

## Pharmacodynamics & Receptors

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- What is Pharmacodynamics?
- the study of the physiological effects of drugs (what drugs do to the body)
- study of mechanisms of drug action (drug targets)
- analysis of relationship between drug concentration and effect

## Basic questions

- What is a Drug ?
- What are Drug Targets ?

## Targets for Drug action

- Drugs are chemicals that affect a physiological system in a specific way
- Most drug targets are proteins: receptors, ion channels, enzymes, carriers
- Specificity & reciprocity. In many cases particular classes of drugs bind only to certain targets & individual targets recognise only certain classes of drugs
- No drugs are entirely specific (ideal) – side effects

## Paul Ehrlich (1854-1915)



Synthesised & tested series of organo-arsenic compounds as treatments for syphilis & sleeping sickness.

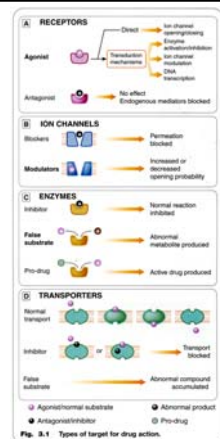
Arsphenamine ("606") marketed by Hoechst in 1910 as *Salvarsan*.

*Nobel Prize for Medicine 1908*

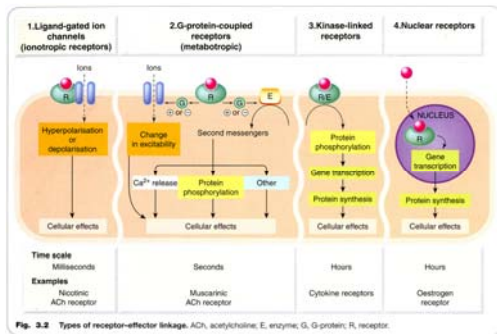
- Drug actions can be explained by chemical interactions
- A drug will not work unless it is bound (to a target)
- Coined the terms "chemotherapy" and "magic bullet"

## Proteins as Drug Targets

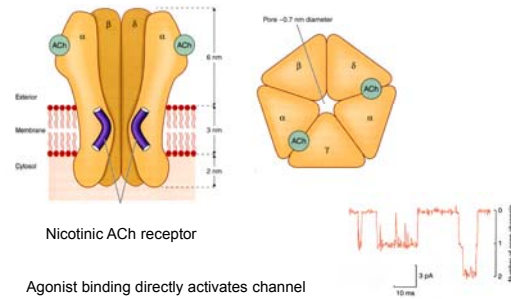
- Receptor – protein molecule that recognises endogenous chemical signal. ACh receptor, GABA receptor,  $\beta$ -adrenergic receptor, cytokine receptor, glutamate receptor.
- Ion channel – membrane protein with integral ion channel. Ligand-gated (ionotropic), voltage-gated, G-protein coupled (metabotropic)
- Enzymes
- Nuclear Receptors
- Transporters



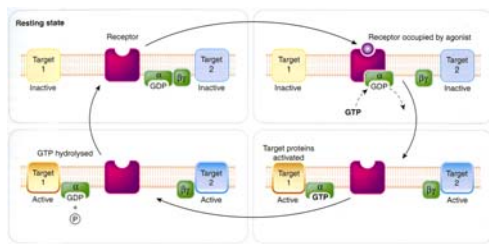
### Coupling of drug binding to cellular effects



### Ligand-gated ion channels



### G-protein coupled receptors

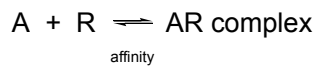


Agonist binding does not directly activate effector (e.g. channel or enzyme) – coupling via G-protein  $\alpha, \beta, \gamma$  subunits, involves hydrolysis of GTP.

