Pharmacodynamics 3

- Part I: Introduction
- Part II: Binding of the ligand to the receptor
  - » interactions between ligand and receptor that govern binding
- Part III: Quantitative description of ligand-receptor binding
  - » binding at equilibrium
  - » association and dissociation kinetics
- Part IV: Relationship between ligand concentration, binding and effect
  - » concentration-effect relationship
  - » mechanisms/models of ligand→binding→effect coupling
  - » Agonists: definition, efficacy, potency
  - » Allosteric modulators
  - » Antagonists: types, quantitative description of their interaction with receptor and agonists
- Part V: Other aspects of receptor function and drug therapy

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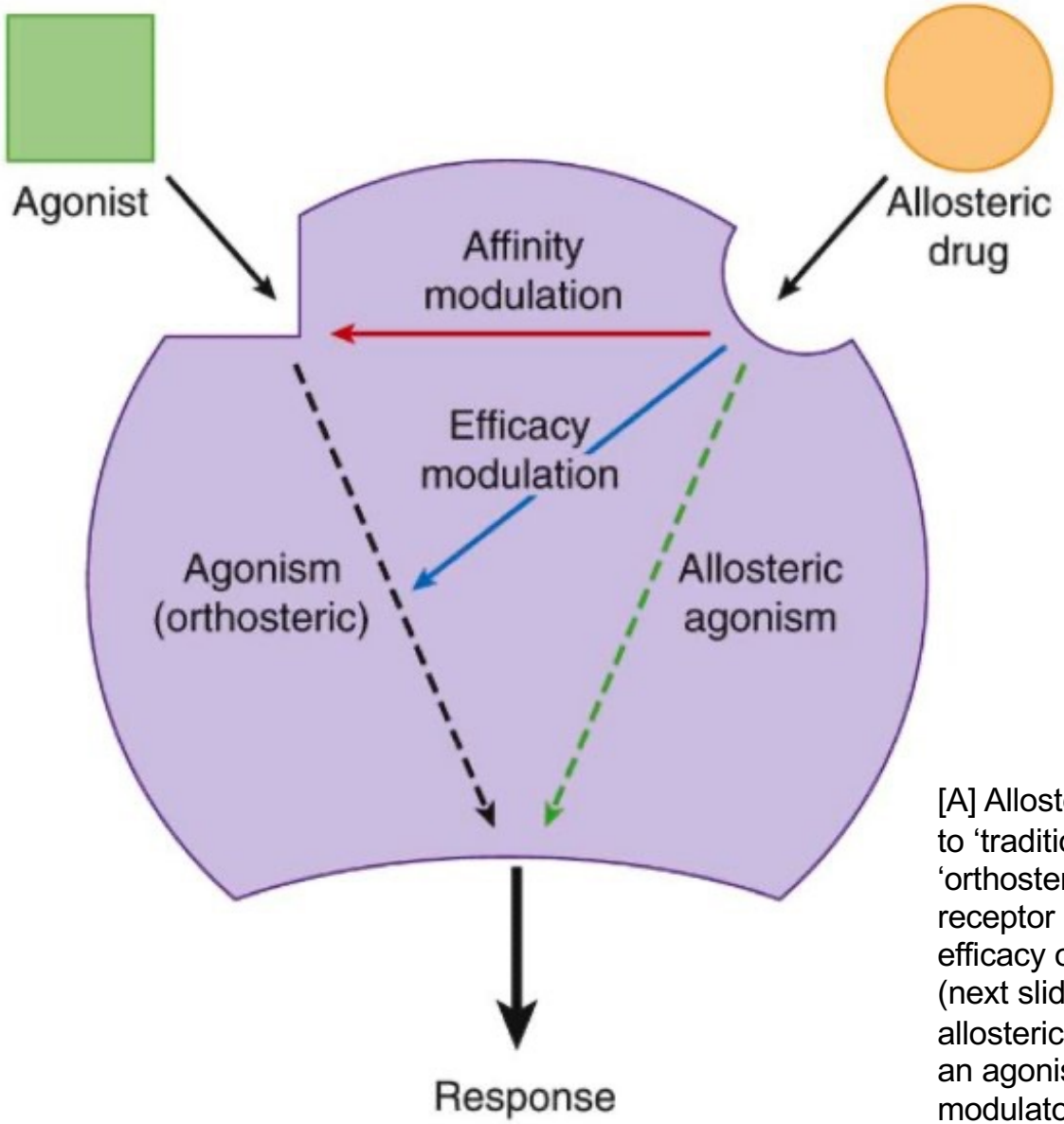
Allosteric modulators

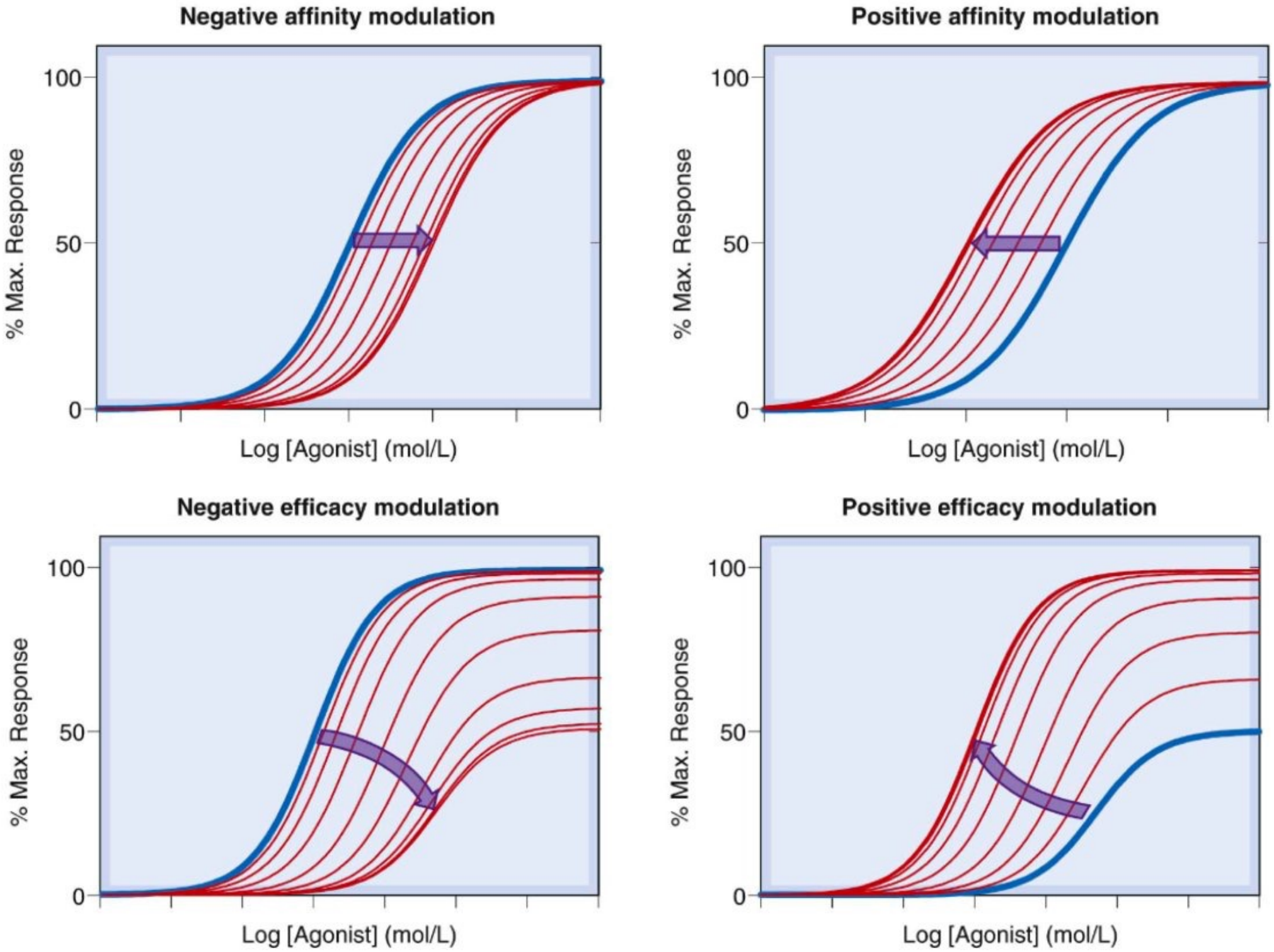
- **allosteric modulators* bind to their own site on the receptor that is different from the binding site of the endogenous agonist**, and produce an effect through a change in protein conformation. Allosteric modulators can affect the affinity of the responsiveness of the receptor to the agonist.
- A prominent example are benzodiazepines (diazepam) acting on $GABA_A$ receptor channels.

(* originally “allosteric” (Monod, 1965) was defined as a specific property of multi-subunit proteins; the current use of this term is quite different from its original meaning).

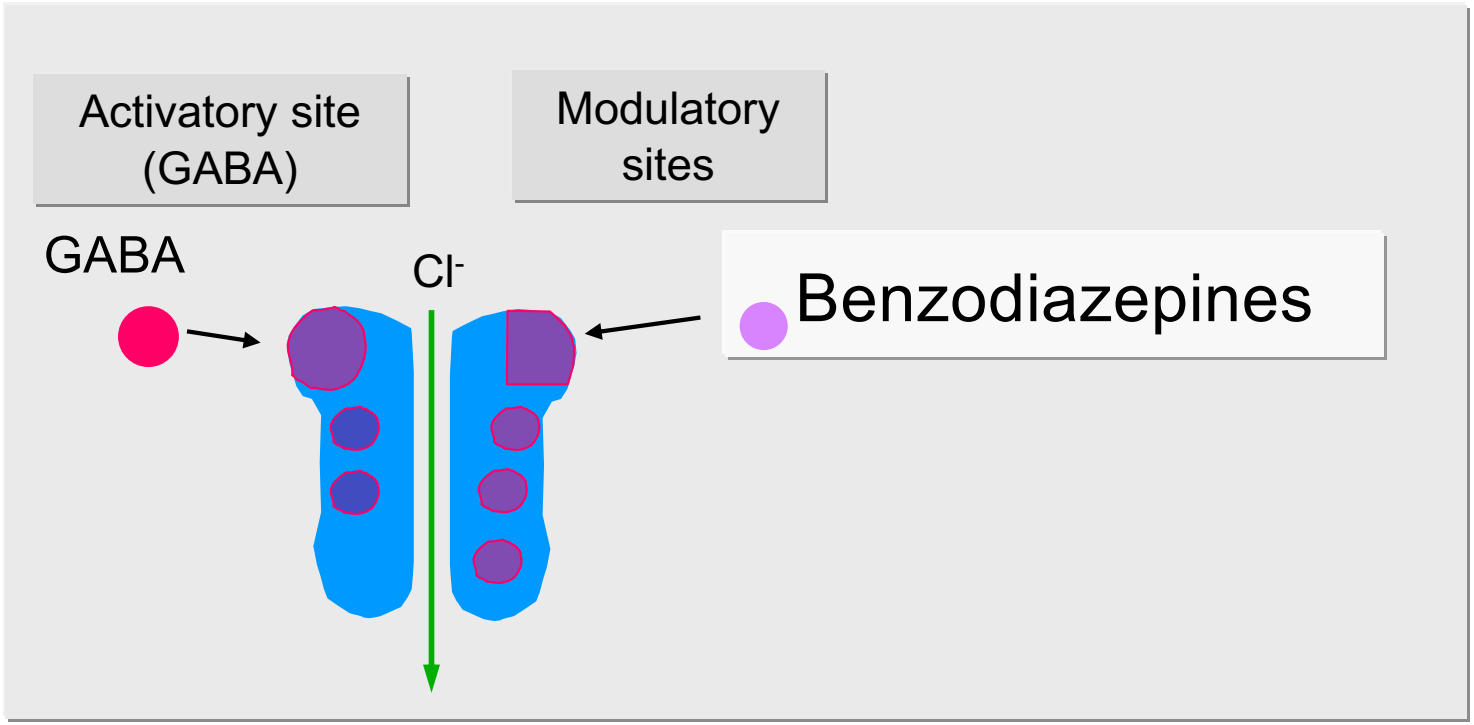
Scheme allosteric drug action



[A] Allosteric drugs bind at a separate site on the receptor to 'traditional' agonists (now often referred to as 'orthosteric' agonists). They can modify the activity of the receptor by (i) altering agonist affinity, (ii) altering agonist efficacy or (iii) directly evoking a response themselves. [B] (next slide) Effects of affinity- and efficacy-modifying allosteric modulators on the concentration–effect curve of an agonist (blue line). In the presence of the allosteric modulator the agonist concentration–effect curve (now illustrated in red) is shifted in a manner determined by the type of allosteric modulator until a maximum effect of the modulator is reached.


Negative affinity modulation
100
50
0
% Max. Response
Log [Agonist] (mol/L)
Positive affinity modulation
100
50
0
% Max. Response
Log [Agonist] (mol/L)
Negative efficacy modulation
100
50
0
% Max. Response
Log [Agonist] (mol/L)
Positive efficacy modulation
100
50
0
% Max. Response
Log [Agonist] (mol/L)

Benzodiazepines are $GABA_A$ receptor modulators



- Benzodiazepines bind to a site that is distinct from the GABA binding site
- Benzodiazepines can not activate GABAA receptors but they change the way the receptors reacts to GABA
- termed ”positive allosteric modulators” (PAMs)

