
Pharmacology

Definition

The study of the manner in which the functions of living systems is affected by chemical agents

- Knowledge of the normal & abnormal functioning of the body is necessary
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How do drugs act/work?

A few drugs act by simple mechanisms related to their chemical or physical properties. e.g.:

- EDTA is a metal chelating agent with high affinity for Pb^{2+} . It is used for treatment of lead intoxication
- Antacids such as $\text{Mg}(\text{OH})_2$ & $\text{Al}(\text{OH})_3$ are bases and act by neutralizing acid after oral administration
- Mannitol: an osmotic diuretic, biologically inert, does not penetrate into cells. Given IV it is filtered in the glomerulus but not reabsorbed. -

Diuresis

Most drugs act by binding to cells

- To produce a biological response drug molecules must exert some chemical influence on one or more constituents of the cell
 - Drug molecules must get very close to constituent cellular molecules for their function to be altered
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But molecules in organisms greatly outnumber drug molecules?

- Drugs must be “bound” to particular constituents of cells and tissues in order to produce an effect
 - Ehrlich summed it up as follows: “*corpora non agunt nisi fixata*” - a drug will not work unless it is bound.
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The major aim of pharmacological research is:

- To understand the nature of these binding sites
 - To understand how the association of a drug molecule with a binding site leads to a biological response
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What are these binding sites?

- Mainly proteins
 - The only exception to protein as target sites is DNA (site of action for some anticancer drugs, some antimicrobials, mutagenic & carcinogenic agents)
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Protein targets for drug binding

- Enzymes
- Ion channels
- Carrier molecules
- Receptors

NB: there are still many drugs whose binding sites are still unknown

ENZYMES

There are many drugs which act by targeting enzymes:

- **Act as substrate analogues** which competitively inhibit the enzyme
 - (a) reversibly e.g. neostigmine which inhibits acetyl cholinesterase
 - (b) irreversibly e.g inhibition of cyclo-oxygenase by aspirin
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■ Act as false substrates

- drug act as a natural substrate
- undergo chemical transformation to form an abnormal product
- inhibit the normal metabolic pathway

e.g.: - fluorouracil (anticancer) replaces uracil as an intermediate in purine biosynthesis

- it can not be converted into thymidylate
 - blocks DNA synthesis and hence cell division
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- Activation of drugs by enzymes
 - some drugs require enzymic degradation to convert them to the active form
 - such drugs are called prodrugs
- e.g.: Proguanil $\xrightarrow{\hspace{1cm}}$ cycloguanil
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ION CHANNELS

- These are pores which are situated on the cell membrane
 - On the outer part of the pores are situated ions:
 - K^+ (potassium channels)
 - Ca^{2+} (Calcium channels)
 - Na^+ (sodium channels)
 - Cl^- (chloride channels)
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- **Some ion channels are directly linked to a **receptor** and open only when the receptor is occupied by an **agonist** (**Ligand-gated ion channels**)**
 - **Some are direct targets of drug action**
 - the simplest type of interaction is physical blockade of the ion channel by a drug:
e.g.: (1) the blockade of the voltage gated sodium channels by local anesthetics
(2) the blockade of sodium entry into renal tubular cells by the diuretic amiloride
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- Channel function can also be modulated by drugs which bind to accessory sites on the channel protein
- The binding of such drugs influence the gating of the channel

examples

- (1) **Dihydropyridines (vasodilators)**
 - **Ca²⁺ channels** open in response to depolarization of the cell membrane
 - The binding of dihydropyridines to the channel may **inhibit or facilitate the opening** depending on the structure of the dihydropyridine
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(2) ATP-dependent K^+ -channels of the pancreatic β -cells

- pancreatic β -cells secrete insulin when [plasma glucose] \uparrow
 - \downarrow [intracellular ATP] \longrightarrow channels open
 - Channels blocked by sulphonylureas
 - Blocking the K^+ -channels \longrightarrow β -cell depolarization
insulin secretion \longrightarrow
 - Sulphonylureas \longrightarrow do not block the channel physically
 - They modulate its gating by binding to an associated protein, the Sulphonylureas -receptor
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GABA receptor/chloride-channel complex

- **GABA** (natural substrate)
 - Stimulation of the receptor by **GABA** opens the associated Cl⁻ channel leading to inhibition of neurotransmission in the CNS
 - Benzodiazepines/barbiturates - act on sites which are different to the **GABA** binding site
 - Facilitate opening of the Cl⁻ channel by **GABA**
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CARRIER MOLECULES

- Ions and small molecules (glucose) are not sufficiently lipid soluble to cross the cell membrane and get into the cells
- Transport into the cells requires carriers
- Types of carriers:
 - for transport of glucose & amino acids in the gut
 - for transport of ions & organic molecules by renal tubules
 - for transport of Na^+ , Ca^{2+} ions out of the cell etc.
- **These carriers belong to a family of very well defined transporter systems**