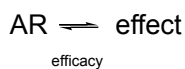
### Drug-Receptor Interactions

- drug binding and receptor activation are distinct steps
- *Affinity* – measure of drug binding
- *Efficacy* – measure of drug activity

### Drug-Receptor Interactions

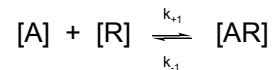


Affinity – measure of propensity of drug to bind



Efficacy (or intrinsic activity) ability of bound drug to change the activity of the receptor

### Drug-Receptor Binding



$k_{+1}$  rate constant for binding step

$k_{-1}$  rate constant for dissociation of complex

at equilibrium

$$k_{+1} [A] [R] = k_{-1} [AR]$$

rearranging

$$\frac{[A][R]}{[AR]} = K_D$$

$$K_D = \frac{k_{-1}}{k_{+1}}$$

The lower the  $K_D$  the higher the affinity of the drug for receptor

## Drug-Receptor Binding

THE MODE OF ACTION OF NICOTINE AND CURARI, DETERMINED BY THE FORM OF THE CONCENTRATION CURVE AND THE METHOD OF TEMPERATURE COEFFICIENTS. BY A. V. HILL, B.A., *Behav. of Trinity College, Cambridge.*

(From the Physiological Laboratory, Cambridge)

It is now clear that there has been a tendency to attribute to a physical rather than to a chemical process, the action of many substances which have an effect upon the response given by a test substance equation. In very few cases, however, has the physical view been worked out in any detail. The action of nicotine and of curari has been investigated by Prof. Langley on the lines of physiological experiment, and he advances the view that these and other similar bodies, in producing their specific effects form reversible chemical combinations with certain constituents—"receptor substances"—of the cells. In the following paper I have tested the mode of action of nicotine and curari, on the binding number of the ligand, by mathematical and physico-chemical methods.



Figure 2. See the text of A.V. Hill's paper published in 1908 (2). See 103 inside the University College London Physiology Department in 1923, shared by students who had received a letter from Hill in 1920 (shown by Ernest Higgins in 1983, from a photograph).

Colquhoun, D. TIPS, 27, 149 (2006)

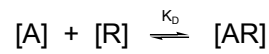
$$p_A = \frac{[AR]}{[AR] + [R]}$$

$$p_A = \frac{[A]}{[A] + K_D}$$

Hill-Langmuir equation

$$B = \frac{B_{\max} [A]}{[A] + K_D}$$

## Derivation of Hill-Langmuir Equation

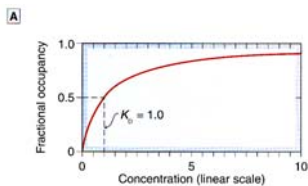


$$\frac{[A][R]}{[AR]} = K_D \quad R_{\text{total}} = [R] + [AR]$$

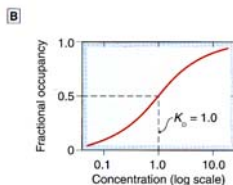
$$p_A = \frac{[AR]}{R_{\text{total}}} = \frac{[AR]}{[R] + [AR]} = \frac{[AR]}{K_D \frac{[AR]}{[A]} + [AR]}$$

$$p_A = \frac{1}{\frac{K_D}{[A]} + 1} = \frac{[A]}{K_D + [A]} = \frac{B}{B_{\max}}$$

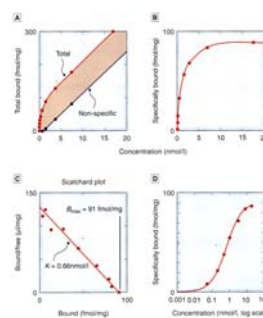
## Hill-Langmuir equation



$$p_A = \frac{[A]}{[A] + K_D}$$



## Calculating $B_{\max}$ & $K_D$ from experimental data



$$B = \frac{B_{\max} [A]}{[A] + K_D}$$

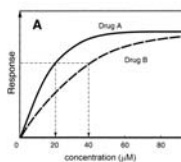
$$[A] (B_{\max} - B) = BK_D$$

$$\frac{B}{[A]} = \frac{B_{\max}}{K_D} - \frac{B}{K_D}$$

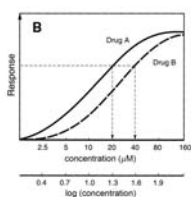
Scatchard equation

$B_{\max}$  total number of binding sites in preparation

## Concentration response curves



Example of two drugs A & B. Drug A is twice as potent as drug B.

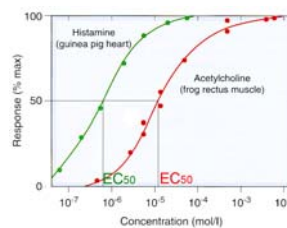


Plotting concentration response on log scale transforms hyperbola to sigmoid curve. Middle section ~linear.

