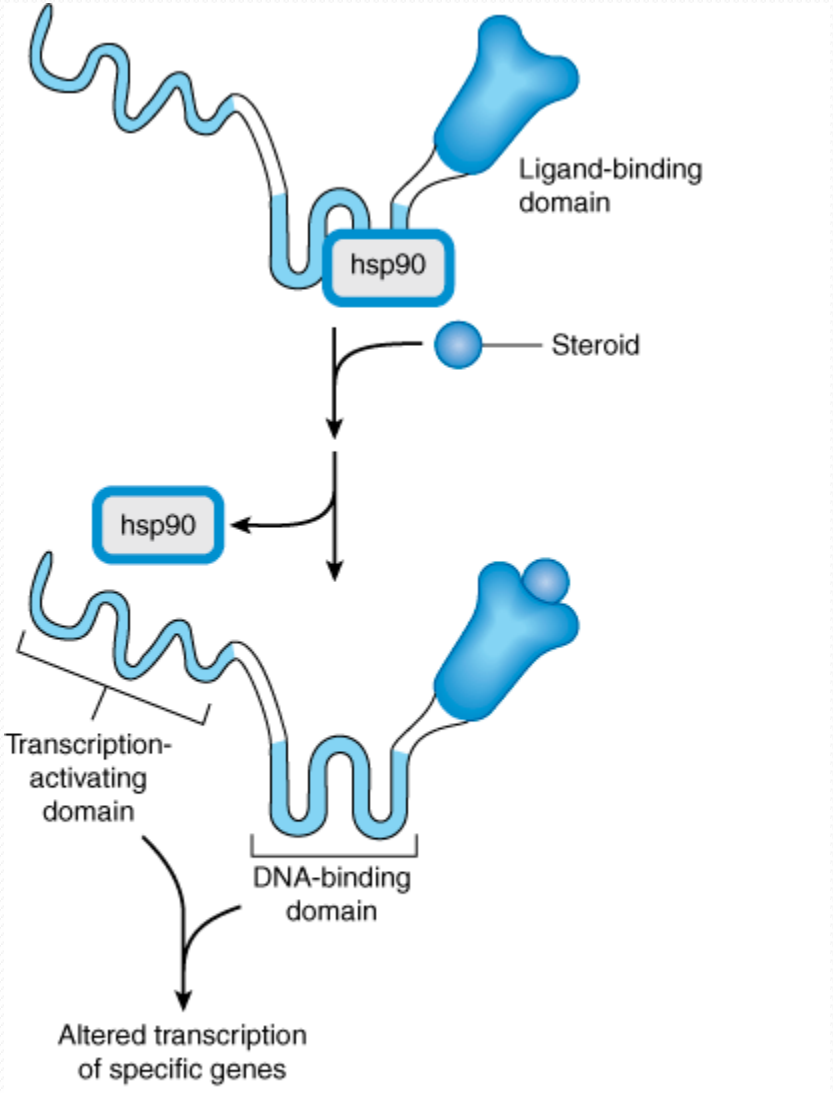
I. Intracellular Receptors for Lipid-Soluble Agents

- These receptors generally regulate gene transcription
- Receptors for **steroids**, **vitamin D**, and **thyroid hormone**
- Ligands sufficiently lipid soluble to cross the plasma membrane and interact with the **intracellular receptor**
- Stimulates the transcription of genes in the nucleus by binding to specific DNA sequences near the gene whose expression is to be regulated

I. Intracellular Receptors for Lipid-Soluble Agents

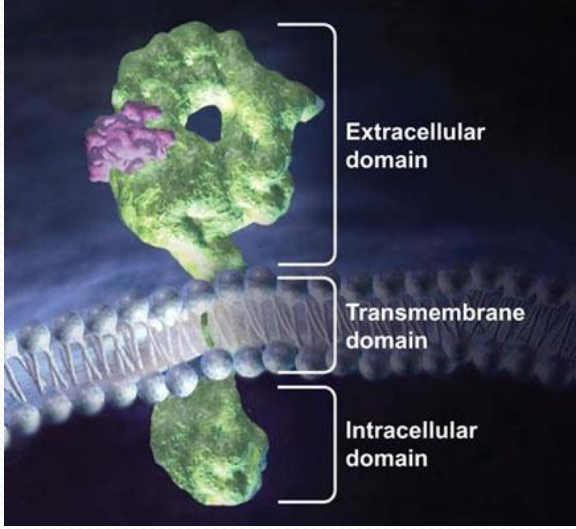
Hormones that act by **regulating** gene expression have two **therapeutic consequences**:

- Effects occur after a period of 30 min. to several hours (*time required for the synthesis of new proteins*).
  - Note, eg. glucocorticoids will not immediately relieve the symptoms of acute bronchial asthma)
- Effects can persist for hours or days after the agonist disappearance
  - (slow turnover of enzymes and proteins, which can remain active after have been synthesized)



- **Mechanism of glucocorticoid action**. The glucocorticoid receptor polypeptide is schematically depicted as a protein with **three distinct domains**.
- A heat-shock protein (hsp90), binds to the receptor in the absence of hormone and prevents folding into the active conformation of the receptor.
- Binding of a hormone ligand (steroid) causes dissociation of the hsp90 stabilizer and permits conversion to the active configuration.