RECEPTORS

- Are recognition sites for drugs
- A receptor produces an effect only when a particular drug is bound to it; otherwise it is functionally inert

Agonists: drugs which activate receptors

Antagonists: (a) drugs which on their own do not activate receptors

(b) displace agonists from their receptors and reduce their biological effect

- Receptors form a key part of the system of chemical communication that all multi-cellular organisms use to coordinate the activities of their cells and organs
 - The term **receptor** is, therefore, reserved for interactions of the regulatory type, where a ligand (chemical molecule) may function as an agonist or antagonist
 - It is limited to molecules/structures which have a **physiological regulatory function**
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Drug specificity

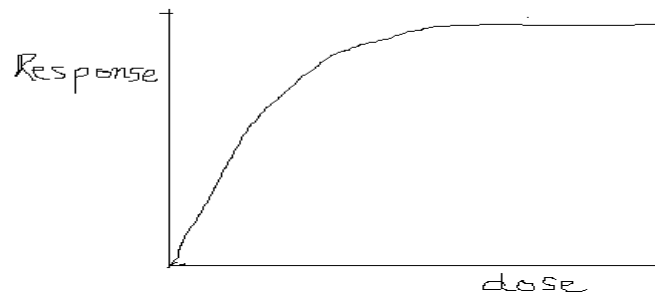
- For a chemical agent/drug to be useful as a therapeutic or scientific tool it must act selectively on particular cells and tissues
 - Conversely: proteins that function as drug targets generally show a high degree of ligand specificity
 - They will recognize only ligands of a certain precise type and ignore closely related molecules
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NO DRUGS ARE COMPLETELY SPECIFIC IN THEIR ACTIONS

If you increase the dose, a drug will affect targets other than the principal one, and lead to side effects

Dose response curves

- A pharmacological response is a function of the dose/concentration
- The relationship between dose & response is represented graphically by dose-response curves



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- There are two types of responses:
 - (a) quantal or 'all-or-none responses
 - (b) graded responses

Quantal responses:

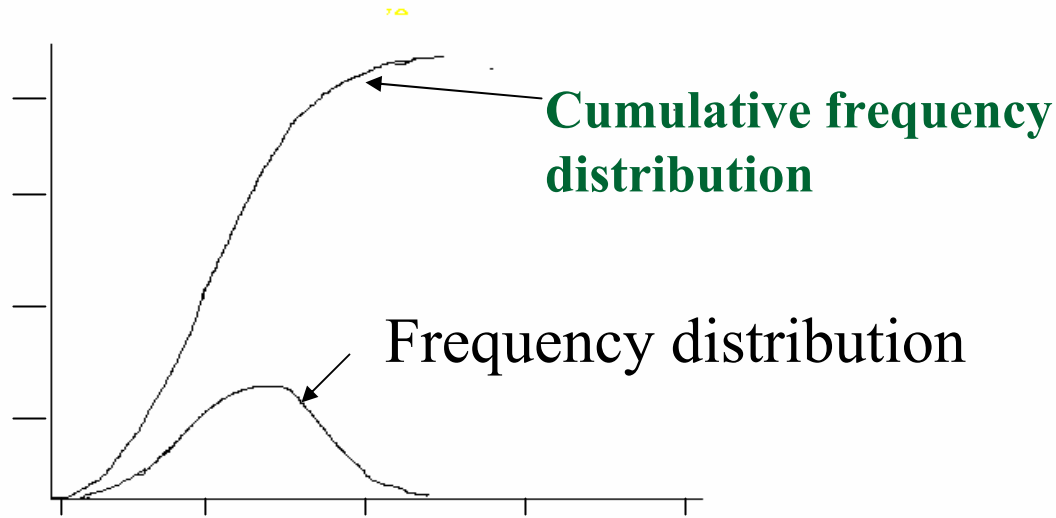
- analgesia for headache
 - digitalis to stop heart
 - sleep or lethal dose for anaesthetics
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- Responses are represented as cumulative percentage of subjects exhibiting a defined effect
 - Quantal relationships can be defined for both toxic & therapeutic effects
 - This allows the calculation of therapeutic index
 - A safe drug has a large therapeutic index.
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Frequency distribution Curve

Y axis = number responding

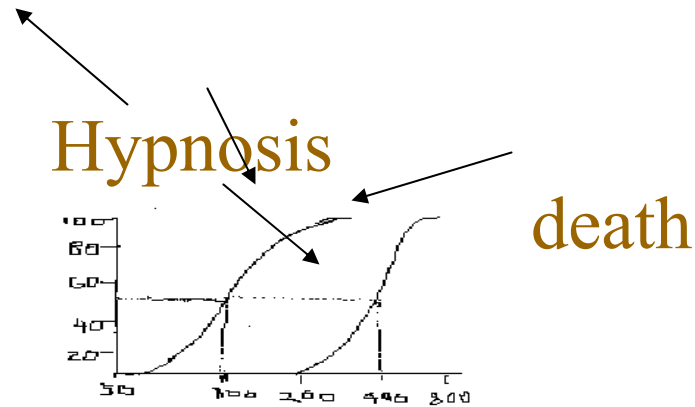
X-axis = conc (mg/L)