Drugs acting in similar ways with different potencies give parallel shifts e.g. in this case shift is  $\log(2) = 0.3$  log units

## Hill Equation

Empirical relationship between concentration and response



$$y = \frac{y_{\max} [x]^n}{[x]^n + EC_{50}^n} \quad \text{General form}$$

$$\text{response} = \frac{100 \times [\text{agonist}]^n}{[\text{agonist}]^n + EC_{50}^n}$$

$EC_{50}$  – concentration for 50% effect  
 $n$  (or  $n_H$ ) – Hill coefficient

Drug potency is sometimes defined as the reciprocal of the  $EC_{50}$

## Cumulative dose response from a population

Physiological end point (30% increase in heart rate)

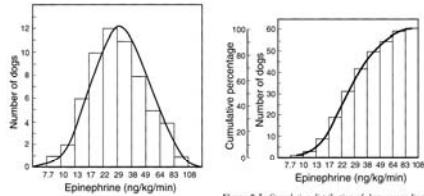


Figure 8-4. Distribution of dogs responding to various infusion rates of epinephrine. (Histogram indicating normal distribution, see text.)

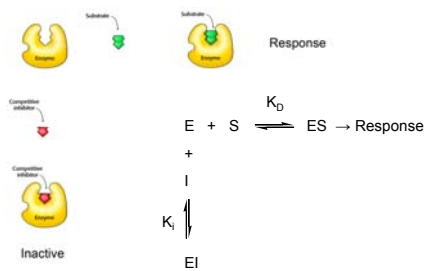
Figure 8-5. Cumulative distribution of dogs responding to various rates of epinephrine infusion. (Data from Fig. 8-4. The fitted curve describes distribution similar to an LDR curve.)

The steepness of the curve (n) reflects the heterogeneity of the population. Steep curve (large n) – homogeneous population.

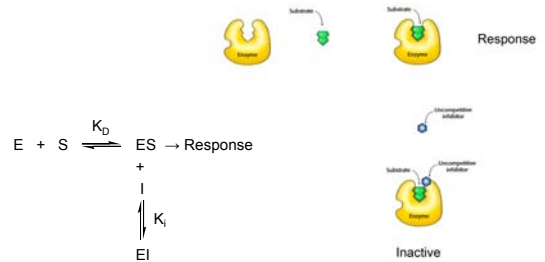
## Receptor Antagonism

- Competitive Antagonism
  - Inhibitor and agonist compete (cannot bind at the same time)
- Uncompetitive Antagonism
- Mixed Antagonism
- - Non-competitive Antagonism

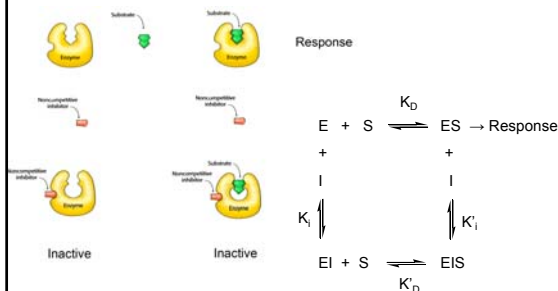
### Competitive antagonism (competitive inhibition)



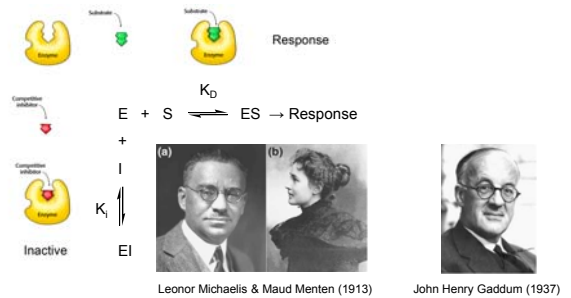
### Uncompetitive Antagonism



### Non-competitive antagonism



### Competitive antagonism (competitive inhibition)



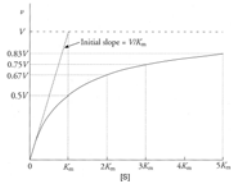
## Michaelis-Menten Equation

$$v = \frac{k_{cat}e_0[S]}{K_M + [S]}$$

Fundamental equation of enzyme kinetics  
Relates reaction rate,  $v$ , to substrate concentration and the Michaelis constant  $K_M$