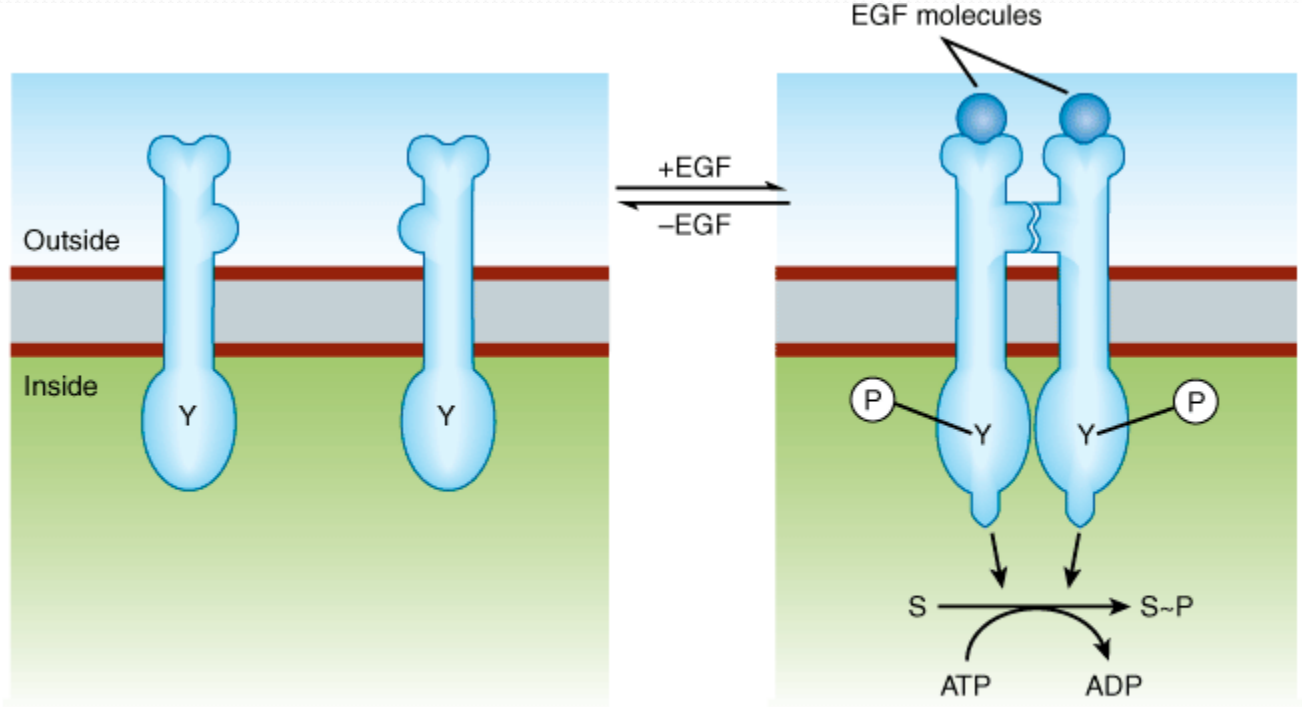
II. Ligand-Regulated Transmembrane Enzymes Including Receptor Tyrosine Kinase



- Mediates the first step in signaling by **insulin**, **growth factors** (**EGF, PDGF, TGF-β**), **atrial natriuretic peptide** (**ANP**), and many other trophic hormones
- Extracellular **hormone-binding domain** and a cytoplasmic **enzyme domain**, which may be a:
  - protein tyrosine kinase
  - serine kinase
  - guanylyl cyclase

II. Ligand-Regulated Transmembrane Enzymes Including Receptor Tyrosine Kinase

- **Binding of ligand to the receptor's extracellular domain cause**:
  1. change in the receptor conformation….
  2. …..receptor molecules bind to one another bringing together the tyrosine kinase domains that become enzymatically active…
  3. …..the domains phosphorylate each other as well as additional downstream signaling proteins
- E.g, insulin, uses a single class of receptors to trigger increased uptake of glucose and amino acids and to regulate metabolism of glycogen and triglycerides in the cell



Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology, 11th Edition:* http://www.accessmedicine.com

Upon binding of EGF (circle), the receptor converts from its ***inactive monomeric state*** *(left) to an* ***active dimeric state*** *(right), in which two receptor* ***polypeptides bind noncovalently. The cytoplasmic domains become phosphorylated (P) on*** *specific tyrosine residues (Y) and their* ***enzymatic activities are activated****, catalyzing phosphorylation of different substrate proteins (S)…..modulation of different biochemical processes*

II. Ligand-Regulated Transmembrane Enzymes Including Receptor Tyrosine Kinase

- The intensity and duration of action of EGF, PDGF, and other agents that act via receptor tyrosine kinases are limited by a process called receptor **down-regulation??**
- The **total number** of **cell-surface receptors is reduced** and the **cell's responsiveness** to **ligand is correspondingly diminished**
- **Genetic mutations** cause **excessive growth factor–induced cell proliferation** and are associated with an **increased susceptibility to certain types of cancer**
- Drugs: inhibitor of tyrosine kinase receptors…..tx of cancer