Quantal conc-effect & dose-effect curves

Y-axis = percent of individuals responding

X-axis = dose



Therapeutic index

Median lethal dose

Median effective dose

$$\text{Or } LD_{50}/ED_{50} = 400/100 = 4$$



Graded responses

- Responses are often described as a percentage of maximal response
 - A linear concentration scale yields a rectangular hyperbolic curve
 - A log scale yields a sigmoid curve
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The mechanism of drug-receptor interaction

- The 1st step in drug action, on a specific receptor, is the formation of a reversible drug-receptor complex
 - This reaction is governed by the law of mass action
 - Suppose that a piece of tissue (heart muscle/uterine smooth muscle) contains a total number of N_T receptors for agonist A
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- When the tissue is exposed to the agonist at a conc. X_A and allowed to come to equilibrium, N_A receptors will be occupied
 - The number of vacant receptors = $N_T - N_A$
 - Normally X_A greatly exceeds N_T , so the binding reaction does not generally reduce X_A
 - The magnitude of the response produced by X_A will be related to the number of receptors occupied
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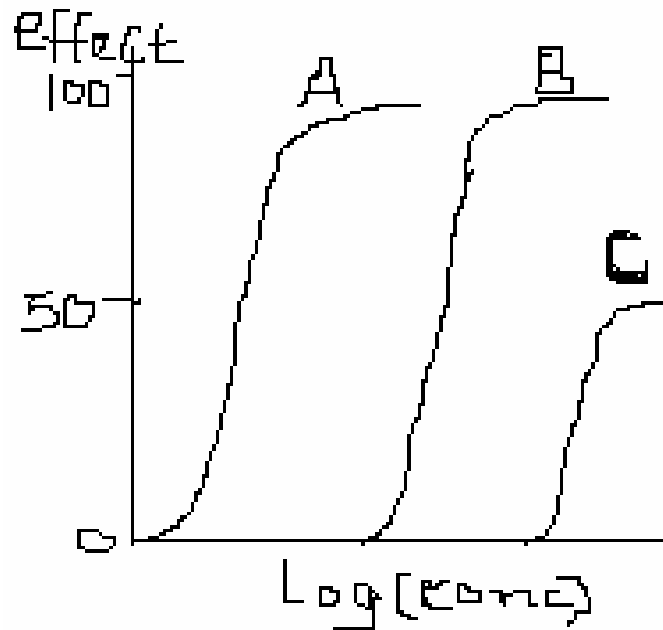
Two important terms of dose-response curves

Potency:

- The location of the concentration curve along the concentration axis
- It is related to the dose of a drug required to produce a given effect

Efficacy:

- The magnitude of effect that can be produced by a drug
- Maximal efficacy is reflected in the plateau of the dose-response curve



- Drug A is more potent than drug B, but both show the same efficacy
- Drug C shows lower potency and lower efficacy than A or B

Competitive antagonist

- Bind reversibly at the agonist binding site
- Its effect can be overcome by increasing concentration of agonist

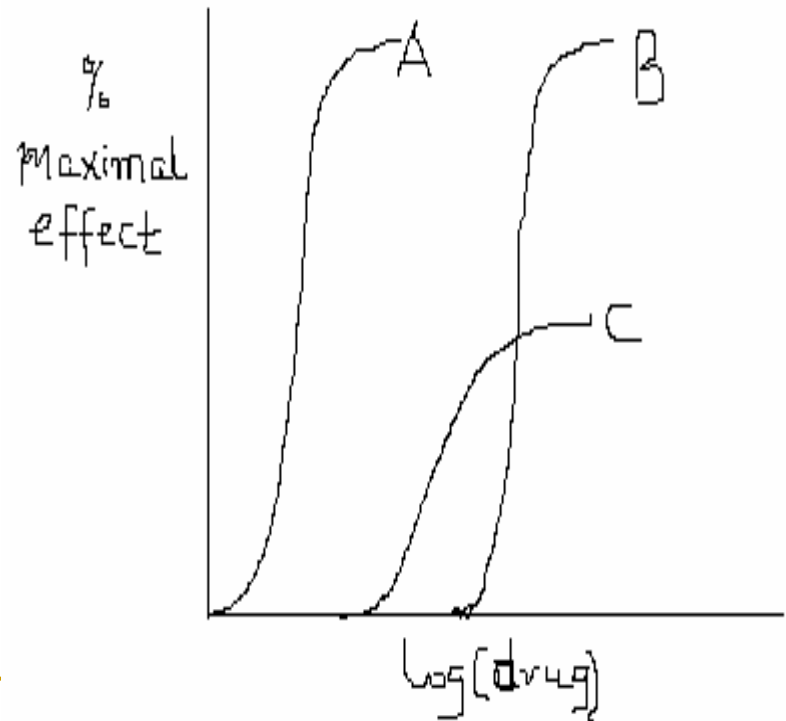
Non-competitive antagonist

- Bind irreversibly to agonist binding site
- The effect is equivalent to removing receptors from the system