5

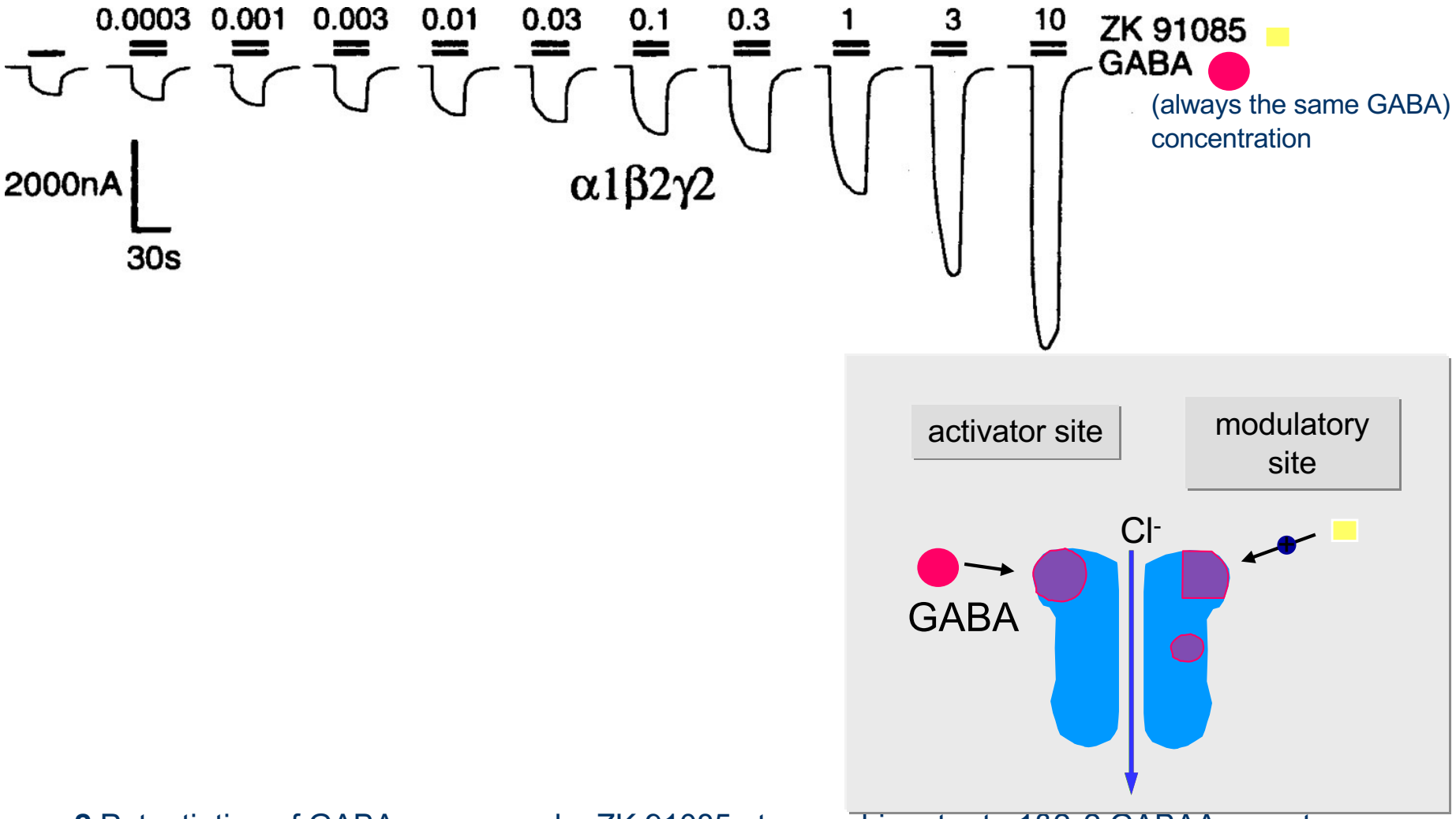
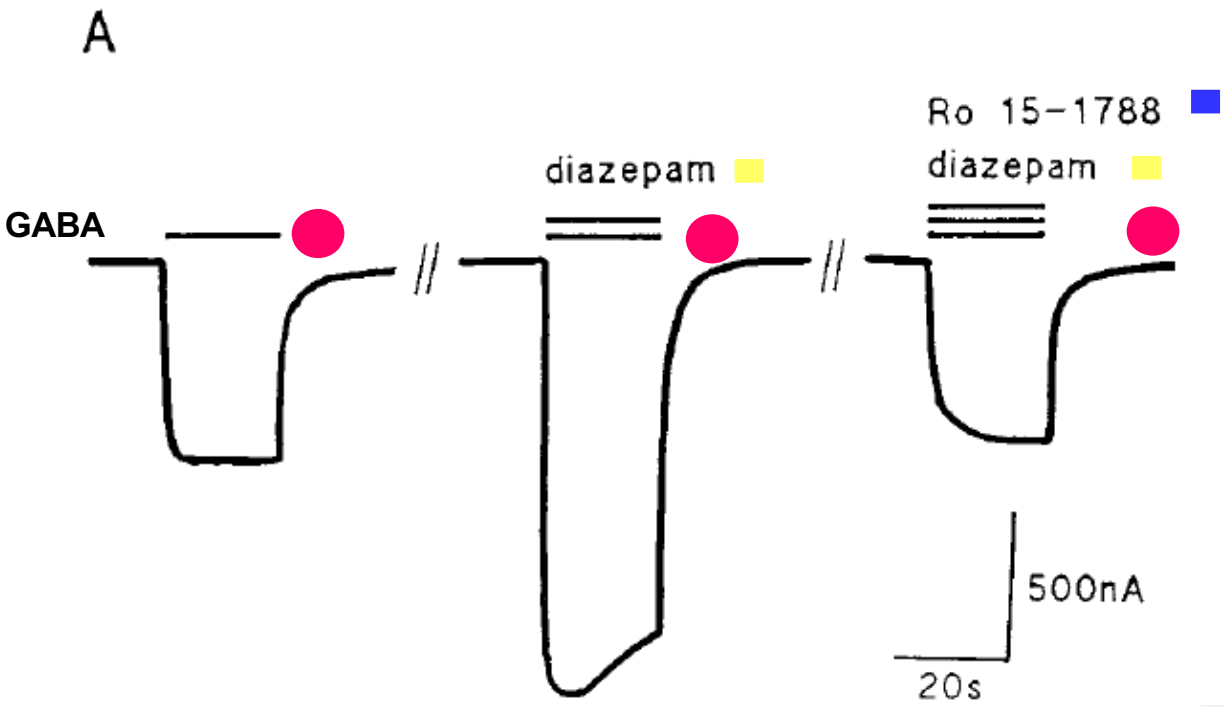
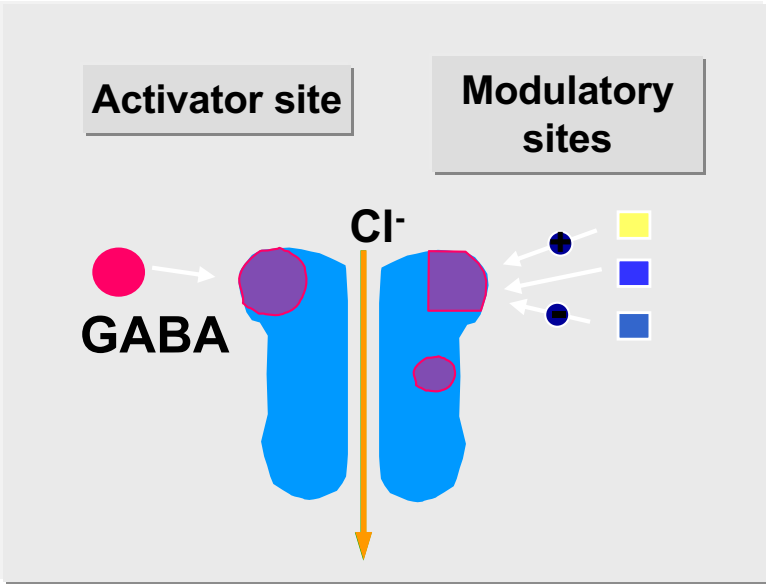


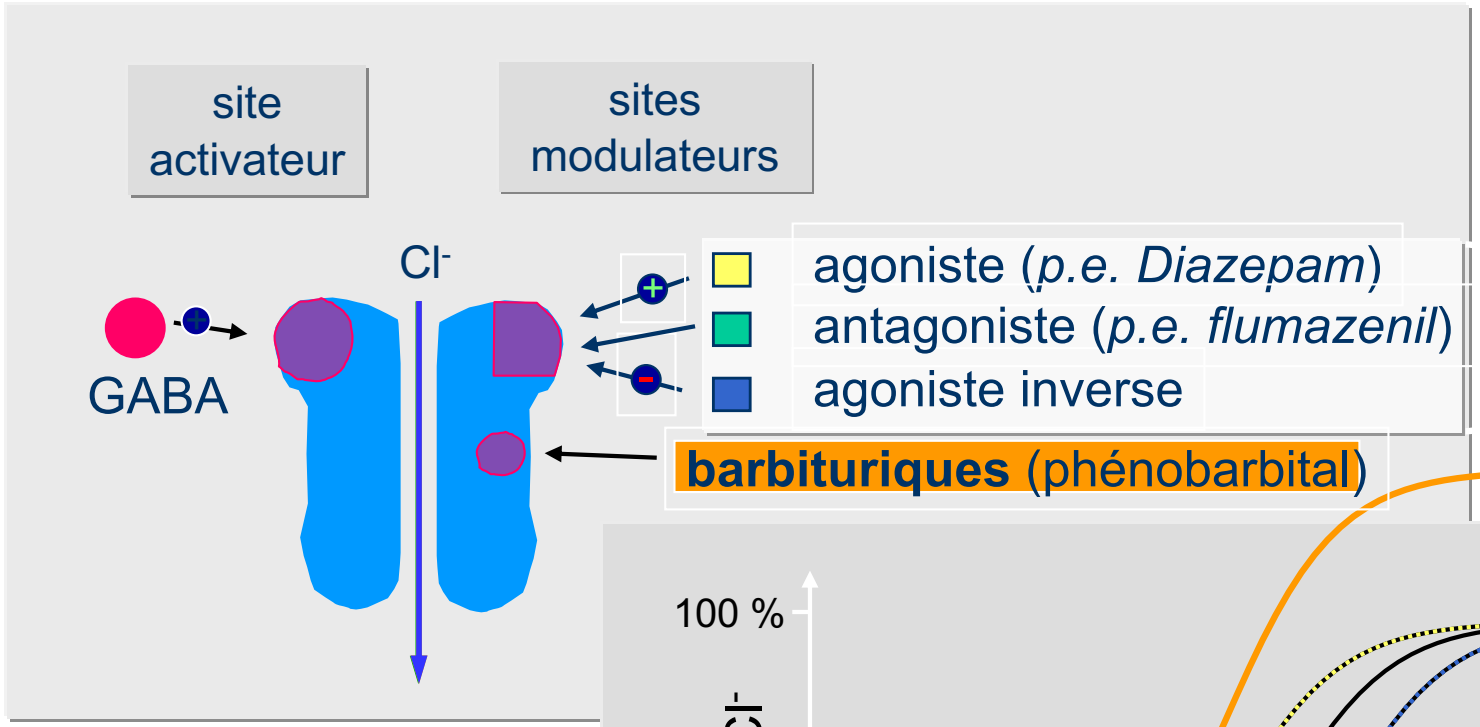
Figure 2 Potentiation of GABA responses by ZK 91085 at recombinant rat a1ß2γ2 GABAA receptors expressed in *Xenopus laevis* oocytes. Application of GABA alone resulted in approximately 5% of the maximal current amplitude. Increasing concentrations of ZK 91085 were coapplied with GABA. Periods of drug application are indicated by horizontal bars above the current records. Concentrations of ZK 91085 are shown in µM.



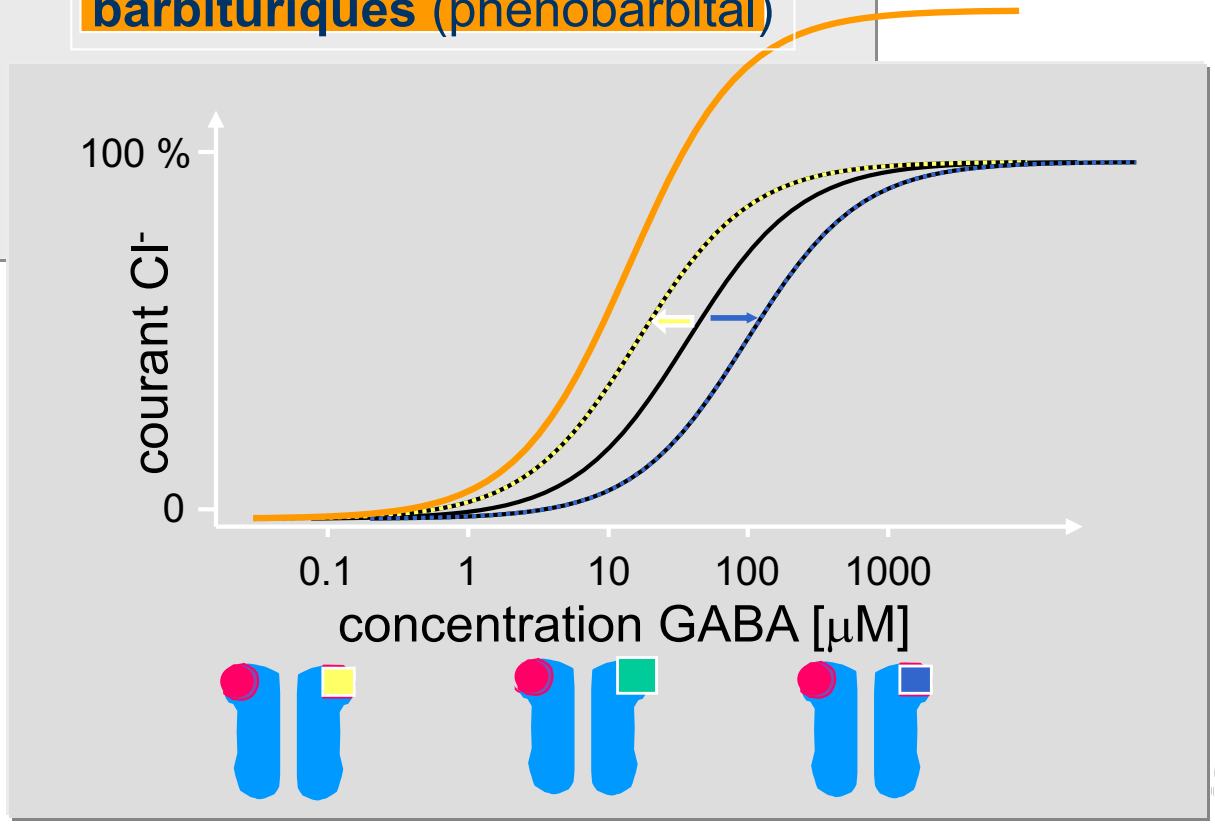


From Buhr et al., JBC 272, 11799–11804, 1997, concentration of GABA and Ro: 1 μM

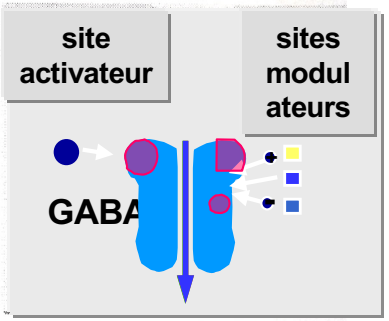
Mechanisms of $GABA_A$ receptor modulators



ligands du site benzodiazépinique



Exercice agonistes/antagonistes récepteur GABA



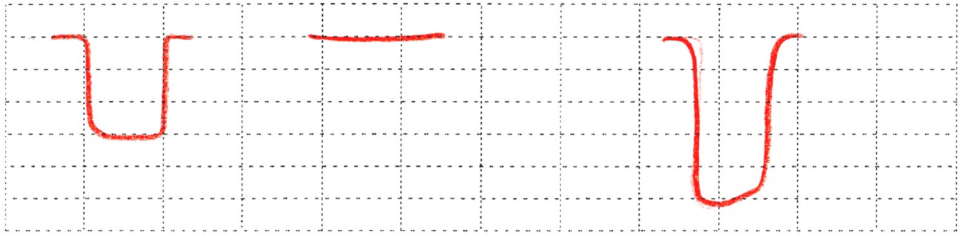
exemple

Expérience

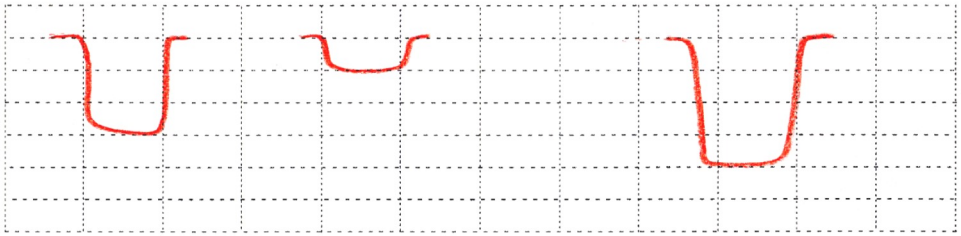
interprétation

(agoniste, etc; site activateur/modulateur)

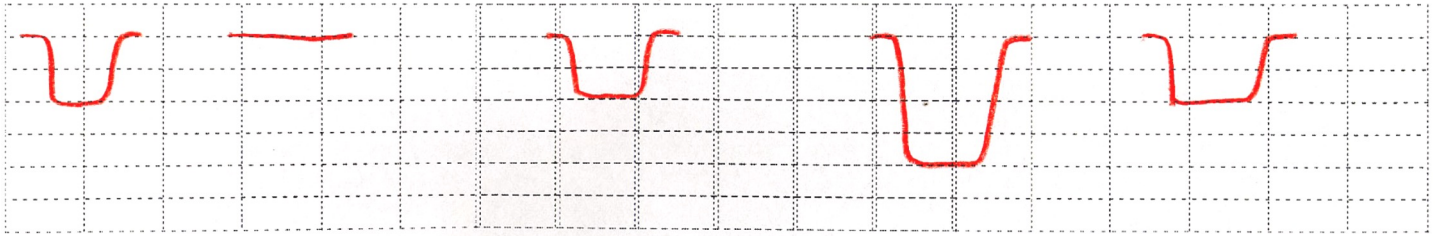
GABA | Substance A | GABA + substance A



GABA | Substance B | GABA + substance B



GABA | Substance C | GABA + substance C | GABA + Diazepam | GABA + Diazepam + substance C