$$v = \frac{V_{max}[S]}{K_M + [S]}$$

Can be written in this form where  $V_{max}$  is the maximum reaction rate



## Maths revision: rectangular hyperbola

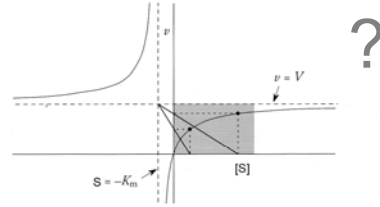


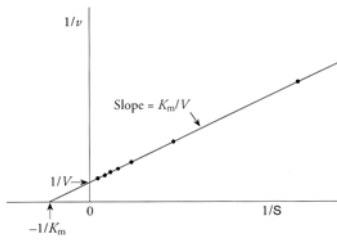
Figure 2.2. Dependence of initial rate  $v$  on the substrate concentration  $S$  for a reaction obeying the Michaelis-Menten equation. The part of the curve from  $S = 0$  to  $5K_M$ , the shaded part of the Figure, is the same as in Figure 2.1, but the range of values shown is much wider and includes physically impossible values, in order to illustrate the relationship of the curve to the two asymptotes, which intersect at the point  $(-K_M, V)$ .

## Double-Reciprocal Plot

Lineweaver-Burke plot

$$v = \frac{V_{max}[S]}{K_M + [S]}$$

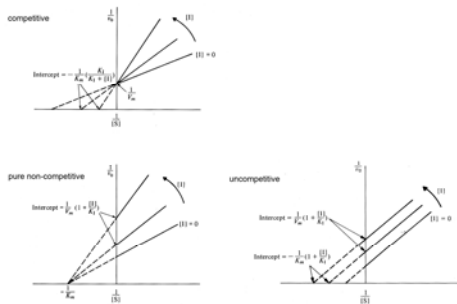
$$\frac{1}{v} = \frac{K_M}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$



## Characterising type of inhibition from double reciprocal plot with and without inhibitor

type of inhibition	apparent $V_{max}$	apparent $K_M$
Competitive	$V_{max}$	$K_M(1 + [I]/K_i)$
Pure non-competitive	$V_{max} / (1 + [I]/K_i)$	$K_M$
Uncompetitive	$V_{max} / (1 + [I]/K_i)$	$K_M / (1 + [I]/K_i)$

## Characterising type of inhibition from Lineweaver-Burk plot with and without inhibitor



## Lineweaver-Burk plot showing inhibition of luciferase enzyme by the volatile anaesthetic halothane

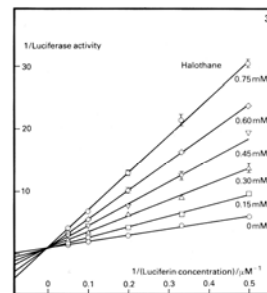
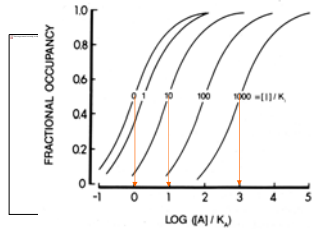


Fig. 3. General anaesthetics inhibit luciferase activity by competing for the natural substrate luciferin.

Franks, NP & Lieb WR (1985) Chemistry in Britain v21, p919

## Schild analysis of competitive antagonism

occupancy of receptor by agonist A, in presence of competitive antagonist B



$$P_A = \frac{[A]}{[A] + K_A}$$

$$P_A = \frac{[A]}{[A] + K_A (1 + [I]/K_I)}$$

$$dr = \frac{[A]}{[A]} = \frac{[I]}{K_I} + 1$$

Schild equation

dose ratio (dr)

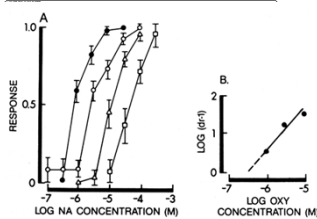
- depends only on  $K_I$  of antagonist, not size of reference response or agonist  $K_A$
- increases linearly with antagonist concentration  $[I]$

## Schild equation holds true if

- The observed response is the same if the occupancy of each site by A is the same, regardless of how many receptors are occupied by antagonist
- Binding of antagonist, I, and agonist, A, are mutually exclusive (competitive inhibition)
- Antagonist does not change receptor conformation (not inverse agonist)
- Antagonist has same affinity for each binding site
- Measurements are at equilibrium