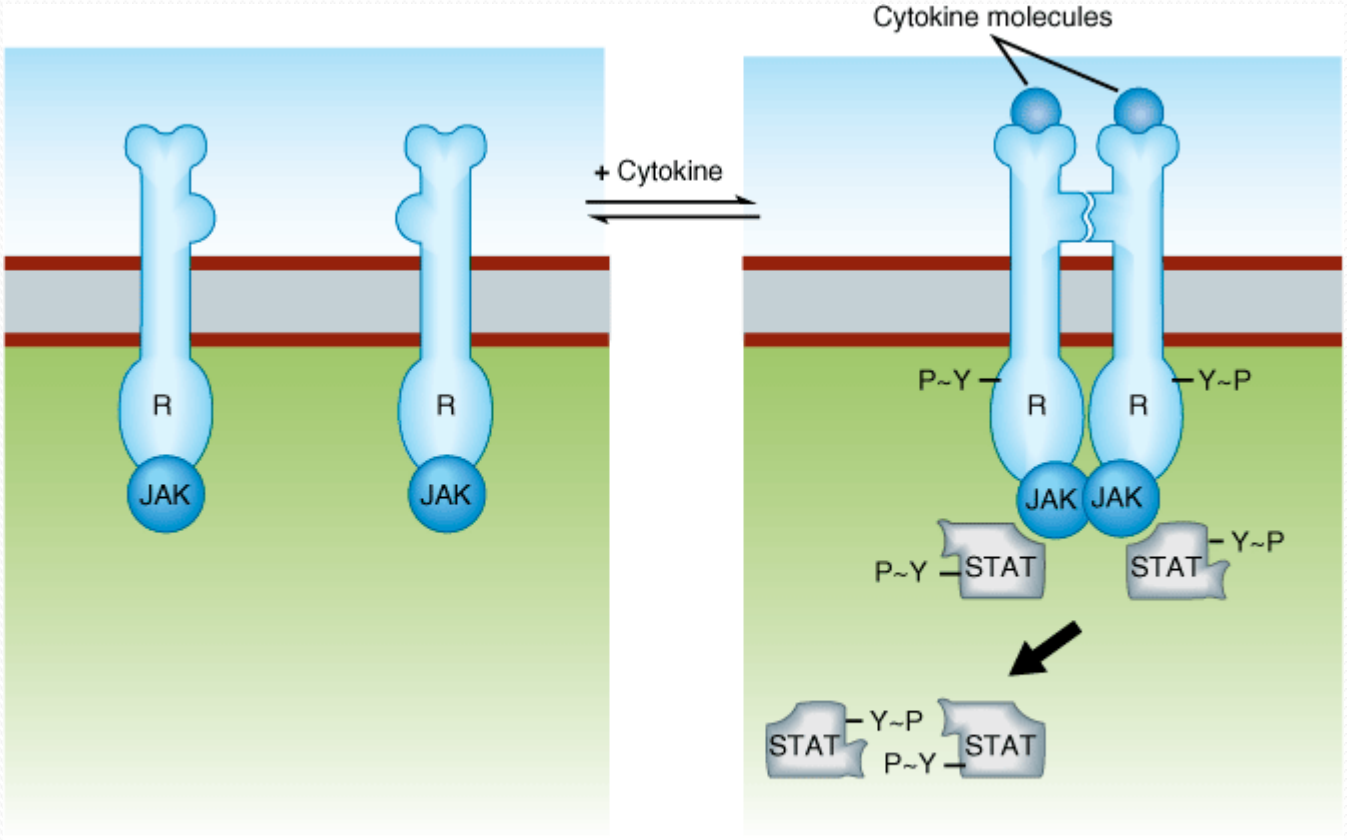
III. Cytokine Receptors

- **Resembl**e mechanism of **receptor tyrosine** kinases,
  - **excep**t that in this case, the **protein tyrosine kinase** activity is **not** i**ntrinsi**c to **the receptor molecule**

- Respond to **heterogeneous** group of **peptide ligands** such as **growth hormone**, **erythropoietin**, **interferons**, and other regulators of growth and differentiation

- A separate protein tyrosine kinase, from the **Janus-kinase (JAK)** family, binds **noncovalently** to the receptor;

- **Cytokine receptors dimerize** after they **bind** the activating **ligand**, allowing the **bound JAKs** to become a**ctivated** and to p**hosphorylate** t**yrosin**e residues on **the receptor**;



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After activation by an appropriate ligand, (JAK) molecules are activated, resulting in phosphorylation of **signal transducers and activation of transcription** (STAT) molecules STAT dimers then travel to the nucleus, where they regulate transcription

III. Cytokine Receptors

- Phosphorylated tyrosine residues bind another set of proteins, called STATs (signal transducers and activators of transcription);
- The **bound STATs** are themselves **phosphorylate**d by **the JAKs**, **two STAT molecules dimerize** (attaching to one another's tyrosine phosphates);
- Finally the **STAT/STAT dimer dissociates from the receptor** and travels to **the nucleus**, where it **regulates transcription of specific genes**

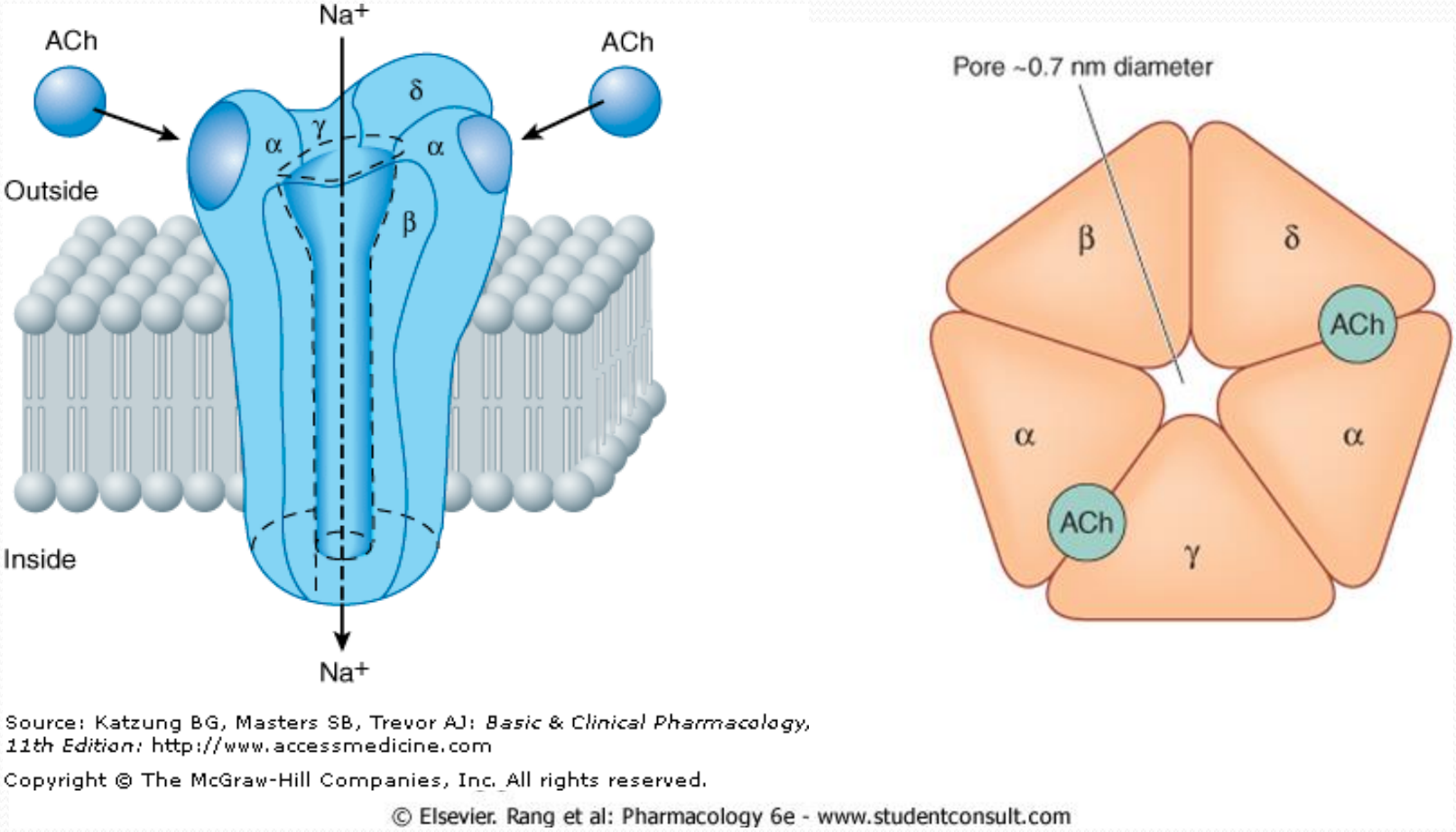
IV. Ligand- and Voltage-Gated Channels (ionotropic)