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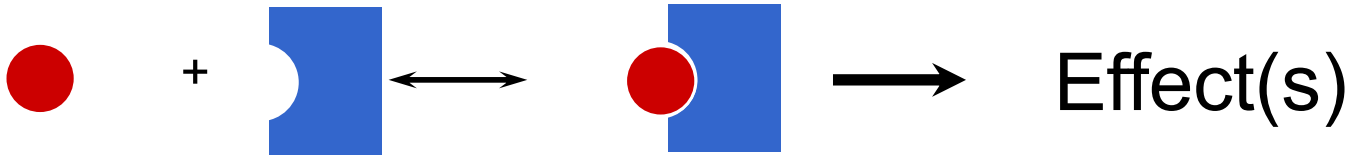
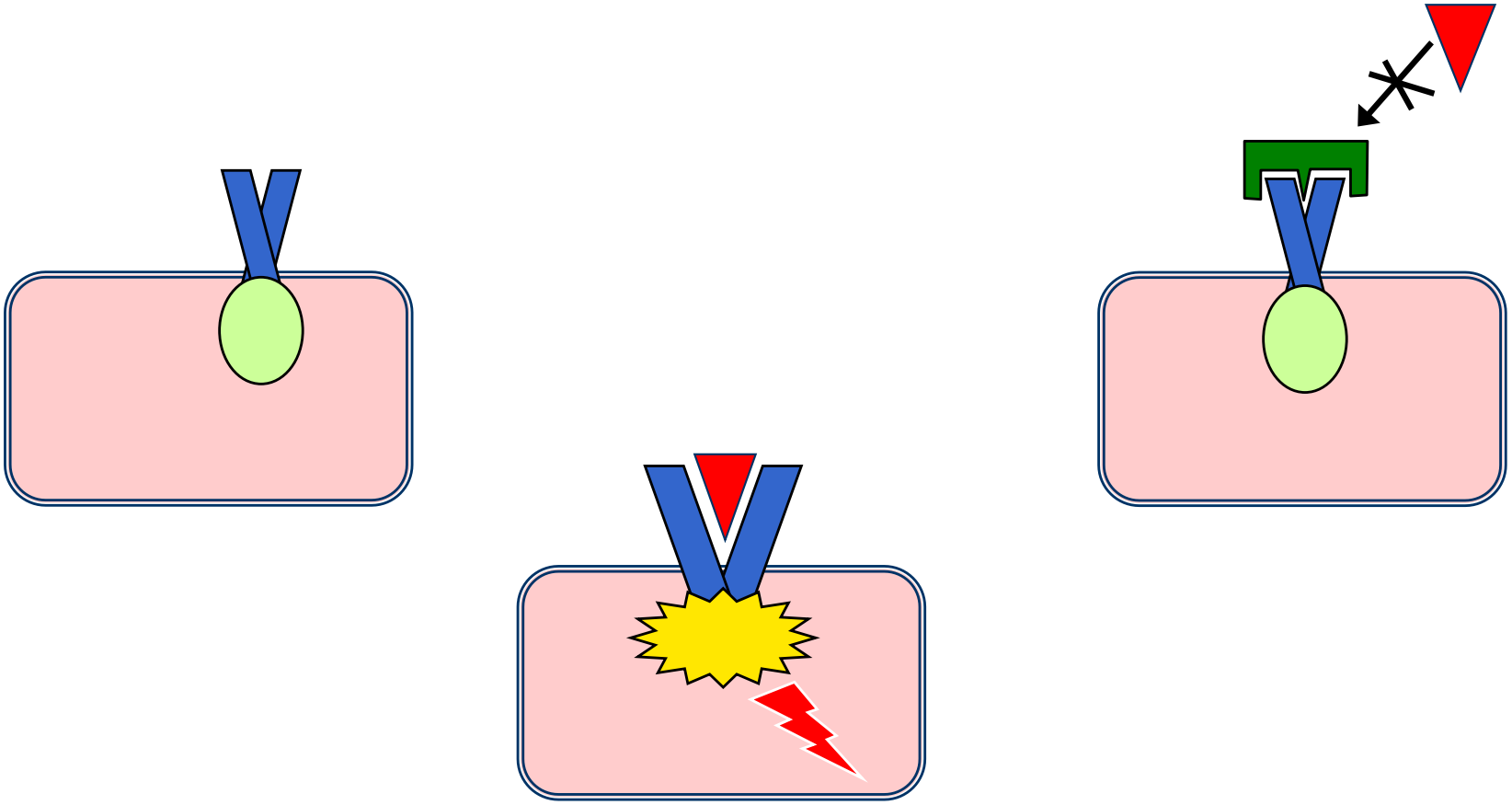


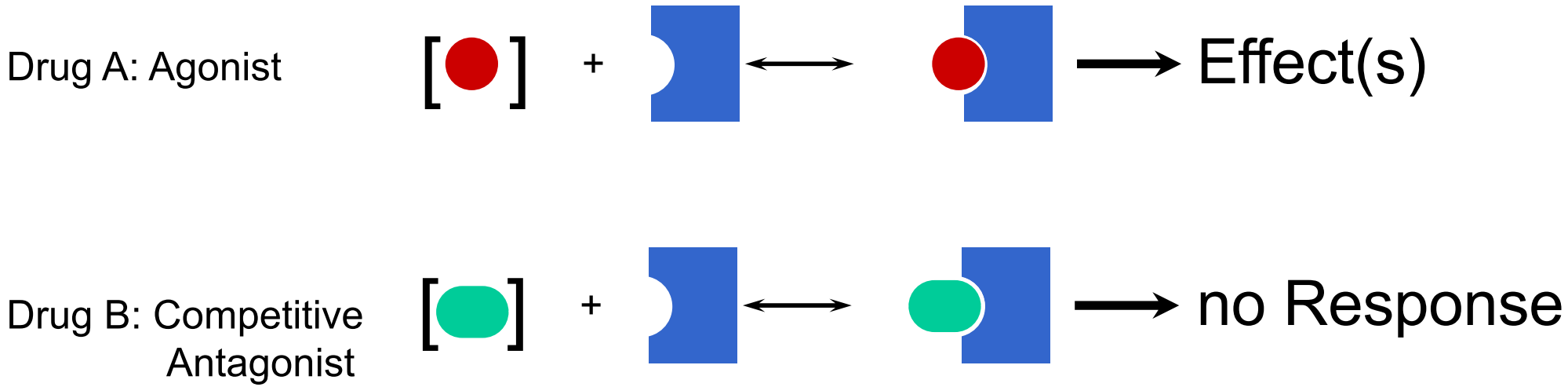
Agonists and antagonists, a first approach

Definitions :

- An **agonist** induces by itself an effect by activating a receptor
- A **competitive antagonist** is a molecule that inhibits the effect of an agonist, but has no effect in the complete absence of an agonist



Agonists and competitive Antagonists, sequential scheme



(these are simplified schemes, assuming that there is no ligand-independent activity of the receptor)

Types of antagonists : definitions

pharmacological antagonism:

- the interaction happens at the same receptor (this is what we are discussing in this class)

chemical antagonism:

- chemical interaction between the two substances in solution; *example: protamin is a basic protein that binds to anticoagulants of the heparin type and can be used to end the effect of heparin.*

pharmacokinetic antagonism:

- Situation in which the "antagonist" effectively reduces the concentration of the agonist at the site of action (changing degradation or elimination); *example: phenytoin induces hepatic metabolism of warfarin, an anticoagulant.*

physiological antagonism:

- effect opposed to that of the agonist, but by acting on a different receptor; *example: in hyperthyroidism, β-blockers are used to reduce the tachycardia that is due to the thyroid hormones; however, the thyroid hormones produce the tachycardia not via β-adrenergic stimulation.*

Types of pharmacological antagonists

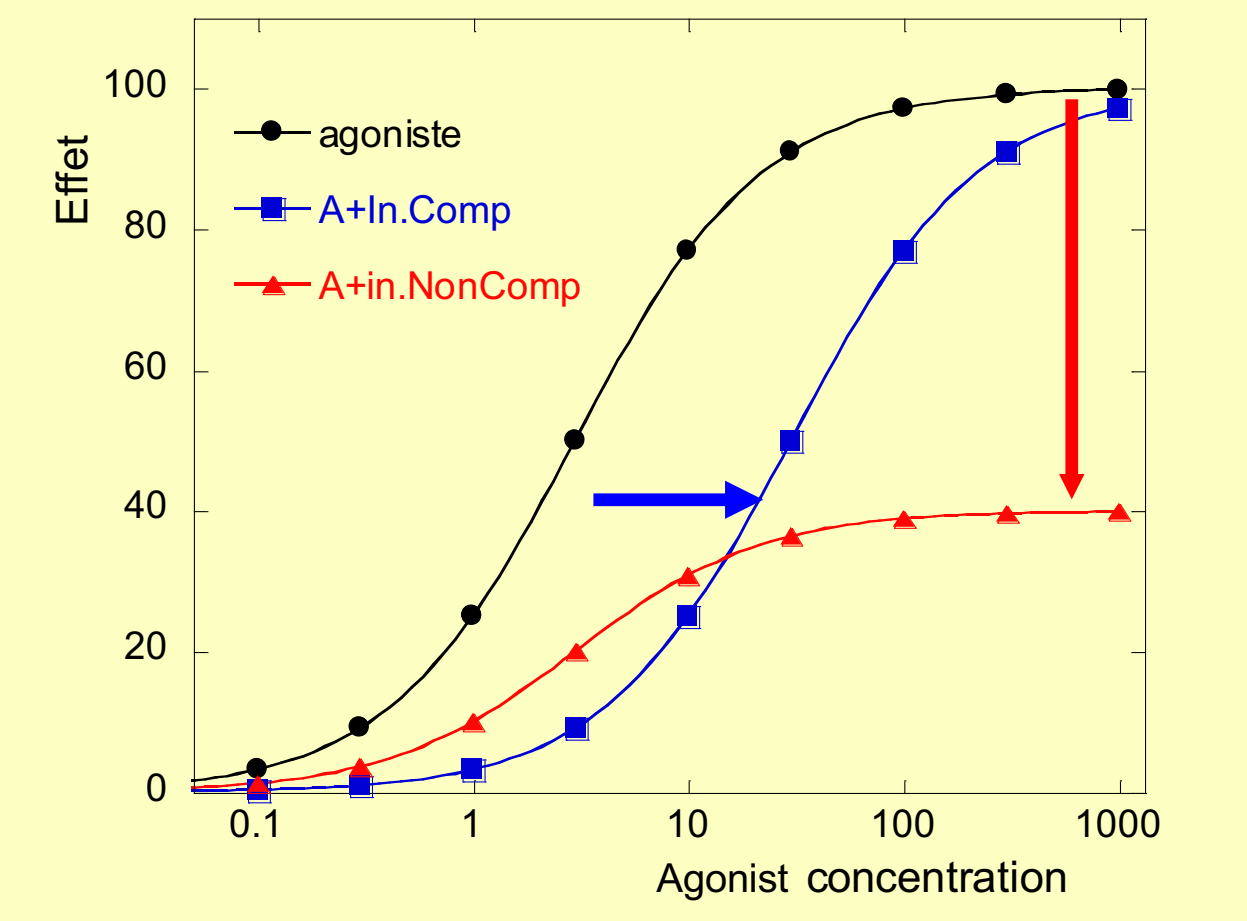
irreversible antagonist:

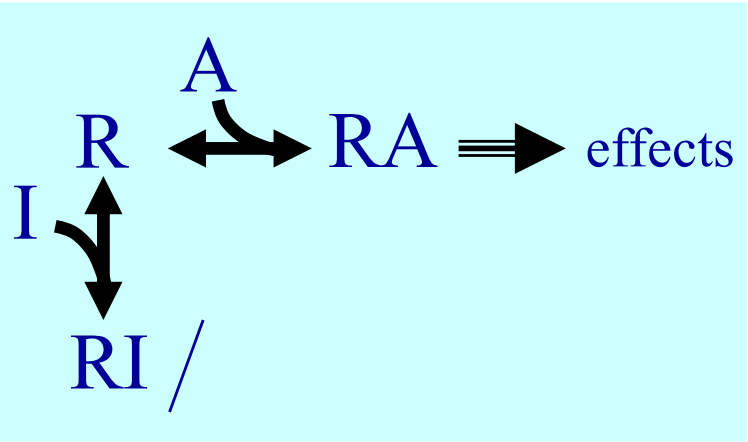
- for example by forming a covalent bond with an active protein (*either in- or outside of the agonist binding site*)
  - *this induces a permanent inactivation of the protein (example: irreversible acetylation of the cyclo-oxygenase of thrombocytes by aspirin)*
  - *the inhibitory effect is irreversible, but in fact limited by the lifetime and replacement rate of the receptor protein*

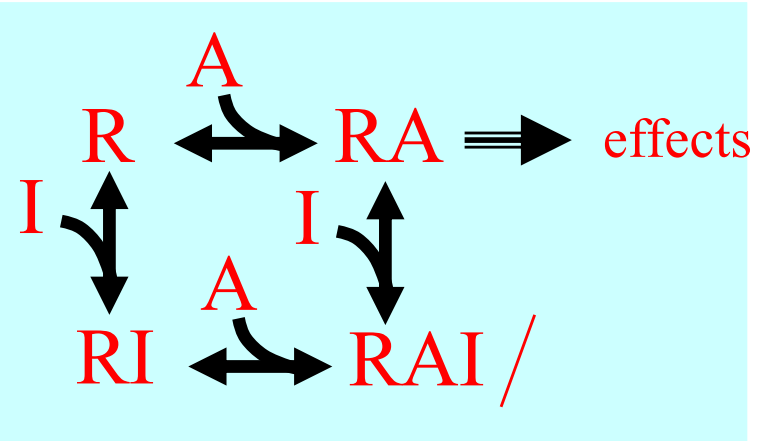
reversible antagonist

- competitive antagonist: a competitive antagonist binds reversibly to the agonist binding site (sometimes called the “active site”) of a receptor. There is a competition between the agonist and the antagonist for occupying the binding site
- non-competitive antagonist : binding to a different site than the agonist binding site, that does not prevent agonist binding but inhibits activation of the receptor. sometimes also called “allosteric antagonists”.

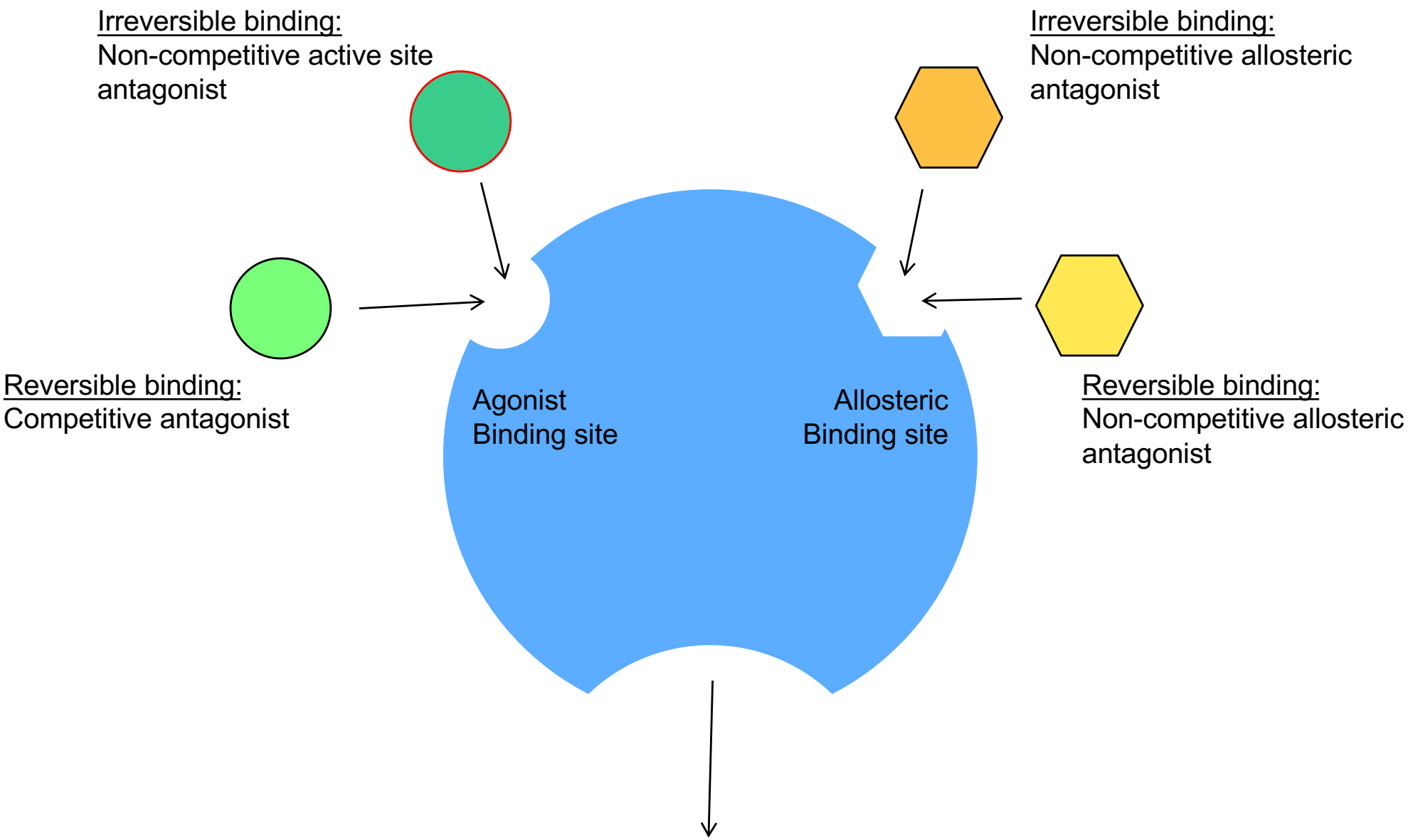
Competitive and non-competitive inhibition by a reversible antagonist



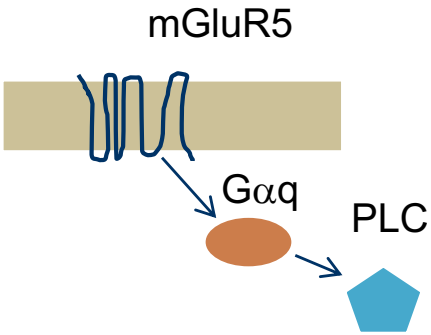




- **competitive inhibition**: reduction of the apparent affinity **Ex: Naloxone**
- **non-competitive inhibition**: reduction of the maximal effect