A = agonist alone

B = agonist + competitive antagonist

C = Agonist + non-competitive antagonist



Binding of drugs to receptors:

- Obeys the law of mass action
- At equilibrium, receptor occupancy is related to drug concentration by the Hill-Langmuir equation
- The higher the affinity of the drug for the receptor, the lower will be KD , and the lower the concentration at which it produces a given level of occupancy
- The same principles apply when two or more drugs compete for the same receptors; each reduces the apparent affinity of the other.

Application of the Hill-Langmuir equation for competitive

antagonism

$$P_A = \frac{X_A}{X_A + KD}$$

This equation can also be written as

$$P_A = \frac{X_A/KD}{X_A/KD + 1}$$

This equation can be extended to describe occupancy when two or more competing drugs are present

- The receptor can bind only one drug molecule at a time
- Applying the same law of mass action, as before, the occupancy equation becomes:

$$P_A = \frac{X_A/KD}{\cancel{X_A/KD} + X_B/K_A + 1}$$

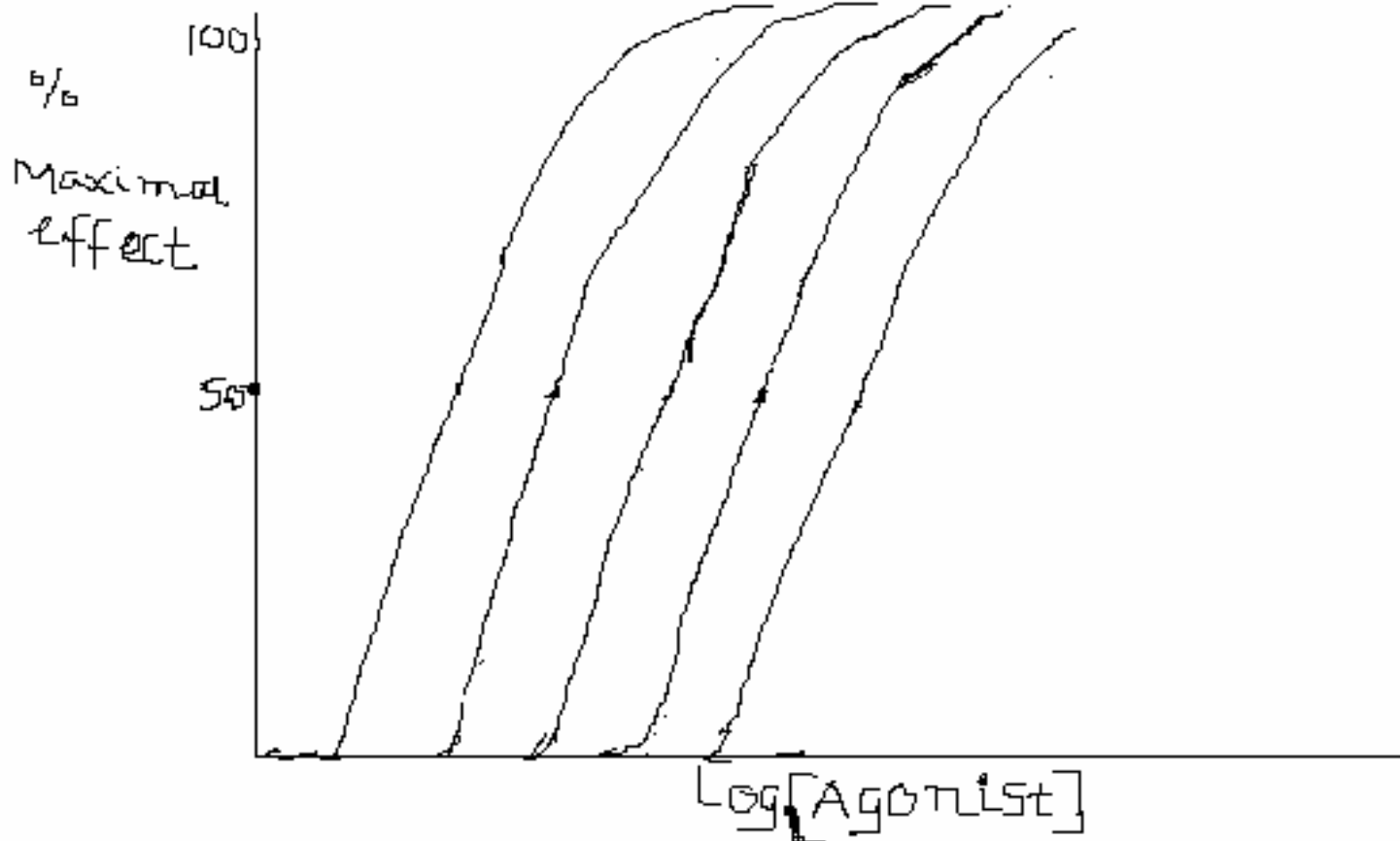
X_B = concentration of antagonist

K_A = dissociation constant for antagonist

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- Adding drug B (competitive antagonist) reduces the occupancy by drug A if the conc of A is kept constant