Ligand-gated channels

- Many of the most useful drugs in clinical medicine act by mimicking or blocking the actions of endogenous ligands that regulate the flow of ions though plasma membrane channels

- Examples: receptors for **acetylcholine**, **serotonin**, **γ-aminobutyric acid**, & excitatory amino acids (**glycine, aspartate, glutamate**)

- **Receptors transmit signal across the plasma me**mbrane by
  - **increasing transmembrane conductanc**e of the **relevant io**n and
  - altering the electrical potential across the membrane (e.g, AChR)



Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology, 11th Edition:* http://www.accessmedicine.com

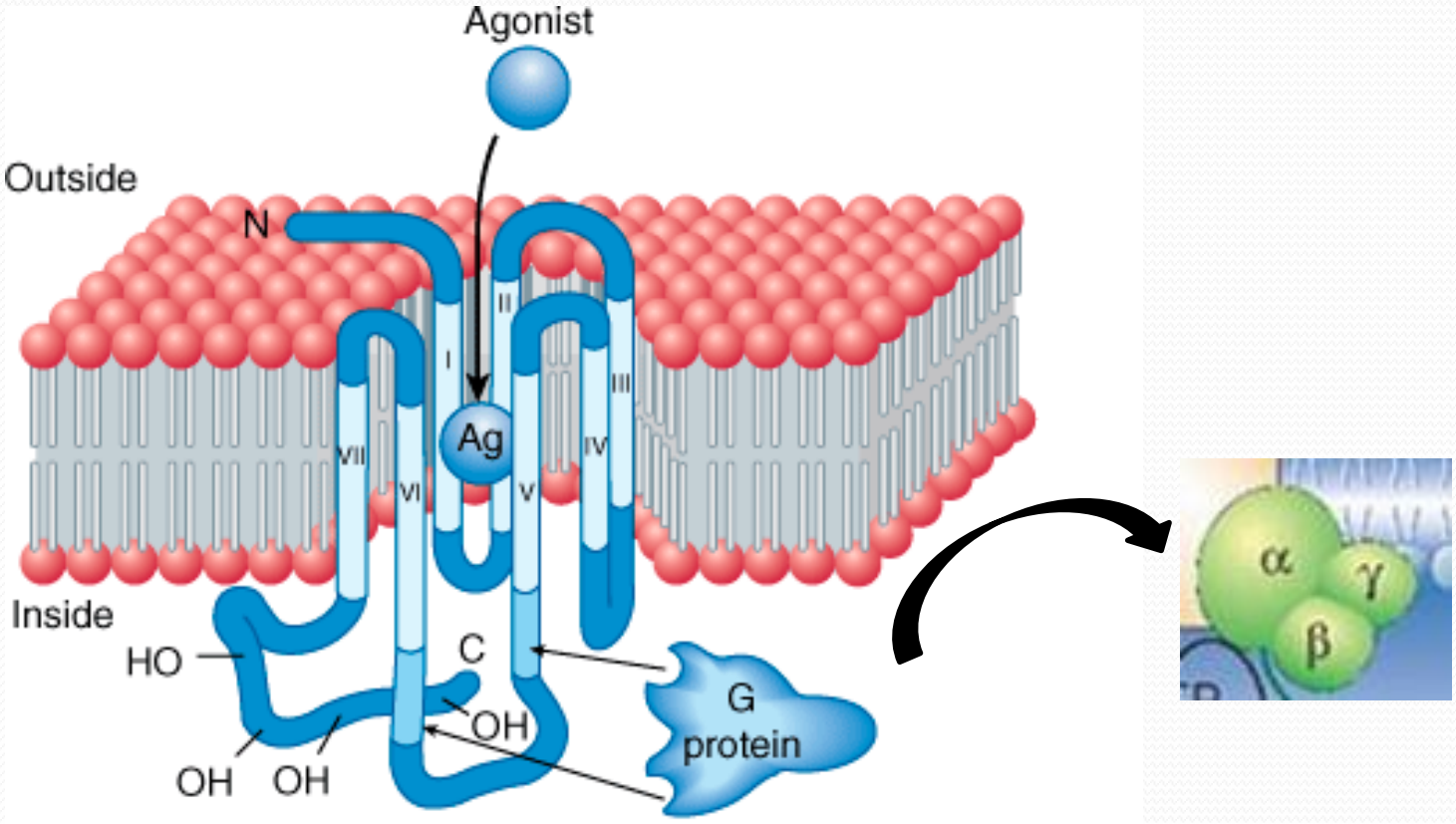
Nicotinic acetylcholine receptor: five receptor subunits (α2, β, γ, δ) form a cluster surrounding a central transmembrane **pore**, the lining of which is formed by the M_2 helical segments of each subunit. These contain a preponderance of negatively charged amino acid, **which makes the pore cation selective**. There are two acetylcholine binding sites in the extracellular portion of the receptor, at the interface between the α and the adjoining subunits. When **acetylcholine binds**, the kinked α helices either straighten out or swing out of the way, **thus opening the channel pore**. (Based on Unwin [1993], [1995].)