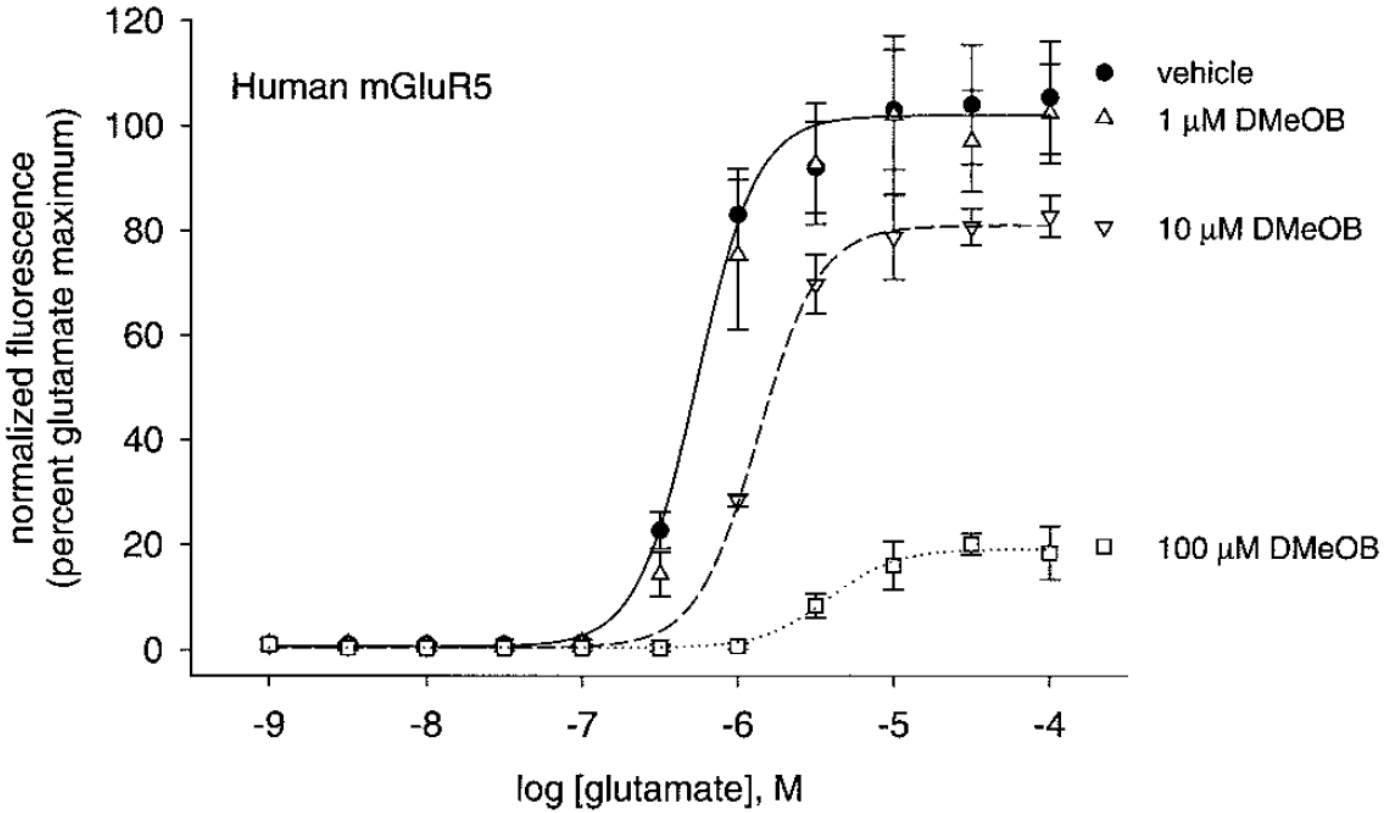

Irreversible binding:
Non-competitive active site antagonist
Irreversible binding:
Non-competitive allosteric antagonist
Reversible binding:
Competitive antagonist
Agonist Binding site
Allosteric Binding site
Reversible binding:
Non-competitive allosteric antagonist
Response

Example: antagonism of mGluR5 receptor



→ Calcium release from intracellular stores

→ *measured by fluorescence*



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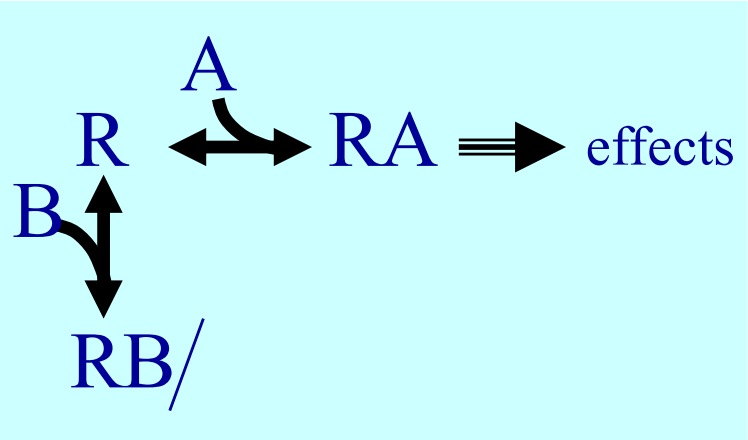
Reversible competitive Antagonism

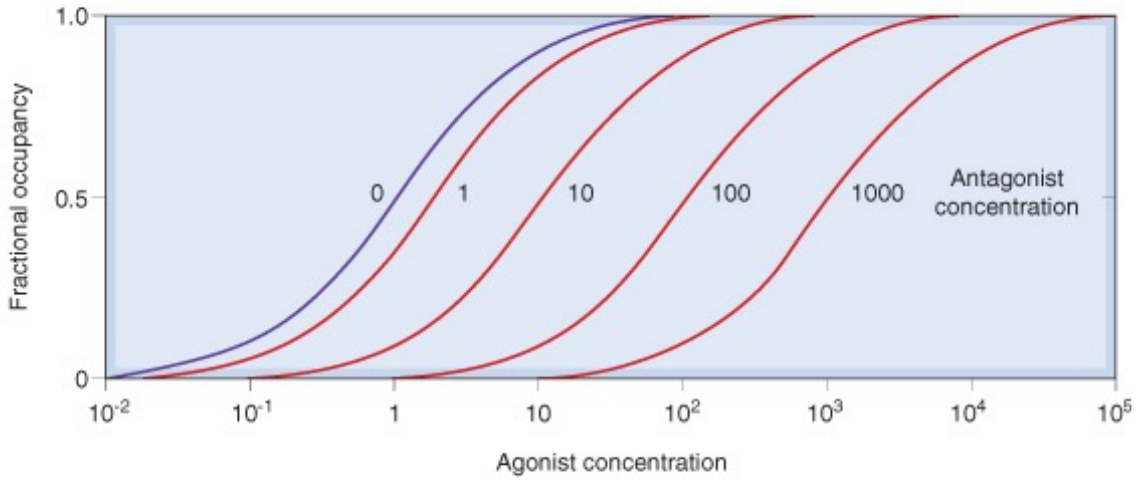


Occupancy of the agonist A in the presence of antagonist B

Suppose that two drugs, A and B, which bind to the same receptor with equilibrium constants K_A and K_B, respectively, are present at concentrations x_A and x_B. If the two drugs compete (i.e. the receptor can accommodate only one at a time), then, by application of the same reasoning as for the one drug situation (equation 8) the occupancy by drug A is given by:

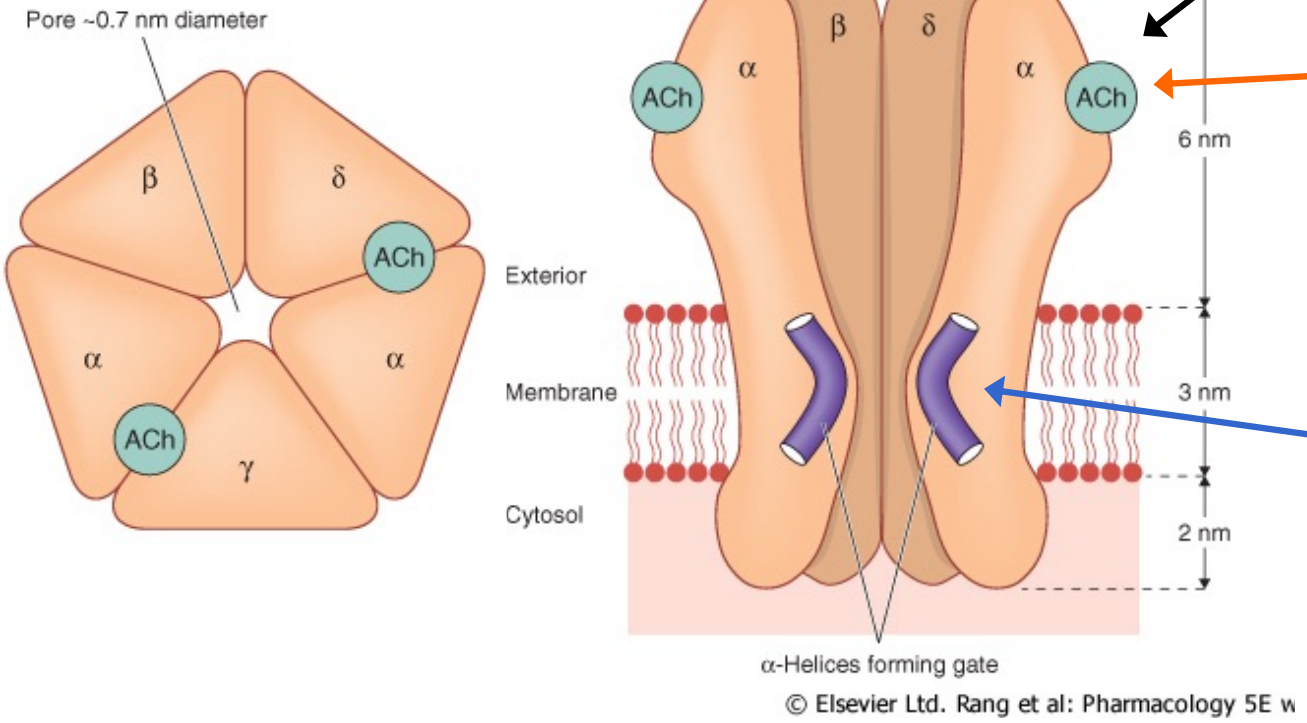
$$pA = \frac{x_A/K_A}{x_A/K_A + x_B/K_B + 1} \qquad (17)$$

adding drug B reduces the occupancy by drug A and shifts the binding curve for A to higher concentrations without changing the slope



Hypothetical agonist concentration-occupancy curves in the presence of reversible competitive antagonists. The concentrations are normalised with respect to the equilibrium constants (K, i.e. 1.0 corresponds to a concentration equal to K, and results in 50% occupancy). A Reversible competitive antagonism.

Example: Pharmacology of the nicotinic acetylcholine receptor



Agonists:
Acetylcholine
Nicotin

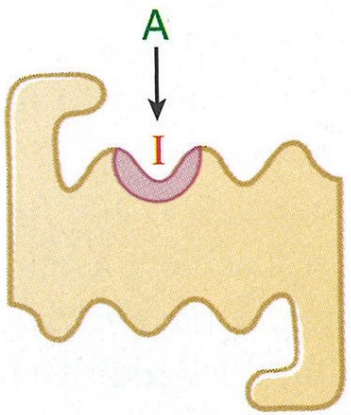
Competitive Antagonists
Tubocurarine
Pancuronium
Vecuronium

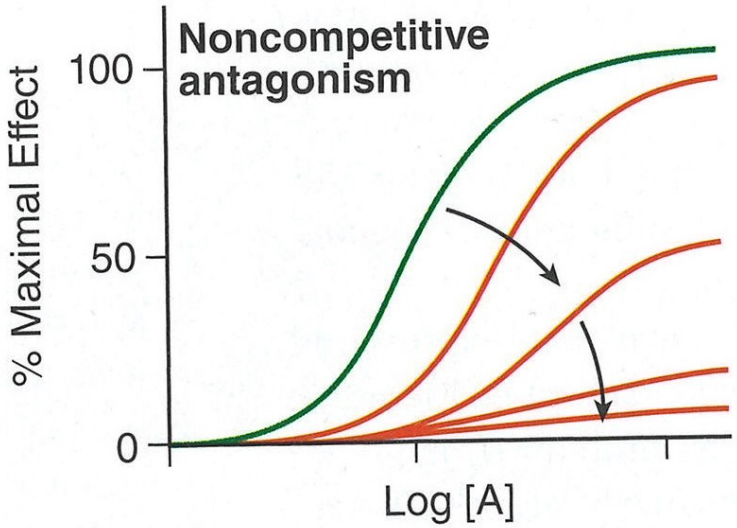
Non-competitive Antagonists :
Neurosteroids
Tetracaine

Figure 3.4 Structure of the nicotinic acetylcholine receptor (a typical ligand-gated ion channel) in side-view (left) and plan-view (right). The five receptor subunits form a cluster surrounding a central transmembrane pore, the lining of which is formed by the M2 helical segments of each subunit. These contain a preponderance of negatively charged amino acids, which makes the pore cation selective. There are two acetylcholine (ACh)-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 swing out of the way, thus opening the channel pore. (Based on Unwin 1993, 1995.)

Other types of non-competitive antagonism

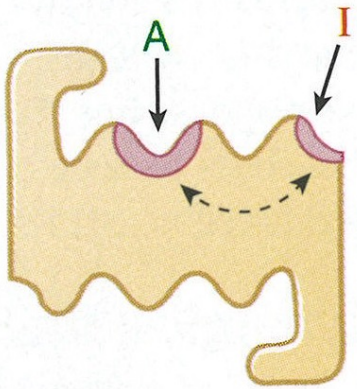
Pseudoirreversible

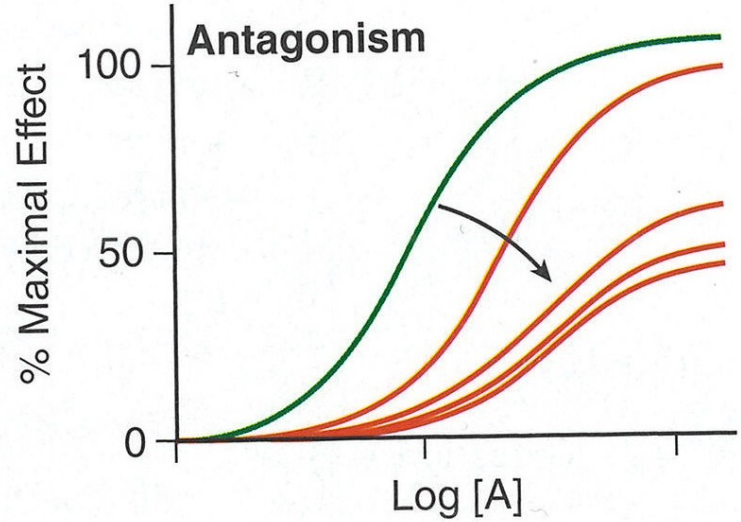




Antagonist binds irreversibly or slowly reversibly to the orthosteric binding site ("A" in the scheme)

Allosteric





Antagonist binds to a modulatory site (I) that interacts with the orthosteric site (A)

Antagonism can be quantitatively described by the IC_{50}

Relationship inhibitor concentration to effect

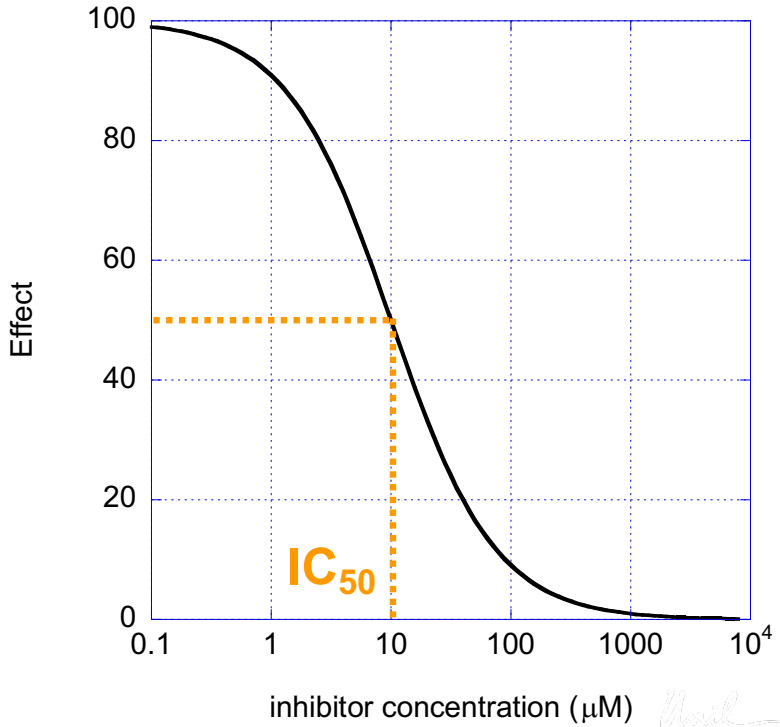
In analogy to the EC_{50}, an IC_{50}, corresponding to the concentration of the inhibitor that inhibits 50% of the response, can be defined :

$$E = \frac{E_0}{1 + x_B / IC_{50}} \quad (18)$$

with

E	effect (response) in the presence of the inhibitor
E_0	effect (response) in the absence of the inhibitor
x_B	concentration of the inhibitor