 - Occupancy of A can be restored by increasing its concentration
 - Suppose the concentration has to increase to X_{A1} , then the dose ratio,
$$r = X_{A1}/X_A = X_B/K_A + 1$$
 - This equation is known as the **Schild equation**
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The Schild equation predicts the following characteristic properties of competitive antagonism:

- The dose ratio, r , depends only on the conc and dissociation constant of antagonist
 - r is independent of agonist dissociation constant
 - The effect of a competitive antagonist is to shift the conc-response curve to the right without changing its slope or maximum
 - r increases linearly with antagonist conc (X_B)
 - The slope of a plot of $(r-1)$ against X_B is equal to $1/K_A$
 - This relationship is independent of the agonist characteristics and **should be the same for all agonists that act on the same population of receptors**
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Parallel curves show plots of agonist effect in the presence of increasing antagonist concentrations

Partial agonists

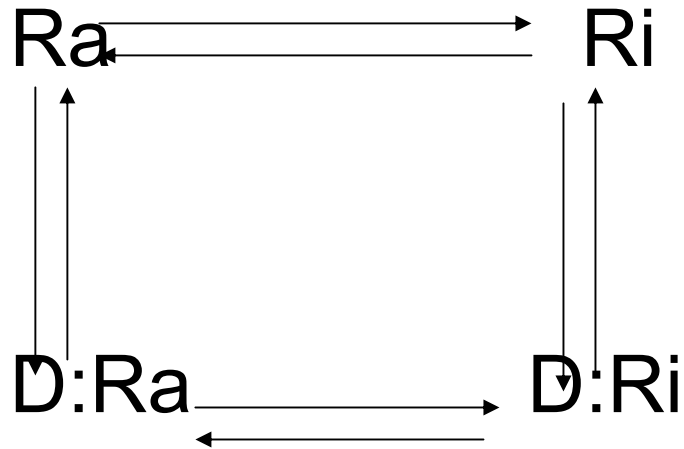
- A partial agonist is a drug that displays efficacy that is intermediate btwn that of an agonist and an antagonist