IV. Ligand- and Voltage-Gated Channels (ionotropic)

- The **time** elapsed between the **binding of the agonist** and the **cellular response** is very rapid; **milliseconds**
- Important for moment-to-moment transfer of information across synapses
- **Voltage-gated** ion channels **do not bind neurotransmitters directly** but are controlled by **membrane potential**;
- such channels are also **important drug targets**:
  - Example: **verapamil** inhibits **voltage-gated calcium channels** that are present in the **heart** and in **vascular smooth muscle**, producing **antiarrhythmic** effects and **reducing blood pressure**

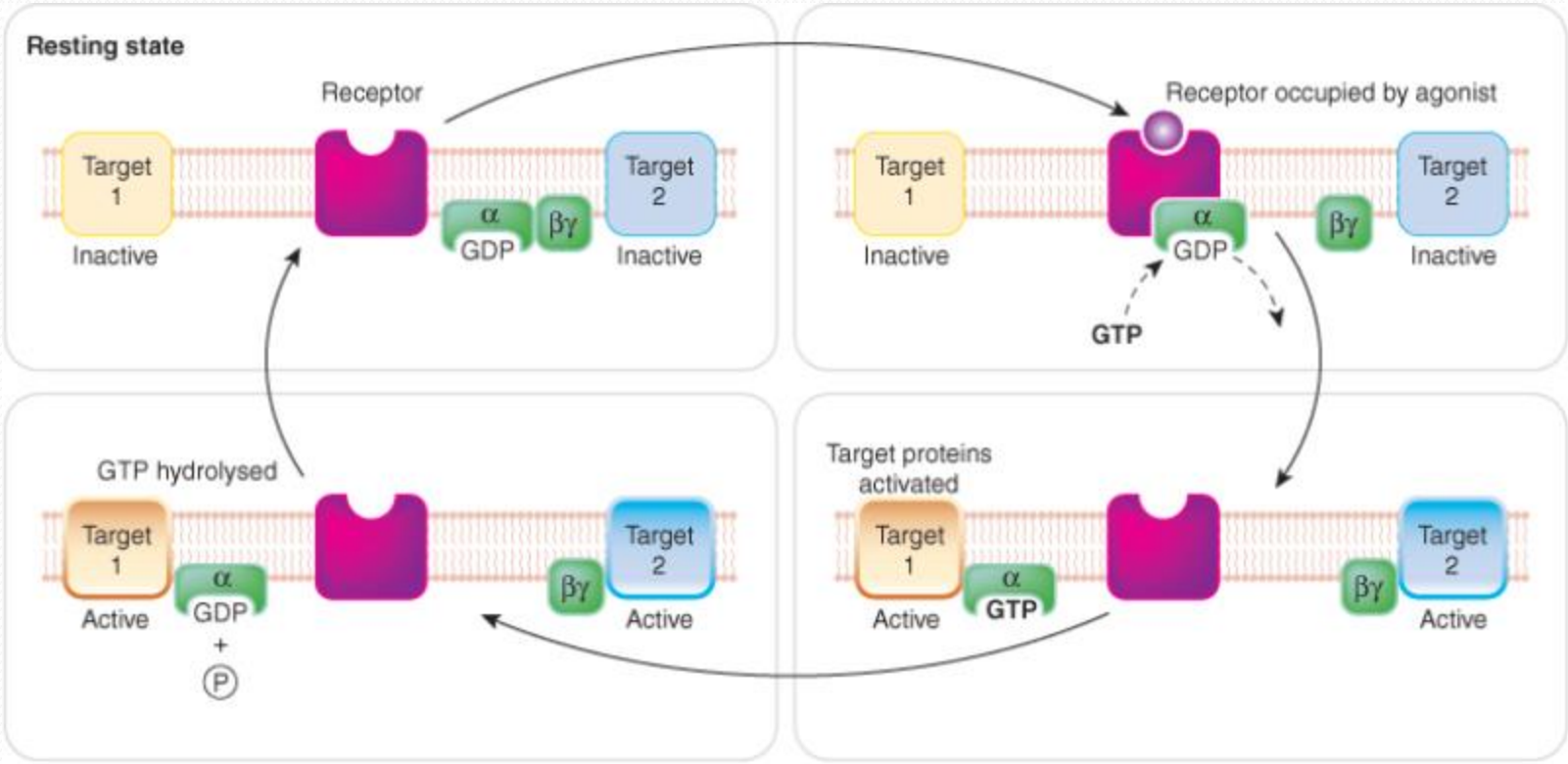
V. G-Proteins and Second Messengers (metabotropic)

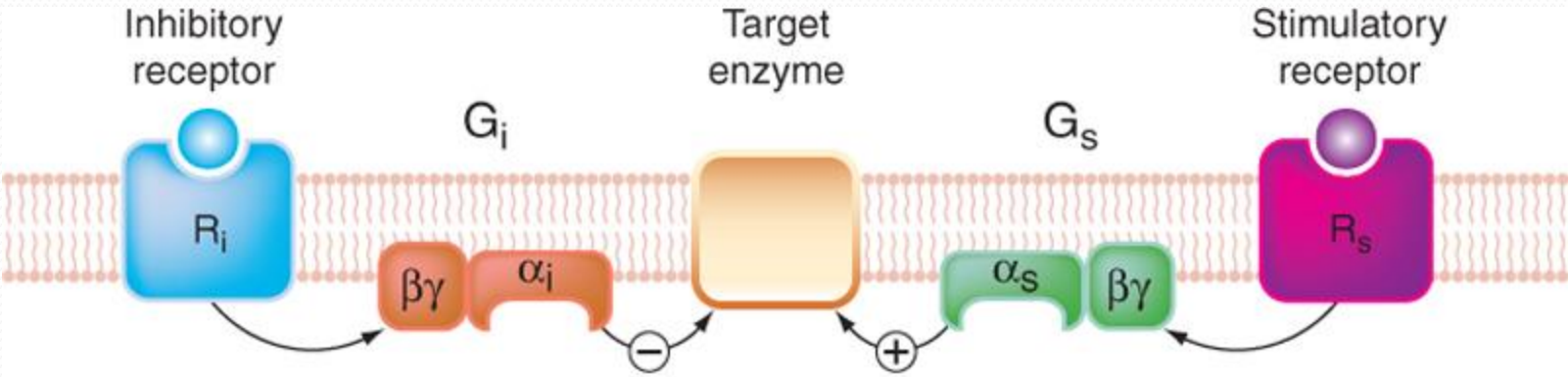
- Many ligands act by increasing intracellular concentrations of second messengers (**cAMP, calcium ion,** or the **phosphoinositides)**
- Ex.
  - **adrenoceptors**,
  - glucagon receptor,
  - thyrotropin receptor,
  - muscurinic receptor
  - and certain subtypes of dopamine and serotonin receptors
- Odorants, and even visual receptors (in retinal rod and cone cells)



Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology, 11th Edition:* http://www.accessmedicine.com

- Polypeptide chain that traverses the plane of the membrane seven times
- Extracellular amino (N) terminal & intracellular carboxyl (C) terminal
- G protein interacts with the third cytoplasmic loop
- The receptor's cytoplasmic terminal tail contains numerous serine and threonine residues whose hydroxyl (-OH) groups can be phosphorylated. This phosphorylation may be associated with diminished receptor-G protein interaction.(desensitization)





V. G-Proteins and Second Messengers (metabotropic)

- Transmembrane signaling system include three separate components:

1. **Detection** of **the ligand** by a cell-surface **recept**or

2. **Activation of a G protein** located on the cytoplasmic face of the receptor

3. The activated **G protein** then **changes the activity of an effector element**, (an enzyme or ion channel)

4. >>>>> this element then changes the concentration of the **intracellular second messenger**

V. G-Proteins Coupled Receptors (metabotropic)

- Many types of G protein have been identified (Table 2–1), each of which mediates effects of a particular set of receptors to a distinctive group of effectors (G_s, G_i, G_g,....)
- Note that an endogenous **ligand** may **bind** and stimulate **receptors** that couple to **different subsets of G** proteins, allowing to elicit **different responses** in **different cells**
- **G_s and other G proteins** use a molecular mechanism that involves:
  - **binding and hydrolysis of GTP**, this mechanism **allows** the **transduced signal** to **be amplified**
  - (NE may encounter its receptor for only few **milliseconds** while GTP-bound G_s may remain active for **tens of seconds**)…spare receptor!

Well-established second messengers

1. Cyclic adenosine monophosphate (cAMP)

- cAMP regulates many aspects of cellular function including:

  - **Mobilization of stored energy** (**carbohydrate** or triglycerides (**TG**) breakdown stimulated by **β-adrenomimetic** catecholamines)

  - **Conservation of water by kidney** (by vasopressin)

  - **Ca^{+2} homeostasis** (regulated by parathyroid hormones)

  - **↑HR & contractility of the heart** (β-adrenomimetics)

Well-established second messengers

1. Cyclic adenosine monophosphate (cAMP)

- cAMP exerts most of its effects by stimulating **cAMP-dependent protein kinases (R_2C_2)**

- When **cAMP binds** to **the R dimer**, **active C chains** are **released** to diffuse through the **cytoplasm and nucleus**,
  - where they transfer phosphate from ATP to appropriate substrate proteins, often enzymes

- The **specificity** of **the regulatory effects of cAMP** resides in the **distinct protein substrates** of the **kinases** that are **expressed in different cells (**e.g liver cells are rich in phosphorylase kinase and glycogen synthase, enzymes that regulate carbohydrate storage and release)

1. Cyclic adenosine monophosphate (cAMP)

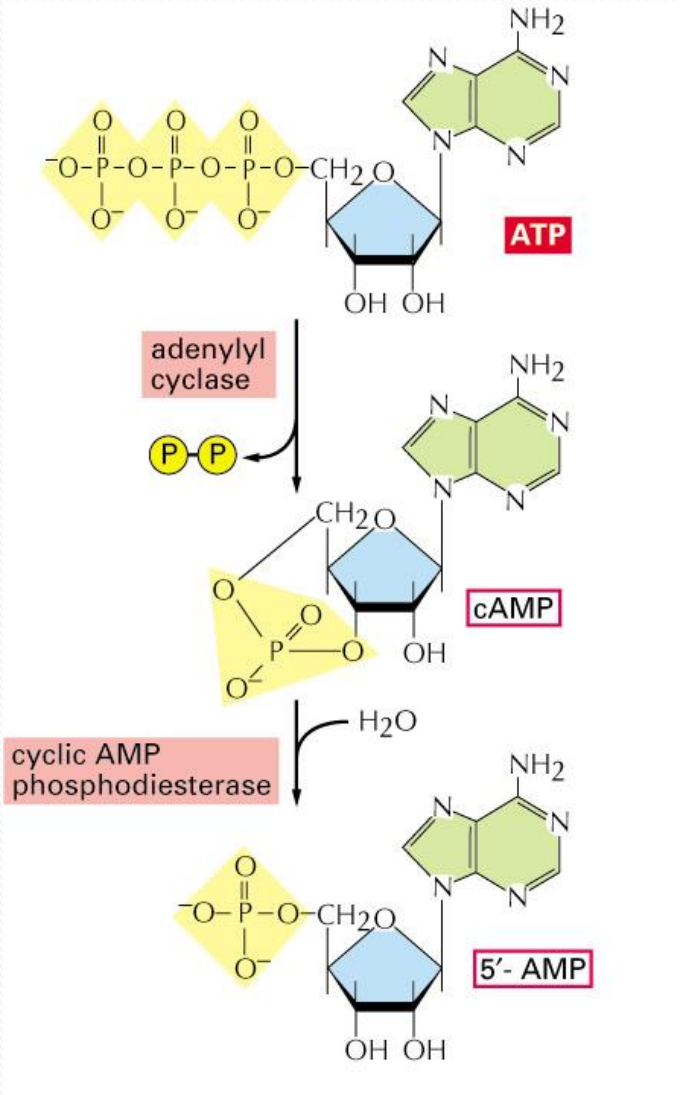
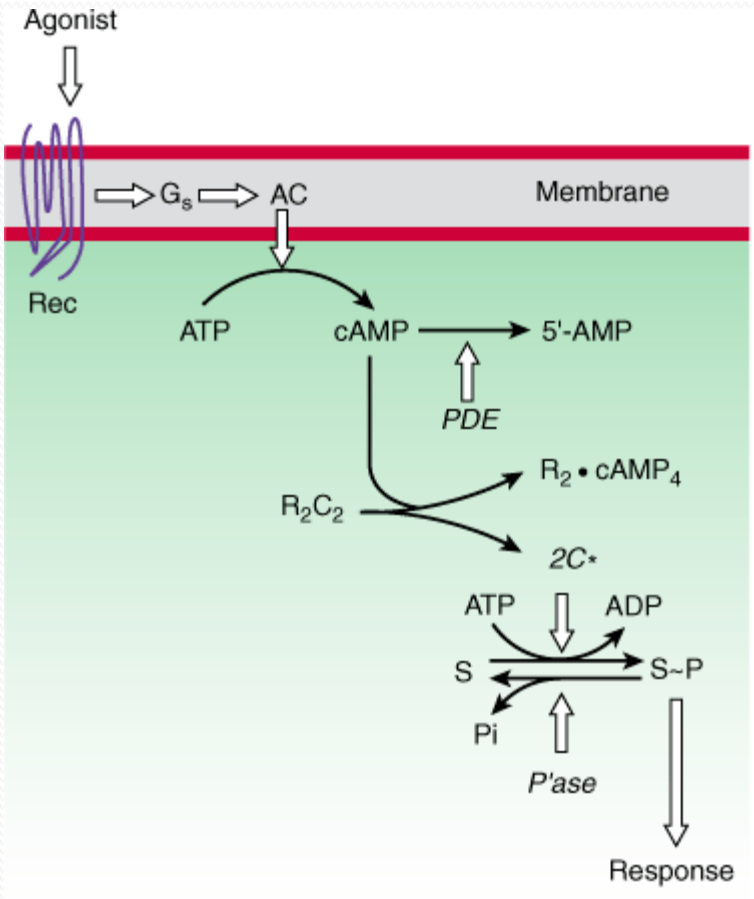