(at a given concentration of agonist)



Example of an inhibition curve

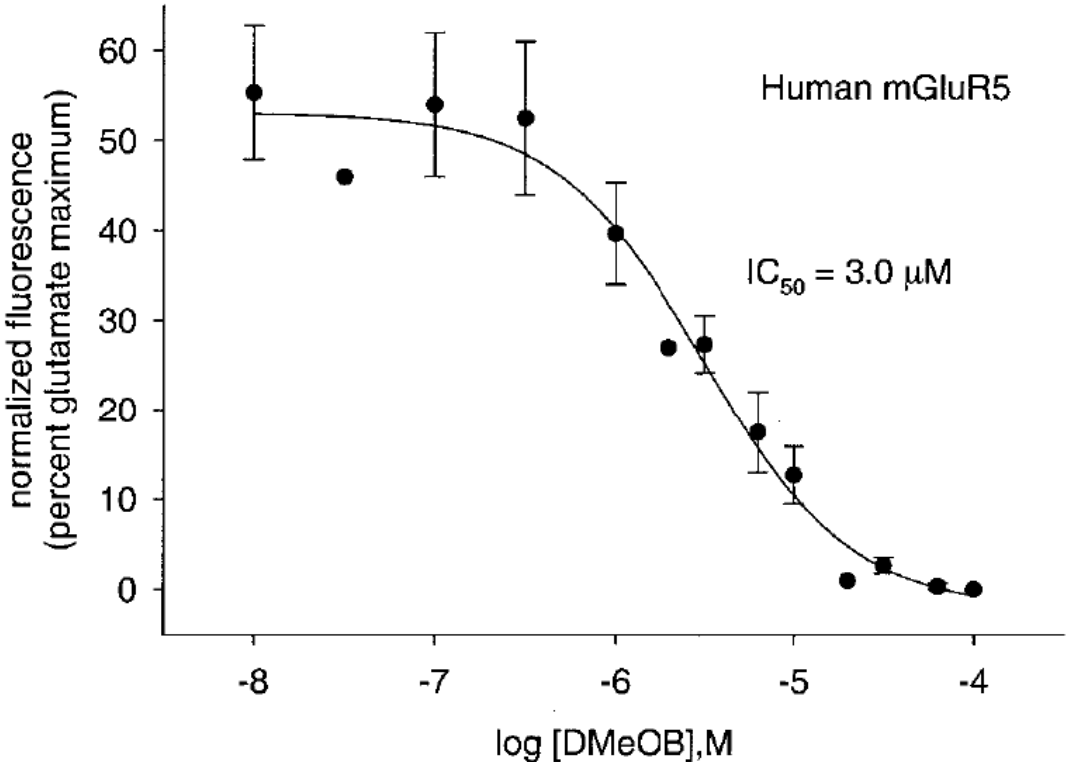


Fig. 8. DMeOB causes the inhibition of glutamate response. Human mGluR5 CHO cells were plated in clear-bottomed 384-well plates in glutamate/glutamine-free medium, loaded the next day with the calcium-sensitive fluorescent dye Fluo-4, and placed in $FLIPR_{384}$. A range of concentrations of DMeOB was added to the cells after 10 s of baseline determination. Five minutes later, a fixed concentration ($\sim EC_{10}$ concentration) of glutamate was added, and the Ca^{2+} response was measured with the use of $FLIPR_{384}$. Concentration-response curves were generated from the mean data of three experiments. Error bars represent S.E.M.

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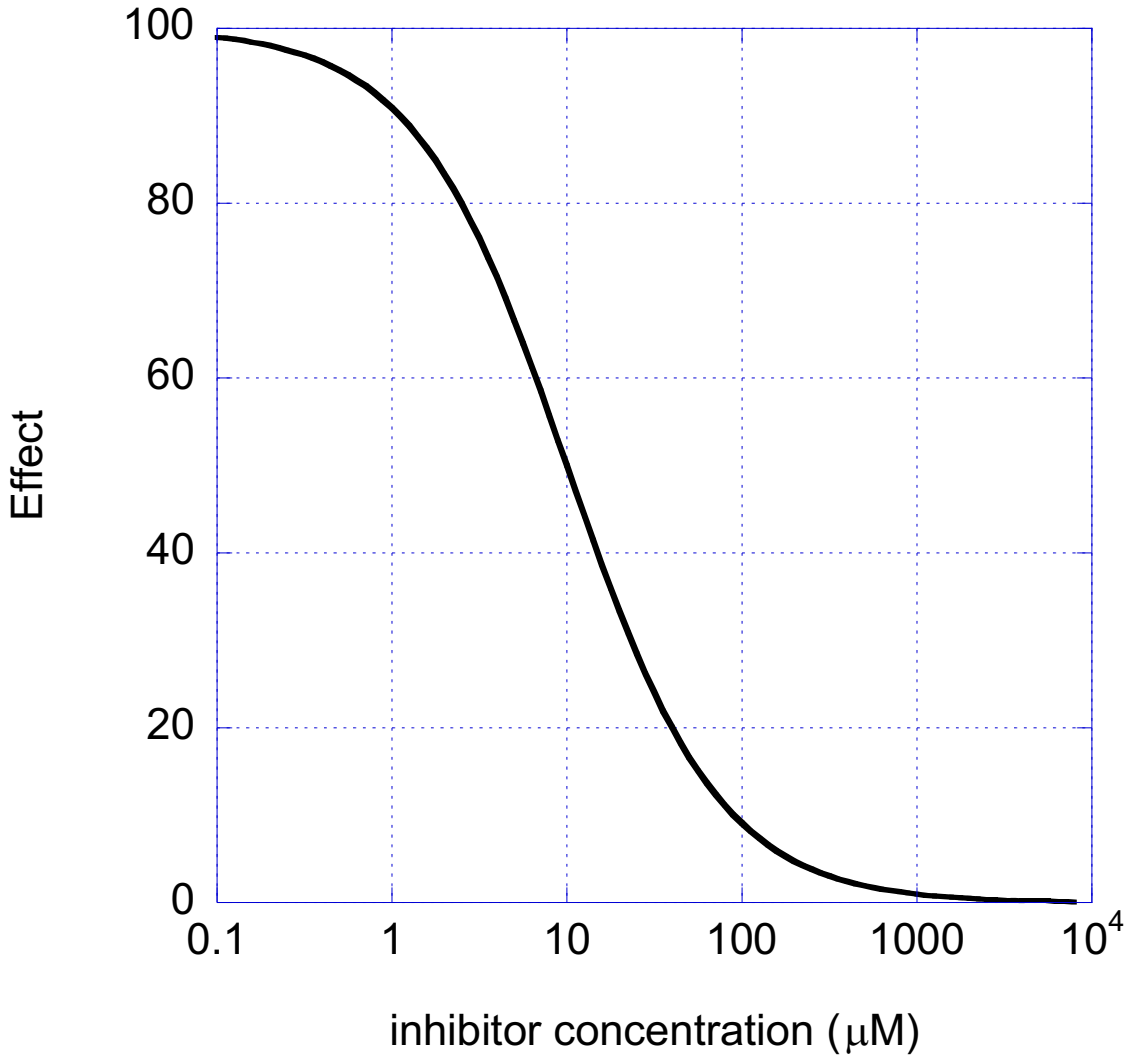
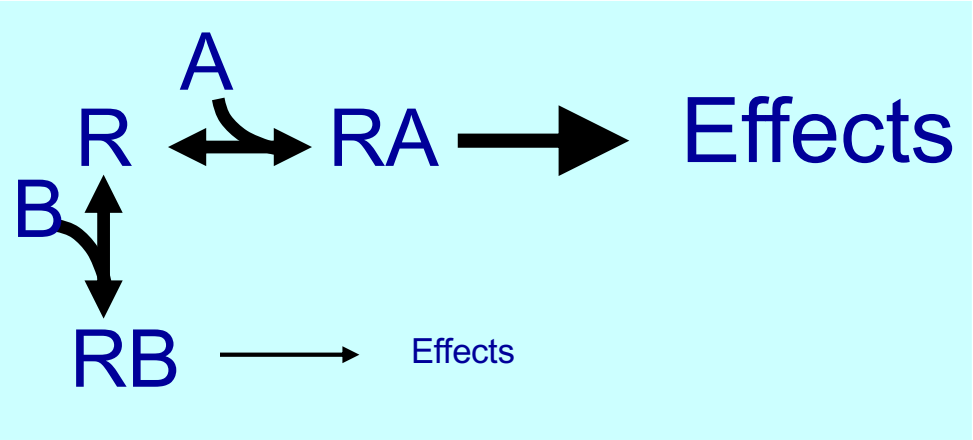


The inhibition curve is measured with increasing concentrations of antagonist, and at one given concentration of agonist (always the same for this curve)

Imagine now that you would carry out an inhibition curve in the same system (receptor, agonist, antagonist), but using a lower agonist concentration, which, in the absence of any antagonist (thus on the left end of the graph), would induce 50% of the effect.

- Draw this curve, for the case of a competitive, and for a non-competitive antagonist
- Will the IC50 be the same as in the existing curve in the graph (obtained at the higher agonist concentration)?

Presence of both a full and a partial agonist



drug A = full agonist
drug B = partial agonist

The full and the partial agonist will compete for the binding site on the receptor and depending on the conditions the presence of the partial agonist may decrease the response.

1. At μ-opioid receptors, Morphine has a Kd of 2.5 nM (K_A), while the antagonist Naloxone has a Kd of 4.4 nM (K_B). Calculate the occupancy of the receptor by Morphine (or 3-methyl-fentanyl)

- in the presence of 10 nM Morphine (or 3-methyl-fentanyl) (x_A) (without antagonist), and
- in the presence of 10 nM Morphine (or 3-methyl-fentanyl) (x_A) and 50 nM Naloxone (x_B)

(*assuming a K_A for China White of 0.4 pM = 0.0004 nM*)

$$p_A = \frac{1}{1 + K_d/x_A} \qquad \textbf{(8)}$$

$$pA = \frac{x_A/K_A}{x_A/K_A + x_B/K_B + 1} \qquad (17)$$

Terms in receptor pharmacology

Orthosteric site: The receptor recognition site to which the endogenous ligand binds

Inverse agonist *: Ligand that inhibits the spontaneous activity of a receptor.

Neutral antagonist *: Ligand that blocks receptor response by competing with agonists or inverse agonists.

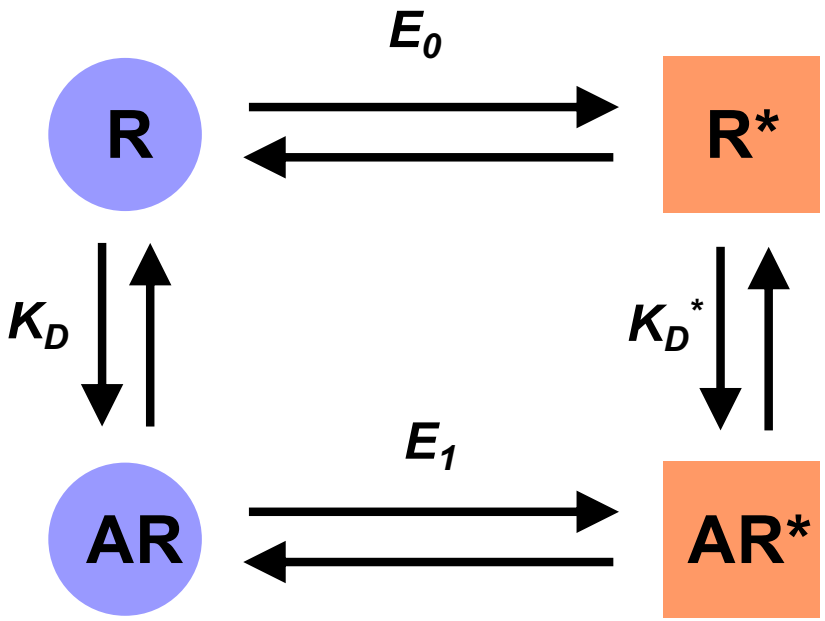
Allosteric site: Any receptor recognition site that is distinct from the orthosteric site.

Allosteric agonist: Ligand that is able to directly activate a receptor by binding to a recognition domain distinct from the orthosteric site.

Allosteric modulator: Ligand that increases or decreases the efficacy and/or affinity of an orthosteric agonist or antagonist by binding to a distinct allosteric site on the receptor.

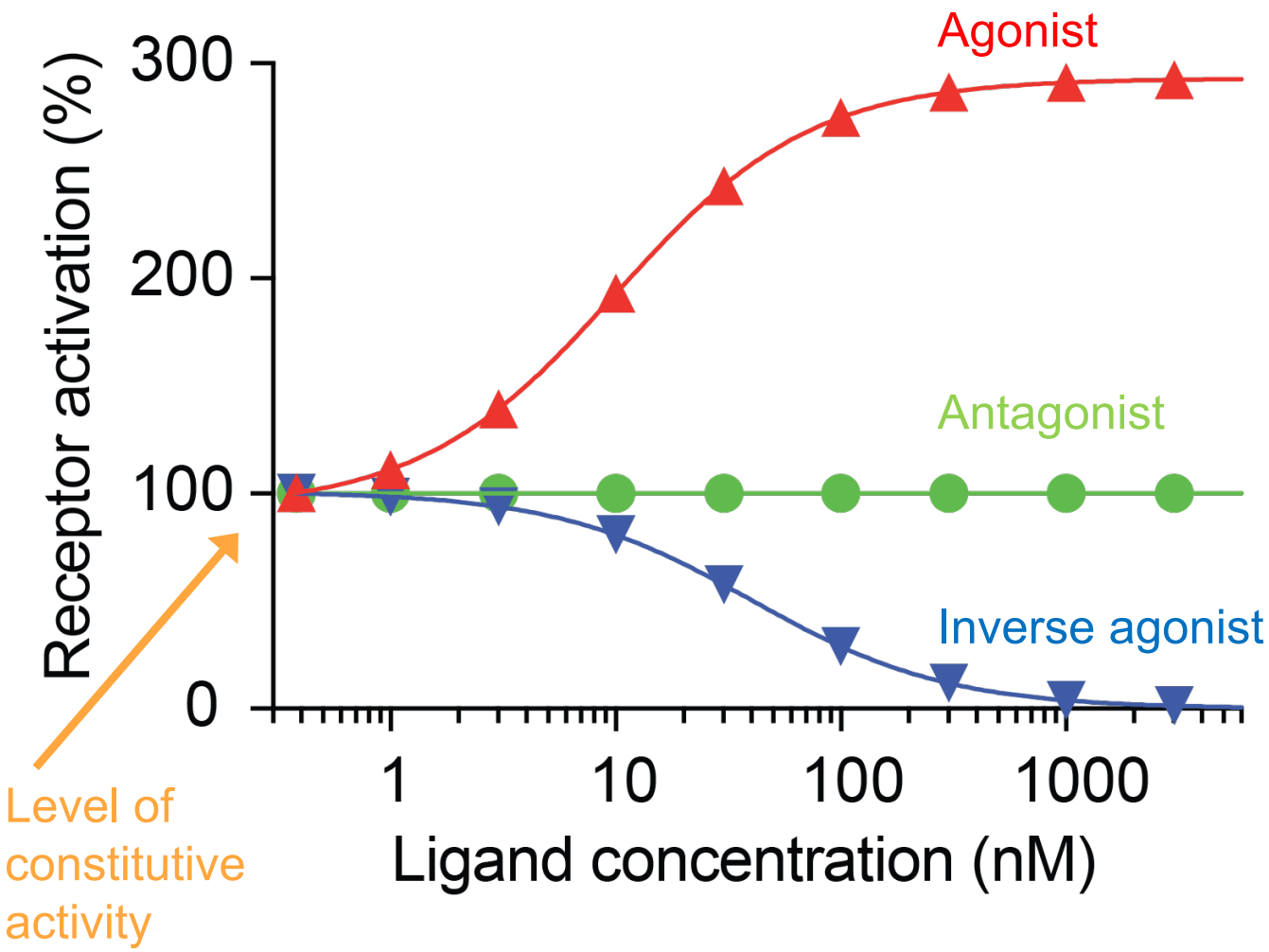
**, On receptors without constitutive (agonist-independent) activity, inverse agonists and neutral antagonists have the same effect*