Mechanism of action of partial agonist

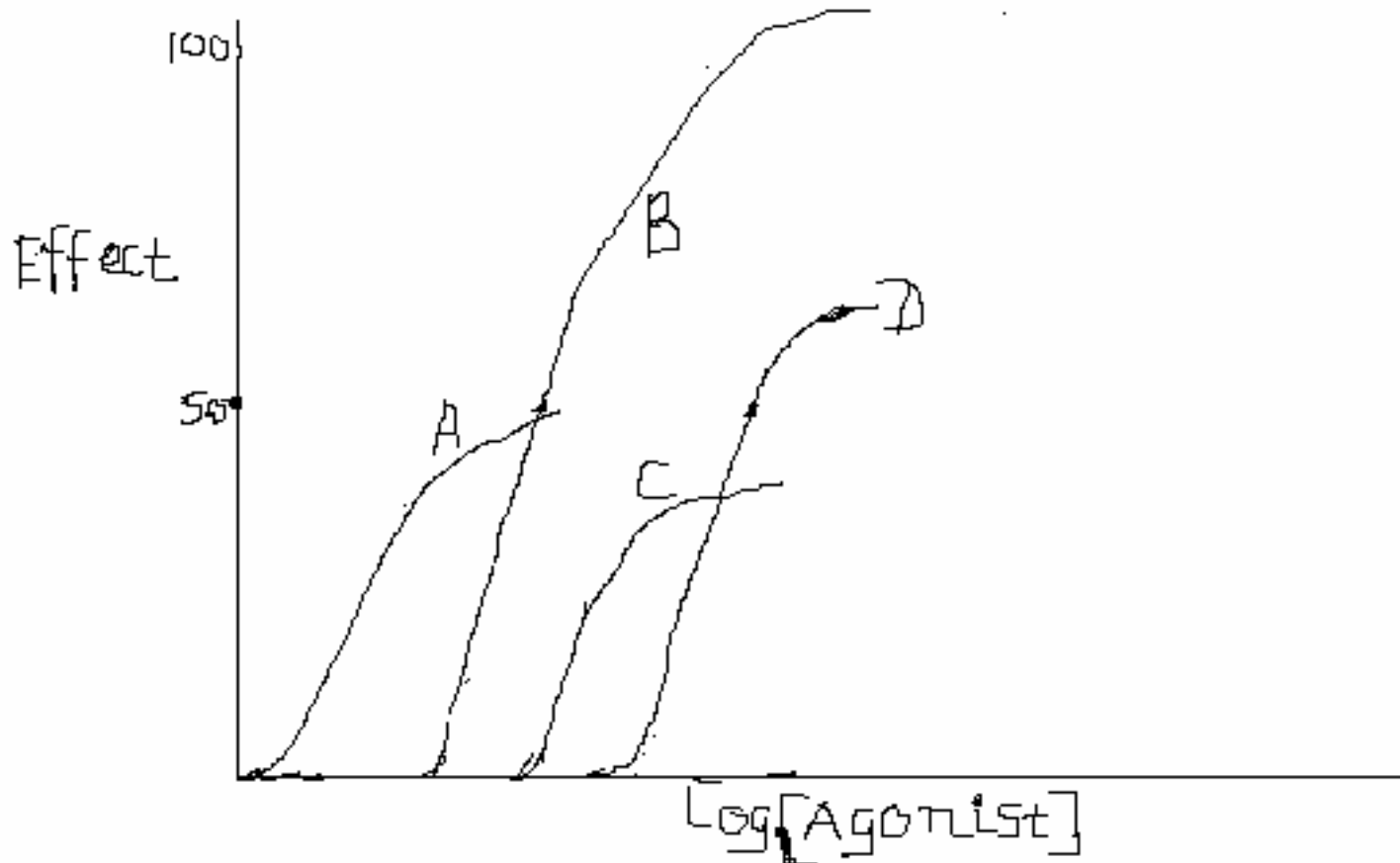
The allosteric theory of drug action

- This theory assumes that a receptor exists in two states, the active state (R_a) and inactive state (R_i)
 - Both forms are capable of binding a drug but with different affinities
 - These two forms exist in an equilibrium, but, while at rest R_i dominates
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- When an agonist binds to the receptor equilibrium shifts to R_a
- A full agonist will completely drive the receptor to the active state

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- A partial agonist is incapable of driving the equilibrium to the active state
 - An antagonist has equal affinity for R_a & R_i
 - it does not change the equilibrium of the resting state
 - it only interferes with the action of agonist by occupying the binding sites
 - If a compound has a higher affinity for R_i than R_a , it is called an inverse agonist (or negative antagonist)
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Responses to a series of partial & full agonists. Potencies vary independent of efficacy

Assignment

There are other types of antagonism to drug action apart from antagonism by receptor block

These include:

Chemical antagonism, Pharmacokinetic antagonism & physiological antagonism

What are these other types of antagonism? Explain.

Receptor classification

- based on pharmacological criteria
 - classified on the basis of the effects of particular drugs
 - Direct measurement of ligand binding to receptors
 - Molecular cloning which has revealed the amino acid sequence of many receptors
 - Analysis of the biochemical pathways that are activated in response to receptor activation
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- All these have led to a lot of confusion in receptor classification

IUPHAR (International Union of Pharmacological Sciences)

- Has set up various expert working groups to produce agreed receptor classifications for the major receptor types, taking into account the pharmacological, molecular & biochemical information available
 - A summary of known receptors is published annually (*Trends in Pharmacological Sciences. Receptor Supplement*)
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Isolation & characterization of receptors

- Before 1970s, when radio-labeling experiments were successfully done for the 1st time, receptors were treated as only theoretical entities
- made isolation of receptors a biochemical reality.
- If a tightly bound radioactive ligand is available, this makes it possible to extract & purify the radioactively labeled receptor material.

Isolation of nAChRs

- **Source** : the electric organs of *Torpedo* sp. Or muscle tissue of electric eels (*Electrophorus* sp.)
- **Reason:** contain large amounts of nAChRs than any other tissue.
- **Source of nAChR ligand:** cobra venom (contains polypeptides which bind with very high specificity to nAChRs)
- These toxins are known as α -toxins
- The best known is α -bungarotoxin, a component of the venom of the Malaysian banded krait (*Bungarus multicinctus*)

The process of isolation by affinity chromatography

- Radiolabeled α -bungarotoxin (receptor ligand) is bound covalently to the matrix of a chromatography column
- The muscle/electric tissue is treated with a non-ionic detergent to dissolve the membrane bound receptor protein
- Extract is passed through the column, which will adsorb the receptor and separate it from other substances in the extract
- The receptor is flushed from the column using a solution containing an antagonist (gallamine)