Figure 15–31. Molecular Biology of the Cell, 4th Edition.



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Well-established second messengers

1. Cyclic adenosine monophosphate (cAMP)

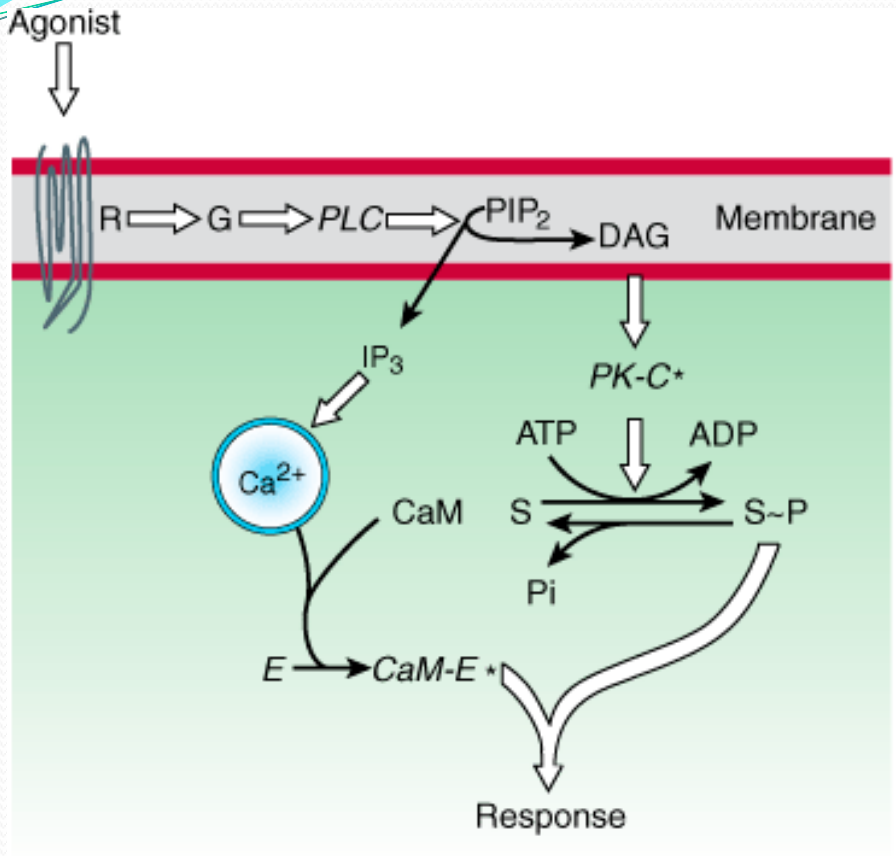
- When the **hormonal stimulus stops**, the intracellular **actions of cAMP** are **terminated** by an **elaborate series of enzymes**:

1. By **phosphodiesterases (PDE)**: cAMP is degraded to 5'-AMP (competitive inhibition of cAMP degradation is one way *caffeine*, *theophylline*, and other *methylxanthines* produce their effects)

2. cAMP-stimulated phosphorylation of enzyme substrates is **reversed** by a group of specific and nonspecific **phosphatases**

2. Calcium and Phosphoinositides

- Another well-studied second messenger system **involves hormonal stimulation** of **phosphoinositide hydrolysis**
- The crucial step is stimulation of a membrane enzyme, phospholipase C (PLC) ⟶ **splits a plasma membrane component (phosphatidylinositol-4,5-bisphosphate (PIP_2))**, **into** two second messengers, **diacylglycerol (DAG)** and **inositol-1,4,5-trisphosphate (IP_3)**
- **DAG** is **confined to the membrane**, where it **activates** a **protein kinase C**
- **IP_3** is **water-soluble** and **diffuses** through **the cytoplasm** → trigger **release of Ca^{2+}** from internal **storage vesicles**
- Ca^{2+} binds to calmodulin ⟶ which regulates activities of other enzymes, including calcium-dependent protein kinases



Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology, 11th Edition:* http://www.accessmedicine.com

Examples:
α1-adrenoceptors,

acetylcholine (M2),

histamine,

vasopressin,

angiotensin,

substance P,
gastrin,
& thrombin

2. Calcium and Phosphoinositides (Cont'd)

- Termination of signaling:

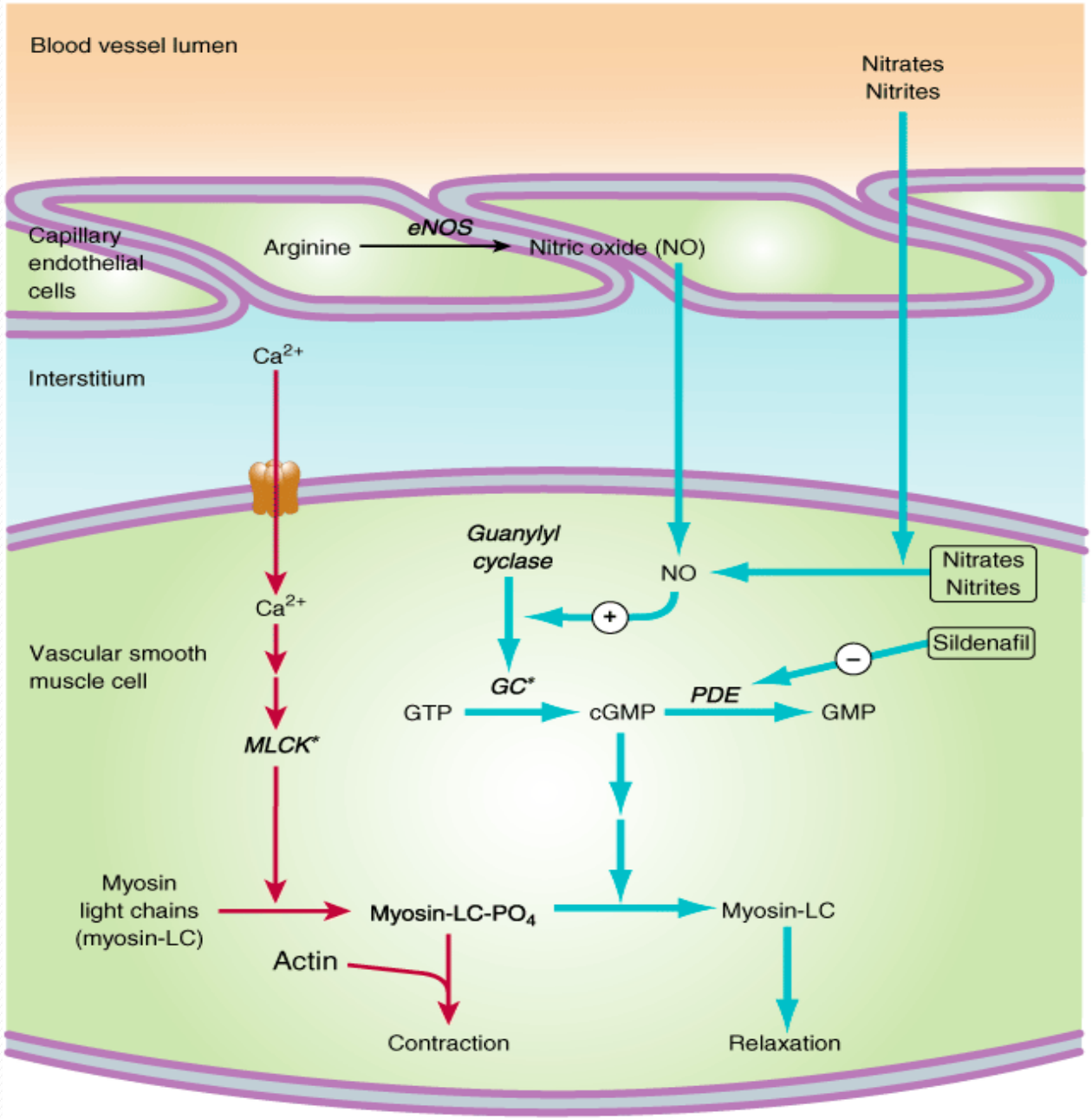
1. **Dephosphorylation** of **IP3**
2. **Phosphorylation** of **DAG** to yield phosphatidic acid phospholipids
3. **Deacylation of DAG** to yield arachidonic acid
4. **Active removal of Ca^{+2}** by Ca^{+2} pumps

3. Cyclic guanosine monophosphate (cGMP)

- Has established signaling roles in only **a few cell types**
- The most characteristic members of this receptor class is **the ANP and NO**
- **Ligand** detected by cell **surface receptors** stimulates **membrane-bound guanylyl cyclase** to **produce cGMP** that acts by stimulating a **cGMP-dependent protein kinase**
- Increased cGMP causes relaxation of vascular smooth muscle by a **kinase** mediated d**ephosphorylati**on of **myosin light chains** (ANP, NO)

- Two different ways:
  - ANP bind to extracellular domain of its receptor → activation of guanylyl cyclase in the intracellular domain

  - NO is generated in vascular endothelial cells in response to natural vasodilator agents such as **acetylcholine and histamine** → NO enters cells →activation of guanylyl cyclase



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3. Cyclic guanosine monophosphate (cGMP) (continued)

- Termination of signaling:
  1. Degradation of cGMP by **phosphodiasterase (PDE)**
  2. Dephosphorylation of kinase substrates

- A number of useful **vasodilating drugs** act by:
  - generating **or mimicking NO**,
  - or by i**nterfering** with the metabolic breakdown of cGMP by **PDE**

Interplay Among Signaling Mechanisms

- Interactions may occur between 2nd messenger systems in which one system may inhibit or stimulate another system
  - The Ca-phosphoinositide and cAMP signaling pathways **oppose one another** in *some cells* (eg, vasopressors act by IP_3-mediated mobilization of Ca^{+2} whereas vasodilators often act by ↑cAMP)
  - While, both systems **act together** to **stimulate glucose releas**e from **the liver**

Receptor regulation

- Receptors sometime are subjected to many regulatory and homeostatic controls
- **Repeated** or **continuous a**dministration of **an agonist** may lead to **changes** in the **responsiveness of the receptor**
- Receptor responses to drugs and hormones often attenuate with time; "***desensitize***" with time. After reaching an initial high level, the response diminishes even in the continued presence of the agonist
- This **"desensitization**" is often **rapidly reversible**; a second exposure to agonist, if provided a few minutes after termination of the first exposure, results in a response similar to the initial response
- HOW? For G-protein mediated response, e.g **β-adrenoceptors**, the mechanism is summarized in figure 2-12