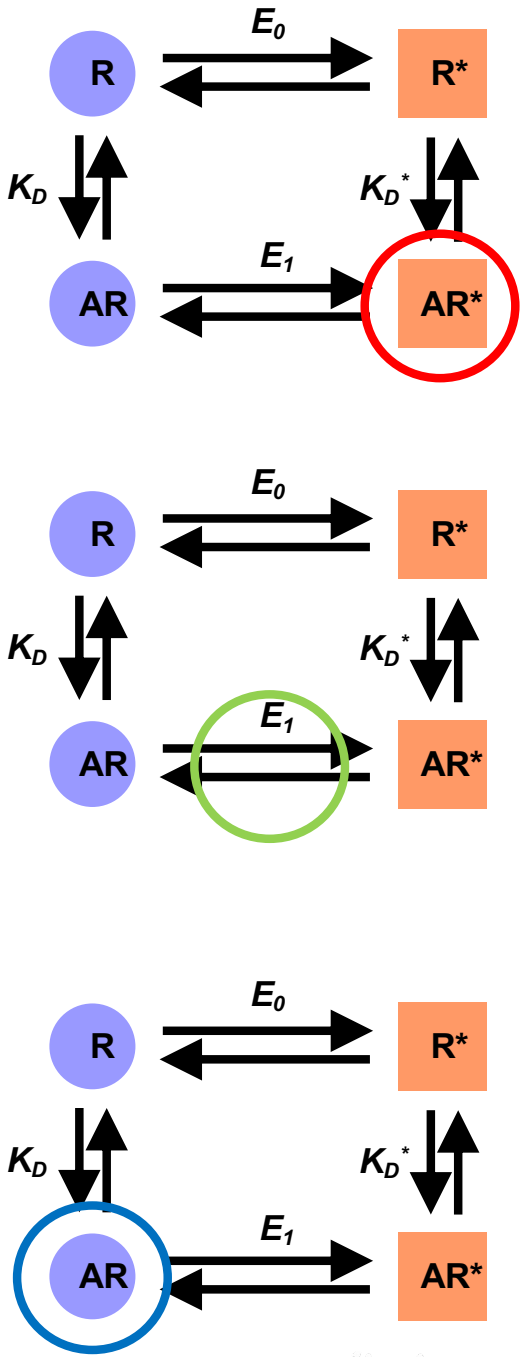
The two-state model explains the action of agonists, inverse agonists and competitive antagonists in terms of the relative affinity of different ligands for the resting and activated states of the receptor



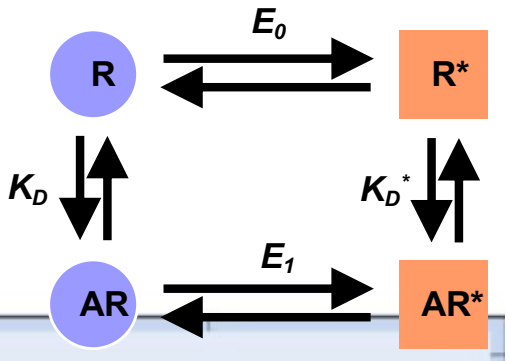
Agonist:	higher affinity for R* than for R *(E1, the relative affinity for R* with respect to R, is a measure of the efficacy of the agonist)*
Inverse agonist:	**higher affinity for R than for R*** *(in case the probability of the R* state is very low, the inverse agonist behaves as a competitive antagonist)*
Competitive Antagonist:	equal affinity for R* and R

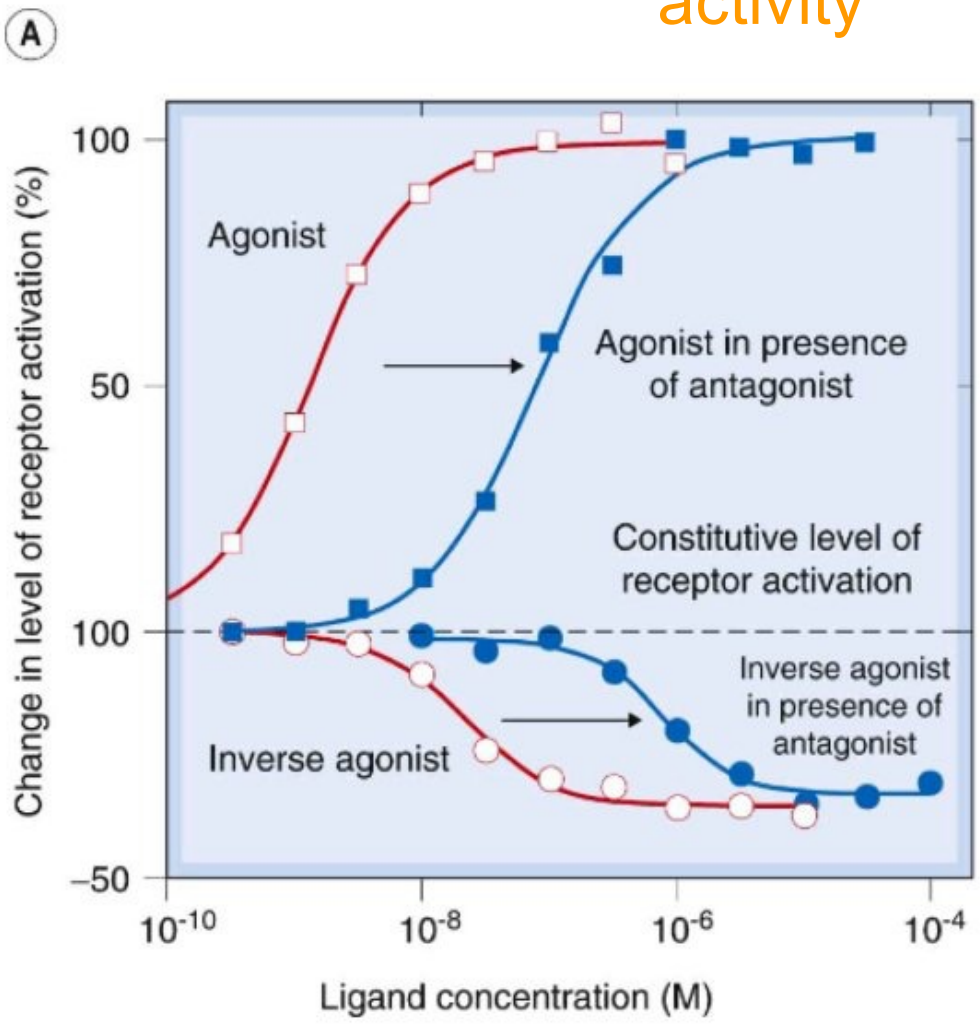
Action of inverse agonist etc. on system with constitutive activity

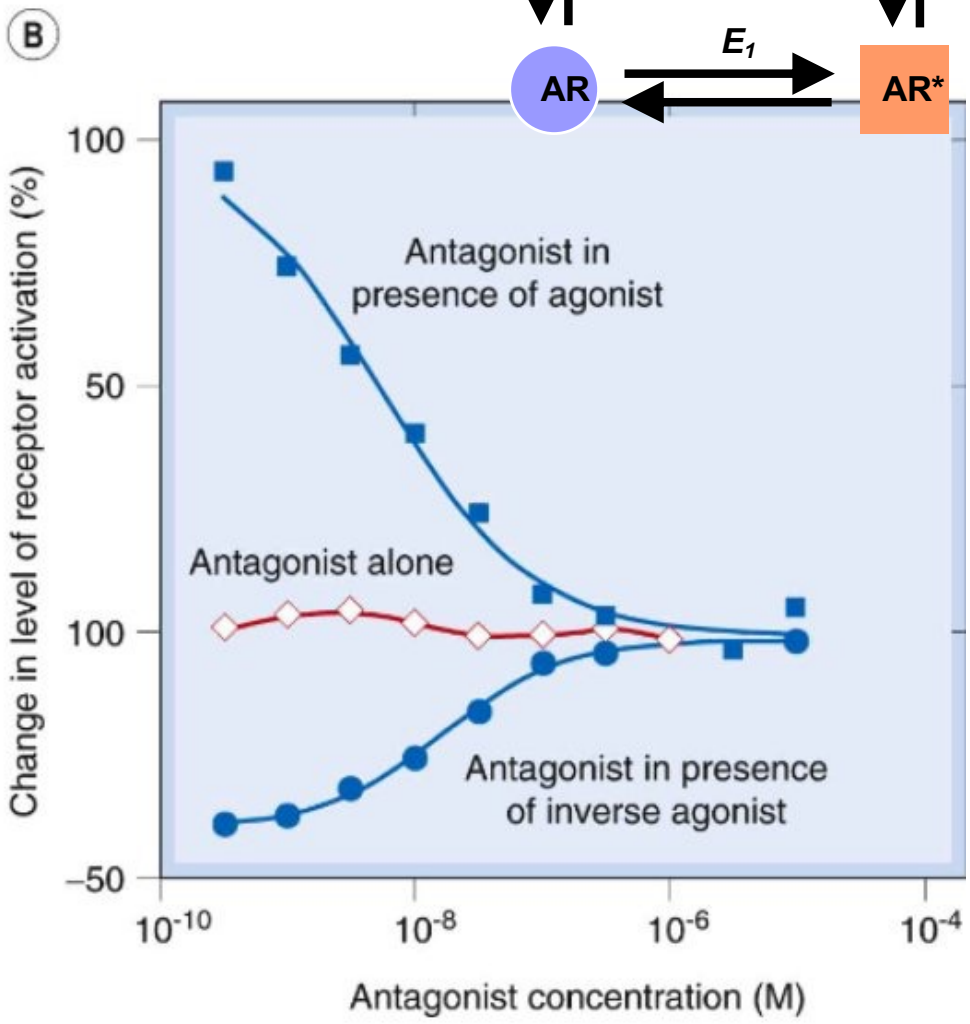




action of agonist, inverse agonist or competitive antagonist in a system that shows constitutive activity

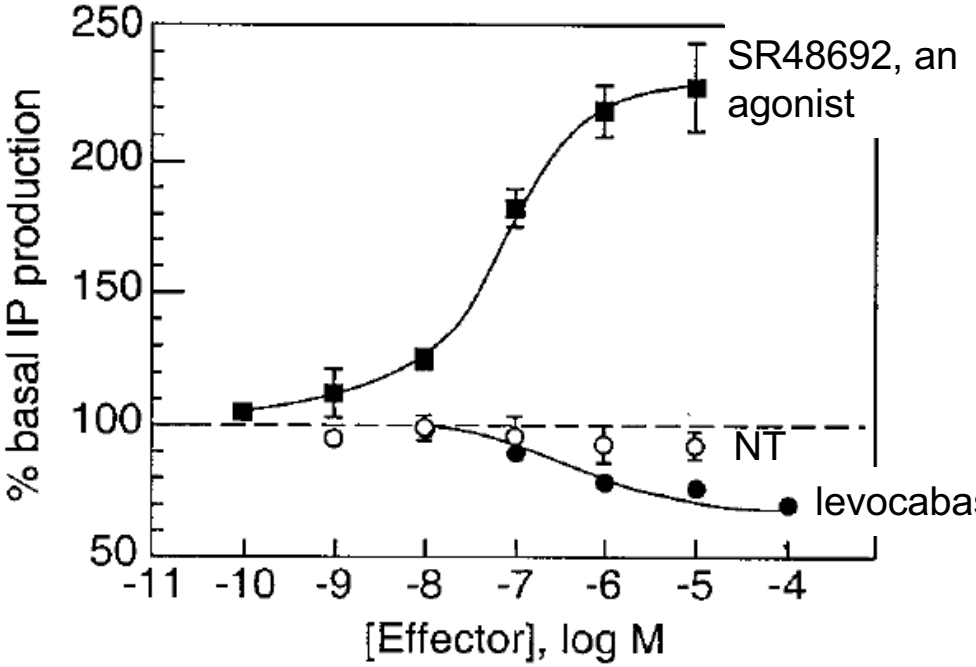






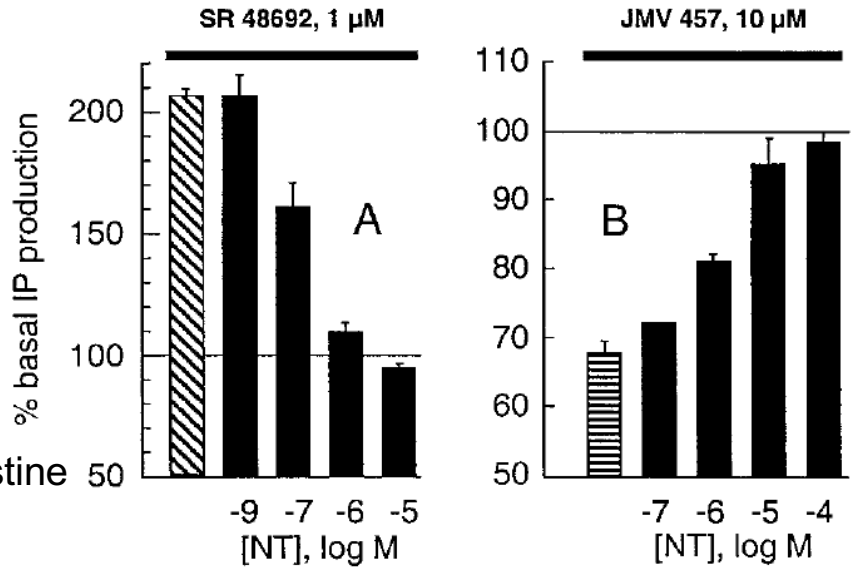
The interaction of a competitive antagonist with normal and inverse agonists in a system that shows receptor activation in the absence of any added ligands (constitutive activation). [A] The degree of receptor activation (vertical scale) increases in the presence of an agonist (open squares) and decreases in the presence of an inverse agonist (open circles). Addition of a competitive antagonist shifts both curves to the right (closed symbols). [B] The antagonist on its own does not alter the level of constitutive activity (open symbols), because it has equal affinity for the active and inactive states of the receptor. In the presence of an agonist (closed squares) or an inverse agonist (closed circles), the antagonist restores the system towards the constitutive level of activity. Figures are from Rang and Dale.

Levocabastine is an inverse agonist and NT is a competitive (neutral) antagonist at the NT2 receptor