Receptor families: structures & signal transduction mechanisms

Type 1: **Channel-linked receptors** **(ionotropic receptors)**

- Membrane bound receptors
- Coupled directly to an ion channel
- Are receptors for fast acting neurotransmitters
- nAChR, GABA_A receptor, glutamate receptor, 5-HT₃ receptor

Type 2: G-protein-coupled receptors (metabotropic receptors or 7- transmembrane spanning receptors)

- Membrane bound receptors
 - Coupled to intracellular effector systems via a G-protein
 - Are the receptors for many hormones & neurotransmitters
 - mAChRs, adrenergic receptors, 5-HT receptors, opiate receptors, peptide receptors, purine receptors.
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Type 3: **Kinase-linked receptors**

- Membrane bound receptors
- Contain an intracellular protein kinase domain (usually tyrosine kinase) within their structure) e.g Insulin receptor & receptors for cytokines and growth factors

Closely related receptors: Receptors linked to guanylate cyclase e.g. the receptor for atrial natriuretic factor (ANF)

Type 4: **Receptors that regulate gene expression (also called **nuclear receptors**)**

- Some are located in the cytosol rather than nuclear compartment
 - e.g. receptors of steroid hormones, thyroid hormones, retinoic acid, Vit D etc.
 - They are soluble cytosolic or intracellular proteins
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G-proteins & their role

- Represent a central command post for communication between receptors & effector enzymes or ion channels.
 - Called G-proteins because of their interaction with guanine nucleotides, GTP and GDP.
 - Consist of 3 subunits $\alpha\beta\gamma$
 - Guanine nucleotides bind to the α -subunit, which has enzymic activity, and catalyses conversion of GTP to GDP
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- All the 3 sub-units are anchored to the membrane through a fatty acid chain, attached to an amino acid residue by prenylation.
 - G-proteins appear to be freely diffusible in the plane of the membrane. This is a key aspect of their function that a single pool of G-proteins in a cell can interact with several different receptors and effectors non-selectively.
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Resting state:

- G-proteins exist as unattached $\alpha\beta\gamma$, trimer attached to GDP through α -subunit.

Receptor occupied by agonist:

- The cytoplasmic part of the receptor undergoes a conformational change and acquire high affinity for $\alpha\beta\gamma$.
- Association of the trimer with the receptor causes the bound GDP to dissociate and to be replaced with GTP (GDP/GTP exchange)

- The G-protein trimer dissociates to α -GTP and $\beta\gamma$, the active forms of the G-protein
 - These diffuse in the membrane and can associate with various enzymes and ion channels, causing activation or inactivation as the case may be
 - Hydrolysis of GTP to GDP by intrinsic GTPase activity of the α -subunit terminates the process
 - Magnitude of GTPase activity is different for different types of effectors
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- Regulation of its GTPase activity by the effector protein means that the activation of the effector is self-limiting
 - This mechanism results in amplification b'cause a single agonist-receptor complex can activate several G-protein molecules in turn, and each of these remain associated with the effector enzyme for long enough to produce many molecules of product.
 - The product is a **second messenger** which causes further amplification before the final cellular response is produced
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Specificity for the different receptor types

- 3 classes of G-proteins, G_s , G_i & G_q
 - Show selectivity to both the receptors and the effectors they couple with
 - They have specific recognition domains which recognize specific binding domains in the receptor and effector molecules
 - e.g. G_s stimulates adenylate cyclase; G_i inhibits adenylate cyclase.
 - The α -subunits of G-proteins differ in structure
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The targets for G-proteins

- Adenylate cyclase, Phospholipase C, phospholipaseA₂, ion channels

Adenylate cyclase/cAMP

- cAMP – nucleotide synthesized within the cell from ATP by the action of adenylate cyclase (membrane bound enzyme)
- cAMP is produced continuously & inactivated to 5'-AMP by phosphodiesterases

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- Many drugs, hormones & neurotransmitters produce their effects by increasing or decreasing the catalytic activity of adenylate cyclase and hence cAMP levels
 - cAMP regulates many aspects of cellular function, e.g:
 - Enzymes involved in energy metabolism
 - Cell division & differentiation
 - Ion transport
 - Ion channels
 - Contractile proteins in smooth muscle
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- All these effects are brought about by a common mechanism - **activation of various protein kinases by cAMP**
 - Protein kinases catalyse the phosphorylation of serine and threonine residues in different cellular proteins, using ATP as the source of phosphate groups, and thereby regulate their function
 - Phosphorylation can either activate or inhibit target enzymes or ion channels
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Phospholipase C/inositol phosphate system