## Schild plot

$$\log(dr - 1) = \log[I] - \log[K_I]$$



$$dr = 2$$

$$\Rightarrow \log(dr - 1) = 0$$

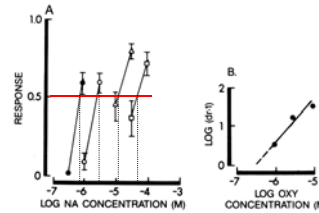
$$\therefore \log[I] = \log[K_I] = -pK_I$$

$$\text{Here } pK_I = 6.5, K_I = 0.316 \text{ mM}$$

NA – noradrenaline; OXY- oxymetazoline (antagonist)

## Schild plot

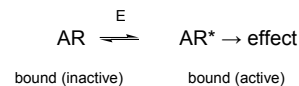
$$\log(dr - 1) = \log[I] - \log[K_I]$$



Null method, works with partial concentration-response curves  
Provided response is the same for given agonist occupancy works for complex mechanisms (eg GPCR)

## Affinity & Efficacy

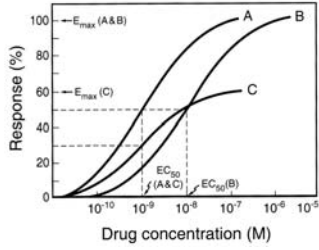
## Efficacy



Efficacy, E, describes transition from receptor with agonist bound (inactive) to receptor with agonist bound (active).

Different agonists (drugs) may have different efficacies

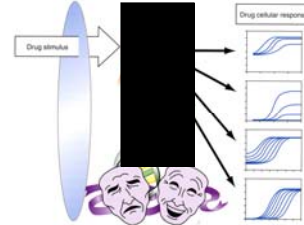
## Full & Partial Agonists



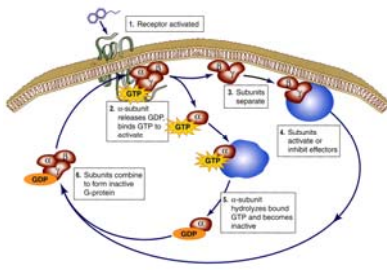
Full agonist  $E_{max} = 100\%$   
 Partial agonist  $E_{max} < 100\%$   
 In this example A & B are full agonists, while C is a partial agonist.  
 B is less potent than A (greater  $EC_{50}$ ).  
 The potency of C is the same as A, but the efficacy of C is less than A.

Classical treatment assumes agonist binding (affinity) and efficacy are discrete entities

## "Classical" Pharmacology



## Inside the Black Box



## Inside the Black Box

Activation of drug-bound receptor  $AR \rightarrow AR^*$   
 (intrinsic efficacy)

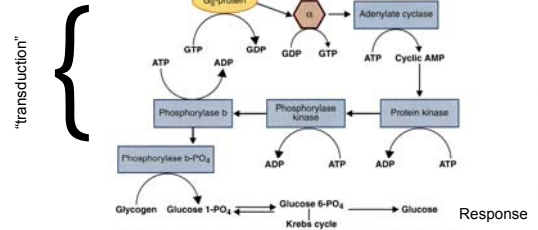
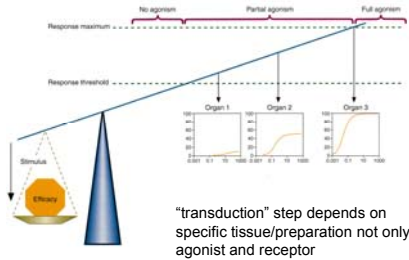
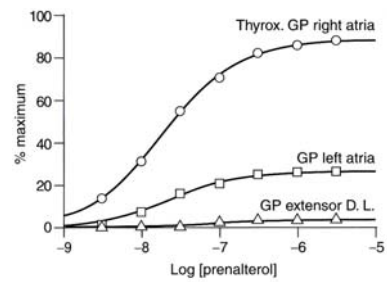


FIGURE 2.8 Stimulus response cascade for the production of blood glucose by activation of  $\beta$ -adrenoceptors.



"transduction" step depends on specific tissue/preparation not only agonist and receptor

Same drug can be a partial agonist or a full agonist in different preparations from guinea pig



Prenalterol;  $\beta$ -adrenoceptor agonist. D.L. – digitorum longus