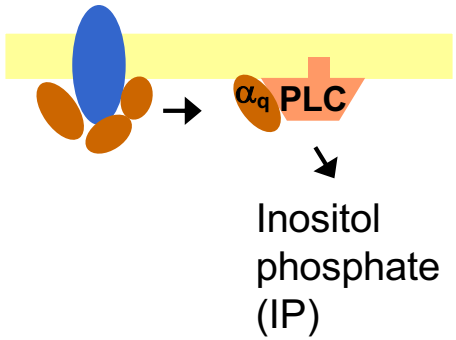


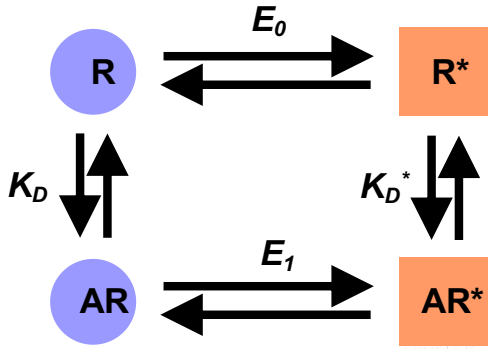
An agonist + NT

An inverse agonist + NT



NT=neurotensin

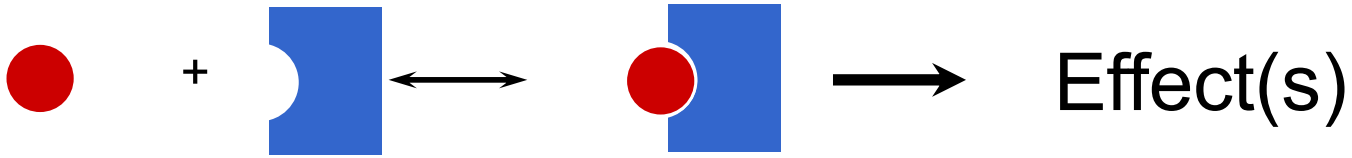




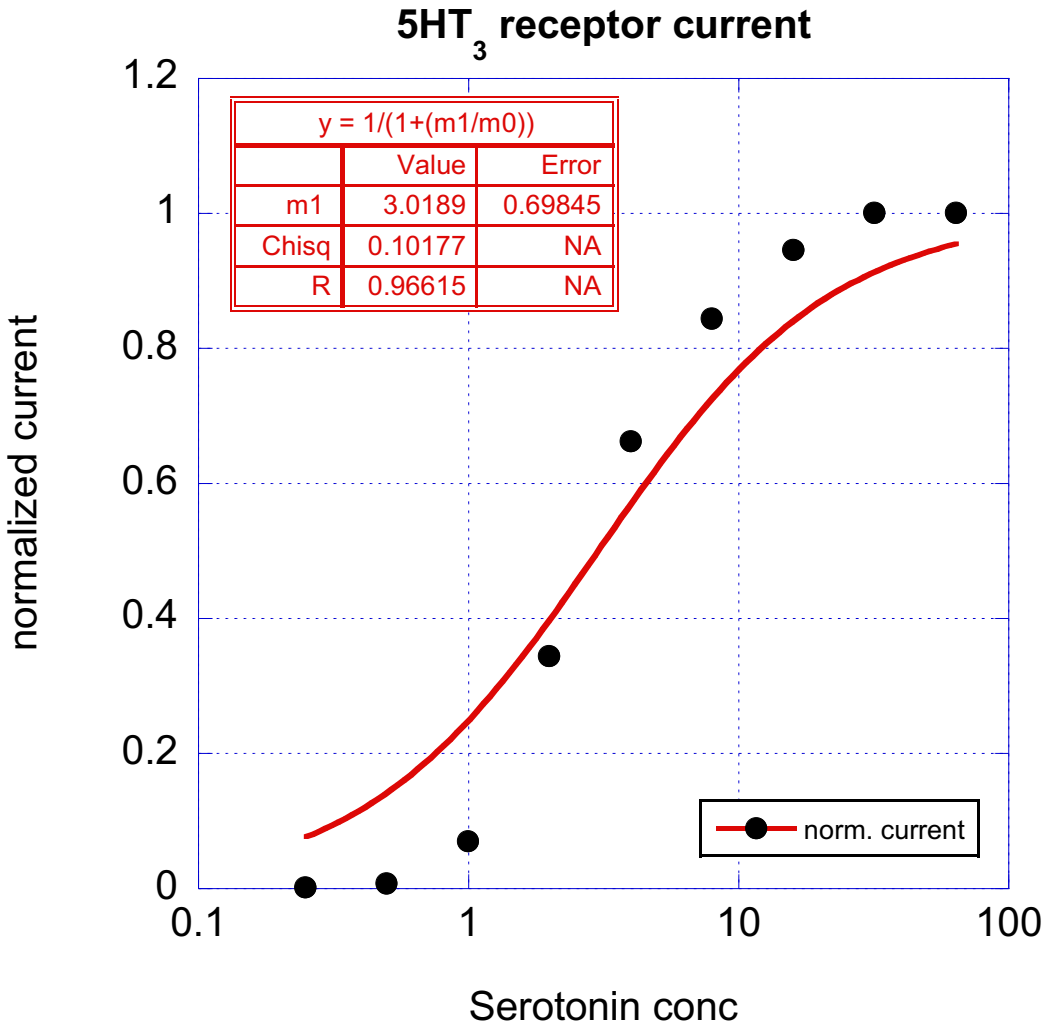
Mol Pharmacol 60:1392–1398, 2001

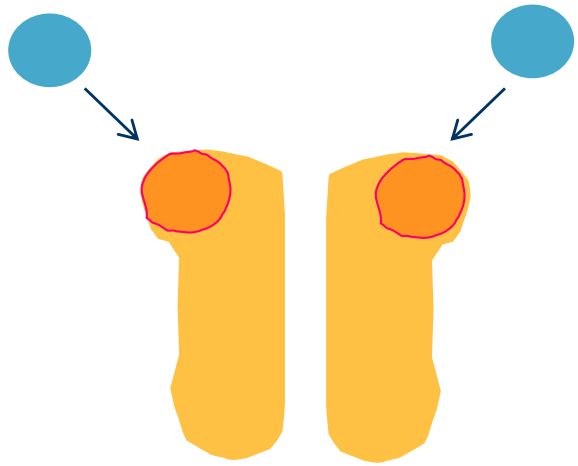
Contents of the part “pharmacodynamics”

- Part I: Introduction
- Part II: Binding of the ligand to the receptor
  - » interactions between ligand and receptor that govern binding
- Part III: Quantitative description of ligand-receptor binding
  - » binding at equilibrium
  - » association and dissociation kinetics
- Part IV: Relationship between ligand concentration, binding and effect
  - » concentration-effect relationship
  - » mechanisms/models of ligand→binding→effect coupling
  - » Agonists: definition, efficacy, potency
  - » Allosteric modulators
  - » Antagonists: types, quantitative description of their interaction with receptor and agonists
- Part V: Other aspects of receptor function and drug therapy



Binding/activation stoichiometry





$$E = \frac{E_{max}}{1 + EC_{50}/x_A} \quad (14)$$

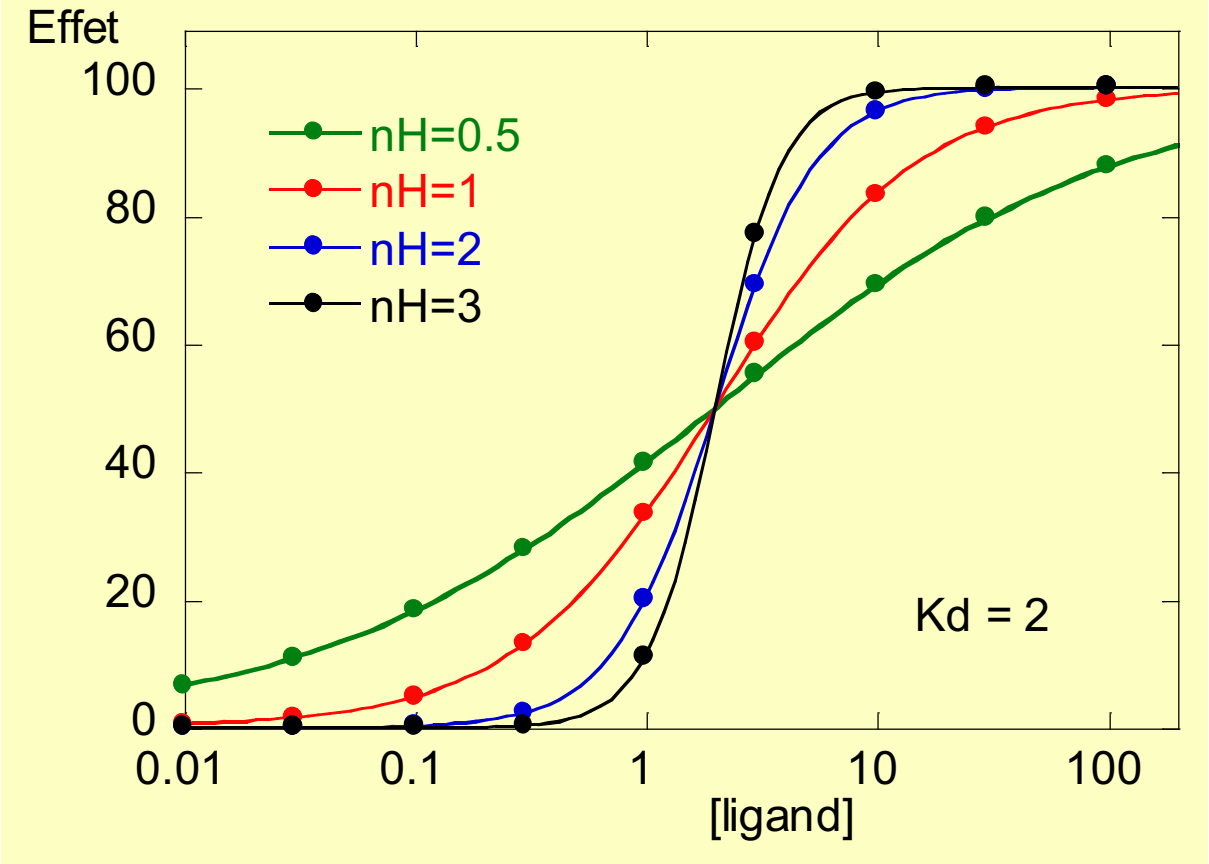
Equation 14 is not able to describe the concentration-current curve of this channel

Binding/activation stoichiometry

- Some proteins require binding of several ligand molecules for receptor activation
- To describe receptor activation in these cases, the Hill coefficient is introduced as shown below. The Hill coefficient allows an estimation of the minimal number of ligand molecules necessary for receptor activation

$$Effect = \frac{Effect_{\max}}{1 + \left(\frac{EC_{50}}{x_A}\right)^{nH}} \quad (22)$$

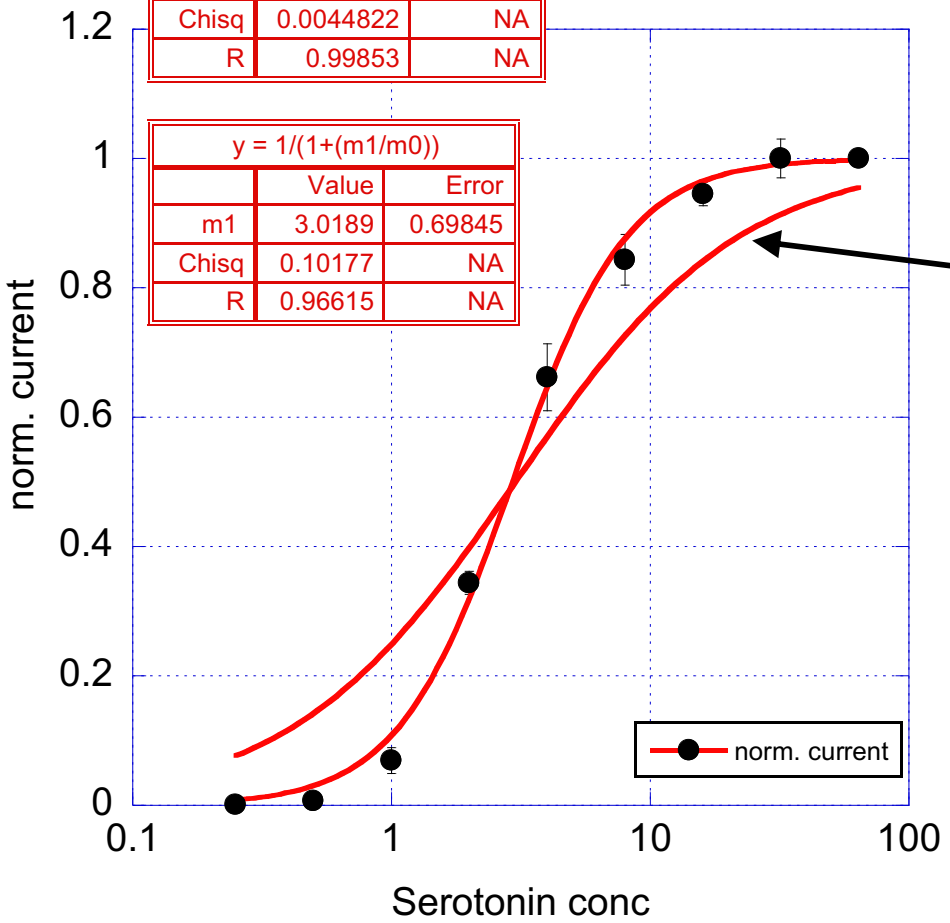
nH = Hill Coefficient



Binding/activation stoichiometry

y = 1/(1+(m1/m0)^m2)		
	Value	Error
m1	2.9385	0.10844
m2	1.9577	0.12441
Chisq	0.0044822	NA
R	0.99853	NA

y = 1/(1+(m1/m0))		
	Value	Error
m1	3.0189	0.69845
Chisq	0.10177	NA
R	0.96615	NA



$$Effect = \frac{Effect_{\max}}{1 + \left(\frac{EC_{50}}{x_A}\right)^{nH}}$$

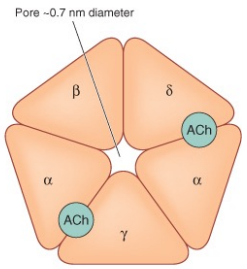
fit with nH=1

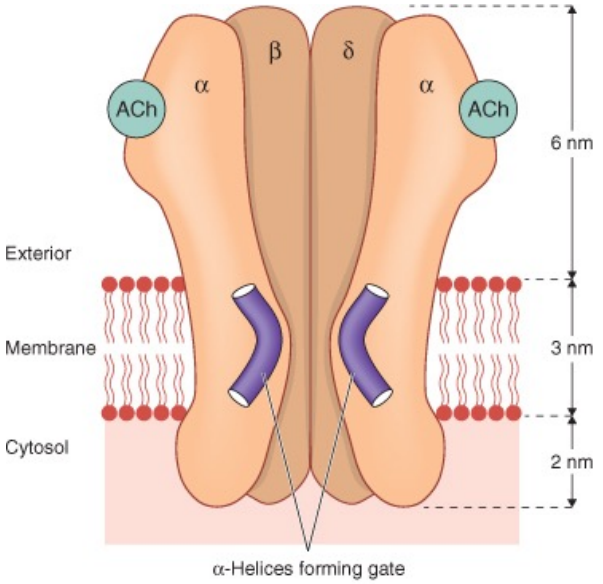
Experiment: concentration-current curve in cells expressing the 5HT-3 receptor

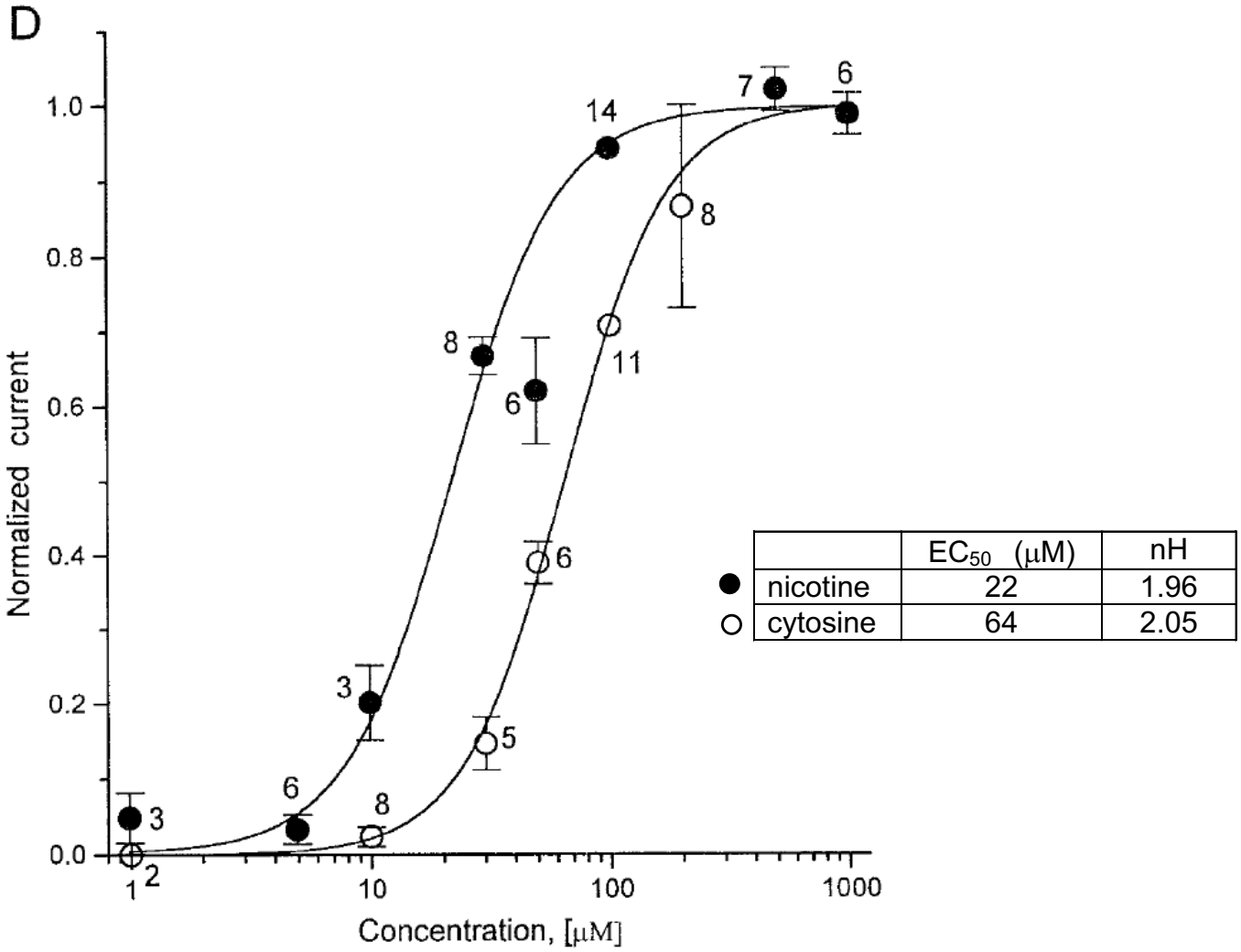
Interpretation of Hill Coefficient >1

- All mechanisms of receptor activation that involve more than one binding site with a conformation change show cooperativity, and the extent of this (steepness of the curve) can provide useful information about the action of agonists.
- The Hill equation which is generally used to fit such data is an empirical description that does not describe any known physiological mechanism, therefore no conclusions about binding and gating can be drawn directly from such fits.
- The Hill slope must be less than the number of agonist molecules that are needed to elicit the response, and may be much less (example: Haemoglobin has 4 binding sites, the Hill slope is ~2.4)
- Cooperativity (Hn>1) in the concentration dependence of the response can arise either from the binding step or/and from the conformational change.