- Phosphatidylinositol is a membrane phospholipid
 - It is the substrate for the membrane bound enzyme phospholipase C_β (PLC_β)
 - It splits PIP₂ into diacylglycerol (DAG) & inositol (1,4,5) triphosphate (IP₃)
 - DAG & IP₃ function as 2nd messengers
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- The activation of PLC_{β} by various agonists is mediated through a G-protein, like adenylate cyclase
 - PIP_2 can be hydrolysed by PLC_{γ} by activation of kinase-linked receptors not dependent on GTP and release IP_3
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Termination of events:

- DAG is phosphorylated by a kinase to form phosphatidic acid
 - IP_3 is dephosphorylated & then re-coupled with Phosphatidic acid to form PIP_2 again
 - Lithium (used for manic depression) blocks the recycling pathway.
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Phospholipase A₂

- Receptor-mediated activation of PLA₂ releases arachidonic acid from PIP₂
 - Arachidonic acid & its metabolites have recently been shown to function as intracellular messengers, controlling K⁺-channel function in certain neurons. It is also a local hormone communicating between cell.
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Inositol phosphate & intracellular Ca^{+2}

- IP_3 releases Ca^{+2} from intracellular stores
- Release of Ca^{+2} mediates a number of pharmacological responses e.g.
 - smooth muscle relaxation
 - increased force of contraction of heart muscle
 - secretion from exocrine glands
 - release of neurotransmitters
 - hormone release
 - cytotoxicity

DAG & Protein kinase C

- Main effect of DAG is to activate a membrane bound protein kinase C (PKC) which phosphorylates serine & threonine residues of a variety of intracellular proteins
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pA_2 values

- Let us revisit the schild equation:

$$r-1 = X_B/K_A \quad (1)$$

- The plot of $r-1$ increases linearly with X_B and the slope is K_A .
- Equation (1) can be expressed logarithmically as $\log(r-1) = \log X_B - \log(K_A)$
- A plot of $\log(r-1)$ vs $\log X_B$ is called the Schild plot
- The plot is a straight line whose slope is 1 and intercept on X-axis is $\log K_A$
- $-\log K_A$ is known as pA_2 (an equivalent of pH & pK)

- pA_2 is, therefore, the negative logarithm of the molar concentration of antagonist required to produce an agonist ratio of 2.
e.g. if pA_2 is 6.5, then $K_A = 3.2 \times 10^{-7}$.
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R.P. Stephenson Postulates & the concept of spare receptors

- (1) An agonist can produce a maximum effect by occupying only a fraction of the total receptor population
- (2) The magnitude of the effect produced by an agonist is some unknown function of receptors occupied
- (3) Each drug-receptor complex provides a biological stimulus (S) to the tissue that is directly proportional to the fraction of receptors occupied

i.e $S = \varepsilon Y$, where Y = fraction of receptors occupied & ε = efficacy

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- The effect, then, is an unknown function of the stimulus. $E = f(S)$

Spare receptors

Drugs that produce a maximal tissue response with an appreciable spare receptor capacity are strong/full agonists

For these fractional receptor occupancy, Y , in the equation $S = \varepsilon Y$ is small and ε , the efficacy is very large.

Partial agonists

- Have low efficacy (ε)
 - Have low or zero spare receptor capacity
 - Bind to the same receptors as full agonists
 - Produce less than maximum response
 - Diminish the effect of full agonists when used together
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Direct measurement of drug binding to receptors

- The binding of drugs to receptors can be measured directly by the use of radioactive drug molecules (^3H , ^{14}C , ^{125}I)

Requirements:

A radioactive ligand (agonist/antagonist)

-must bind with high affinity & specificity

-can be labelled to a sufficient specific radioactivity to enable minute amounts

of binding to be measured.

Procedure:

1. Incubate samples of tissue/membrane fragments with various concentrations of radioactive drug until equilibrium is reached
 2. Remove the tissue/or separate membrane fragments by filtration/centrifugation
 3. Measure its radioactive content
 4. Exclude effect of non-specific binding
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- Assuming that the specific binding follows the Hill-Langmuir equation, then the relationship btwn bound (B) and ligand conc (X_A) is:

$$B = B_{\max} \frac{X_A}{X_A + KD}$$

Where B_{\max} = total number of binding sites in the preparation (fmoles/mg protein)

KD = equilibrium/dissociation constant

Rearranging the equation,

$$B(X_A + KD) = B_{\max} X_A$$

$$BX_A + BKD = B_{\max} X_A$$

Dividing both sides by $X_A KD$ gives,

$$B/KD + B/X_A = B_{\max}/KD$$

Therefore, $B/X_A = B_{\max}/KD - B/KD$

A plot of B/X_A against B (Scatchard plot) gives a straight line from which both B_{\max} & KD can be estimated. The slope = $-1/KD$

B_{\max} = intercept on the abscissa

$\frac{\text{Bound}}{\text{Free}}$

Bound