Example: activation of the nicotinic Acetylcholine receptor







	EC_{50} (μM)	nH
● nicotine	22	1.96
○ cytosine	64	2.05

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Desensitization and tachyphylaxis

- Often, the effect of a drug gradually diminishes when it is given continuously or repeatedly.
  - Desensitization *and* tachyphylaxis *are synonymous terms used to describe this phenomenon, which often develops in the course of a few minutes.*
  - *The term* tolerance *is conventionally used to describe a more gradual decrease in responsiveness to a drug, taking days or weeks to develop*
- The decreased response can be due to:
  - *change in receptors*
  - *loss of receptors*
  - *exhaustion of mediators*
  - *increased metabolic degradation of the drug*
  - *physiological adaptation*
  - *active extrusion of drug from cells*

Desensitization due to changes in receptors

A: Receptors linked to ion channels: time-course of milliseconds, due to conformational change

B: G-Protein-coupled receptors: time-course of minutes, due to phosphorylation, interfering with coupling to 2nd messenger cascades

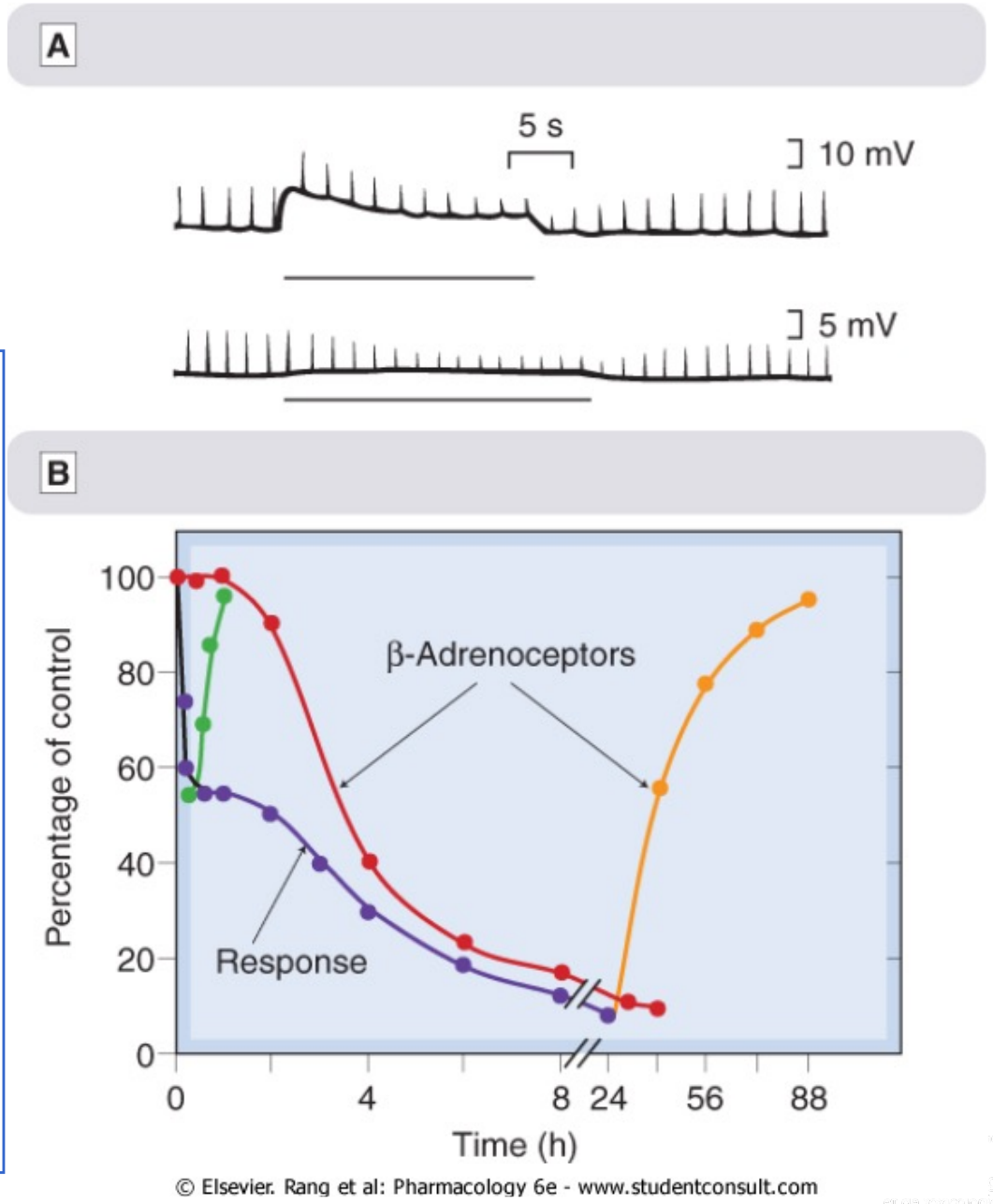
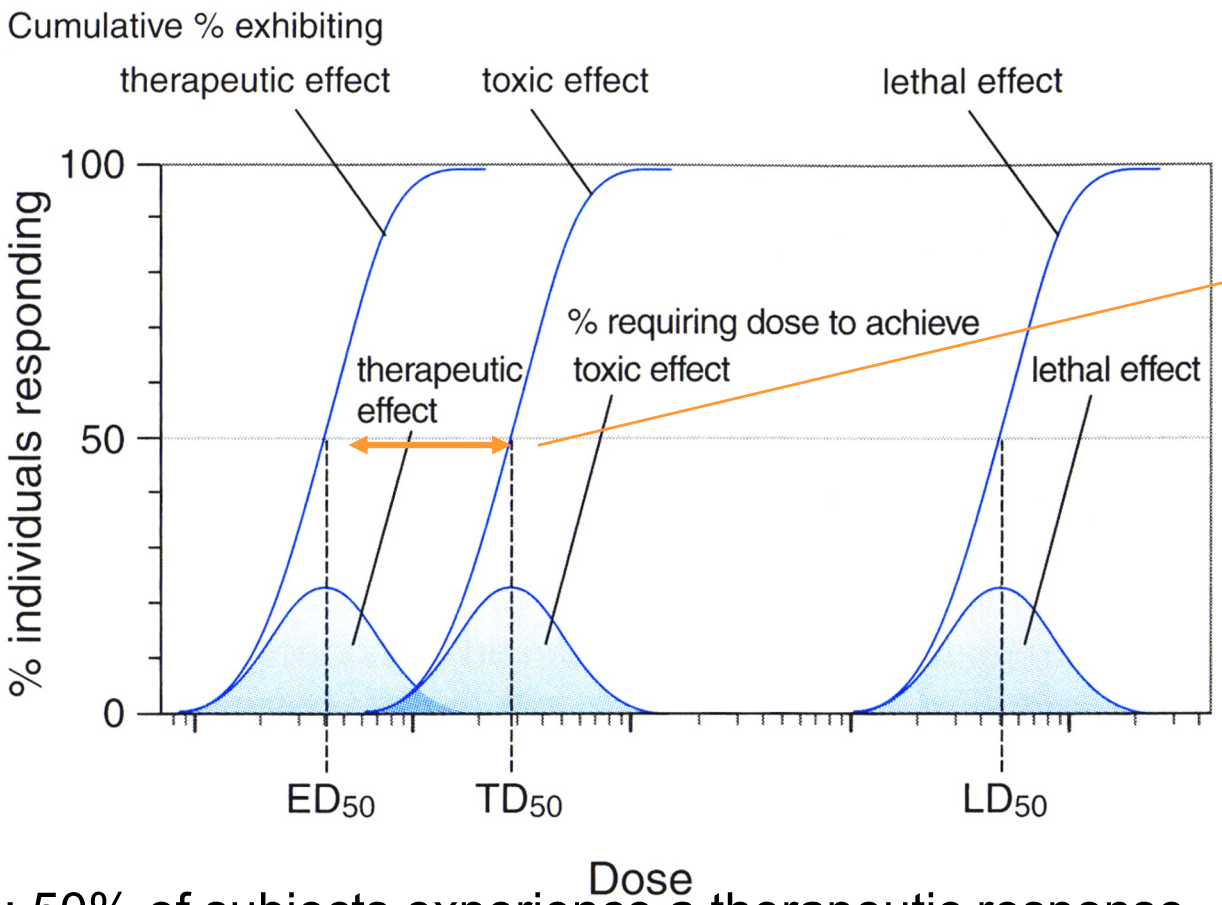


Figure 2-11 Two kinds of receptor desensitisation. Acetylcholine (ACh) at the frog motor endplate. Brief depolarisations (upward deflections) are produced by short pulses of ACh delivered from a micropipette. A long pulse (horizontal line) causes the response to decline with a time course of about 20 seconds, owing to desensitisation, and it recovers with a similar time course. B, β-Adrenoceptors of rat glioma cells in tissue culture. Isoprenaline (1μmol/l) was added at time zero, and the adenylate cyclase response and β-adrenoceptor density measured at intervals. During the early uncoupling phase, the response (blue line) declines with no change in receptor density (red line). Later, the response declines further concomitantly with disappearance of receptors from the membrane by internalisation. The green and orange lines show the recovery of the response and receptor density after the isoprenaline is washed out during the early or late phase. (From: (A) Katz B, Thesleff S 1957 J Physiol 138: 63; (B) Perkins J P 1981 Trends Pharmacol Sci 2: 326.)

Other mechanisms leading to desensitization/tachyphylaxis

- Loss of receptors: internalization of receptors
  - *examples: G-Protein-coupled receptors, hormone receptors*
- Exhaustion of Mediators
  - *e.g. amphetamine, which acts by releasing amines from nerve terminals*
- Altered Drug Metabolism (see lectures by D. Firsov)
  - *e.g. barbiturates, ethanol*

Variability of drug response and therapeutic index



therapeutic index, TI
(therapeutic ratio, "marge thérapeutique")

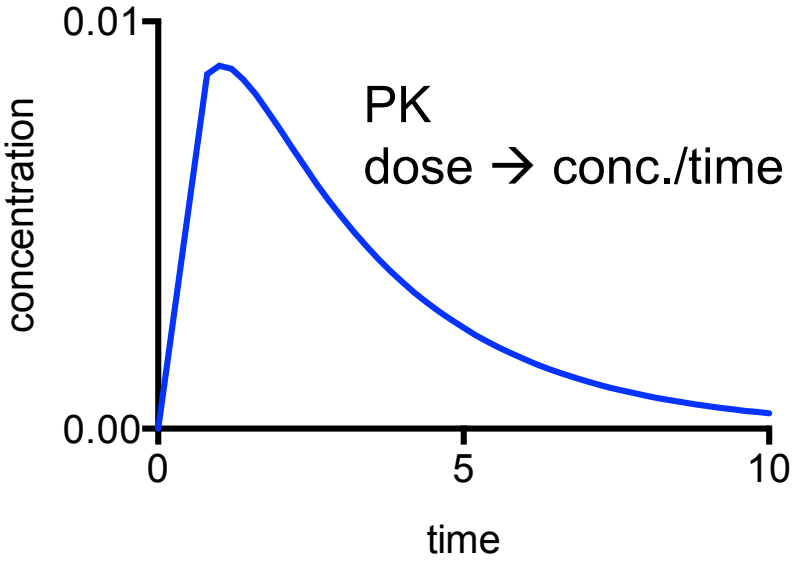
$TI = TD_{50}/ED_{50}$

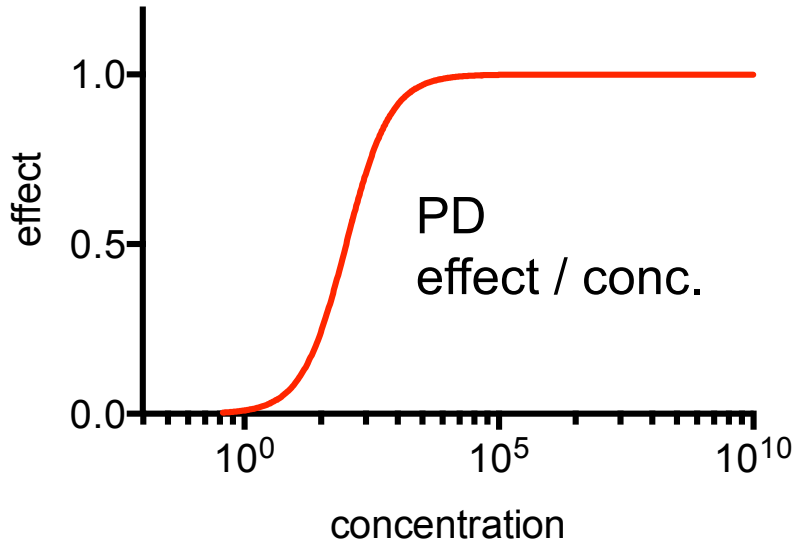
LD_{50} : lethal dose 50
= dose that kills 50% of the exposed animals

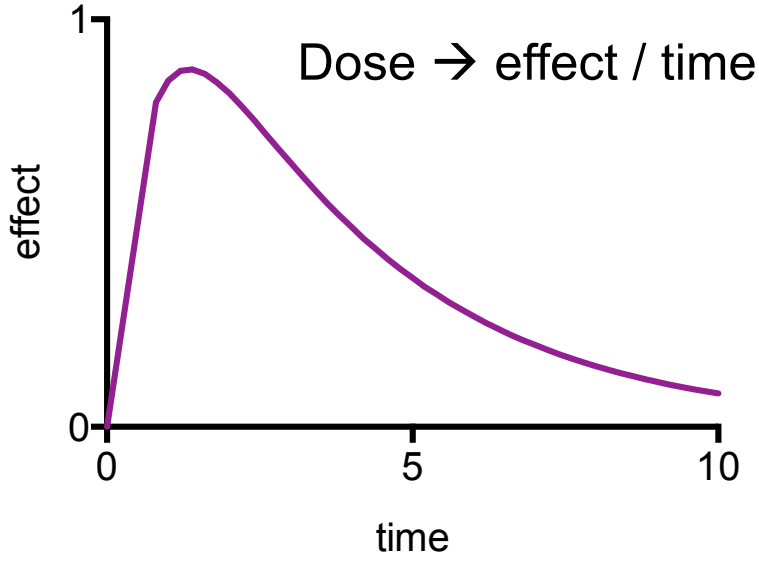
ED_{50} : 50% of subjects experience a therapeutic response
TD_{50} : 50% of subjects experience a toxic response

Figure 2-3. Quantal Dose–Response Curves. Quantal dose-response curves demonstrate the average effect of a drug, as a function of its concentration, in a population of individuals. Individuals are typically observed for the presence or absence of a response (for example, sleep or no sleep), and this result is then used to plot the percentage of individuals that respond to each dose of drug. Quantal dose–response relationships are useful for predicting the effects of a drug when it is administered to a population of individuals, and for determining population-based toxic doses and lethal doses. These doses are called the ED_{50} (dose at which 50% of subjects exhibit a therapeutic response to a drug), TD_{50} (dose at which 50% of subjects experience a toxic response), and LD_{50} (dose at which 50% of subjects die). Note that ED_{50} is the dose at which 50% of subjects respond to a drug; while EC_{50} (as described in the previous figure) is the dose at which a drug elicits a half-maximal effect in an individual subject.

PK/PD modeling







Additional exercises

1. At μ-opioid receptors, Morphine has a Kd of 2.5 nM, while the antagonist Naloxone has a Kd of 4.4 nM. Calculate the occupancy of the receptor by Morphine
   - in the presence of 10 nM Morphine (without antagonist), and
   - in the presence of 10 nM Morphine and 50 nM Naloxone

2. Omeprazol is an inhibitor of the gastric H,K-ATPase that binds to its receptor by forming an –S-S- bridge between a cystein residue of the receptor and an –SH group of the ligand. Its K_{on} for binding to the H,K-ATPase is 2000 $s^{-1}.M^{-1}$.
After how much time will an inhibition of 90% be obtained at an omeprazol concentration of 100 nM?

3. Using an electrophysiology approach you measure ionic currents induced by GABA in cells expressing $GABA_A$ receptors. The currents that you measure during application of the following concentrations of GABA are:

GABA Concentration	0.2 μM	1 μM	2μM	6 μM	12 μM	40 μM	100 μM
current (pA)	440	1750	2800	4700	5650	6500	6800

Draw a concentration-effect curve and determine the EC50 and the maximal effect.

4. Using the agonist A at a concentration of 10 μM you obtain an effect that equals 75% of the maximal effect. Which concentration of the agonist A do you need to apply to obtain the same activation level (75% of the maximal effect) in the presence of 500 nM of a competitive inhibitor whose Ki is 100 nM?