Pharmacodynamics 2

- 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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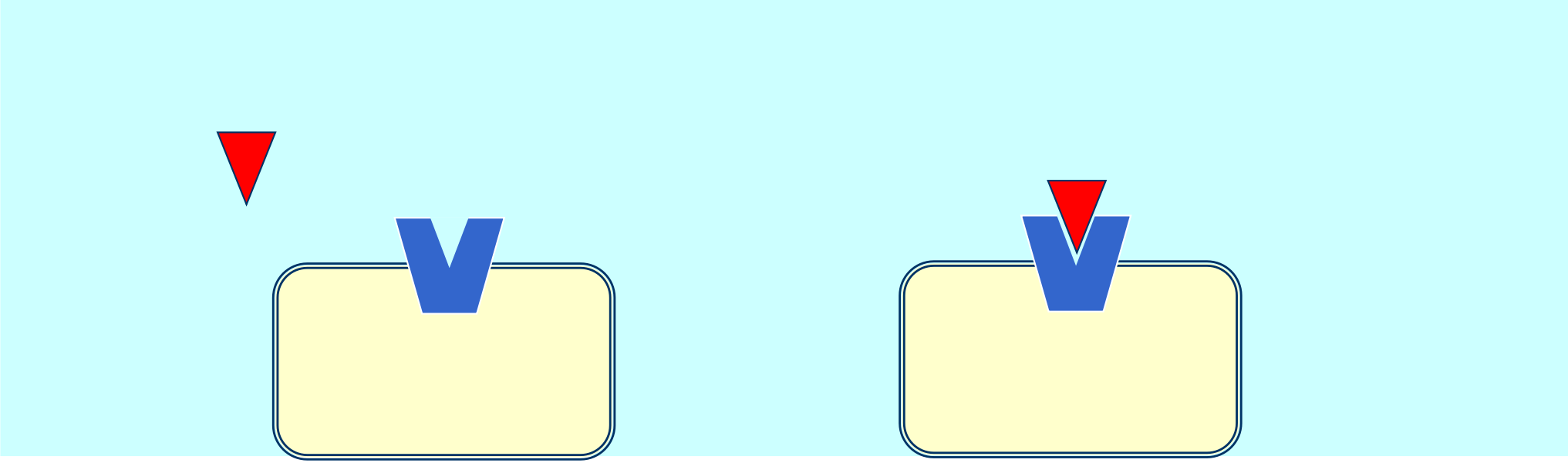
Association and dissociation kinetics



$$L + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} LR$$

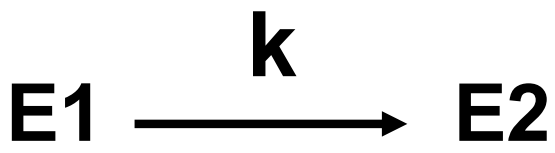
k_{on} : association rate constant [$s^{-1}M^{-1}$]

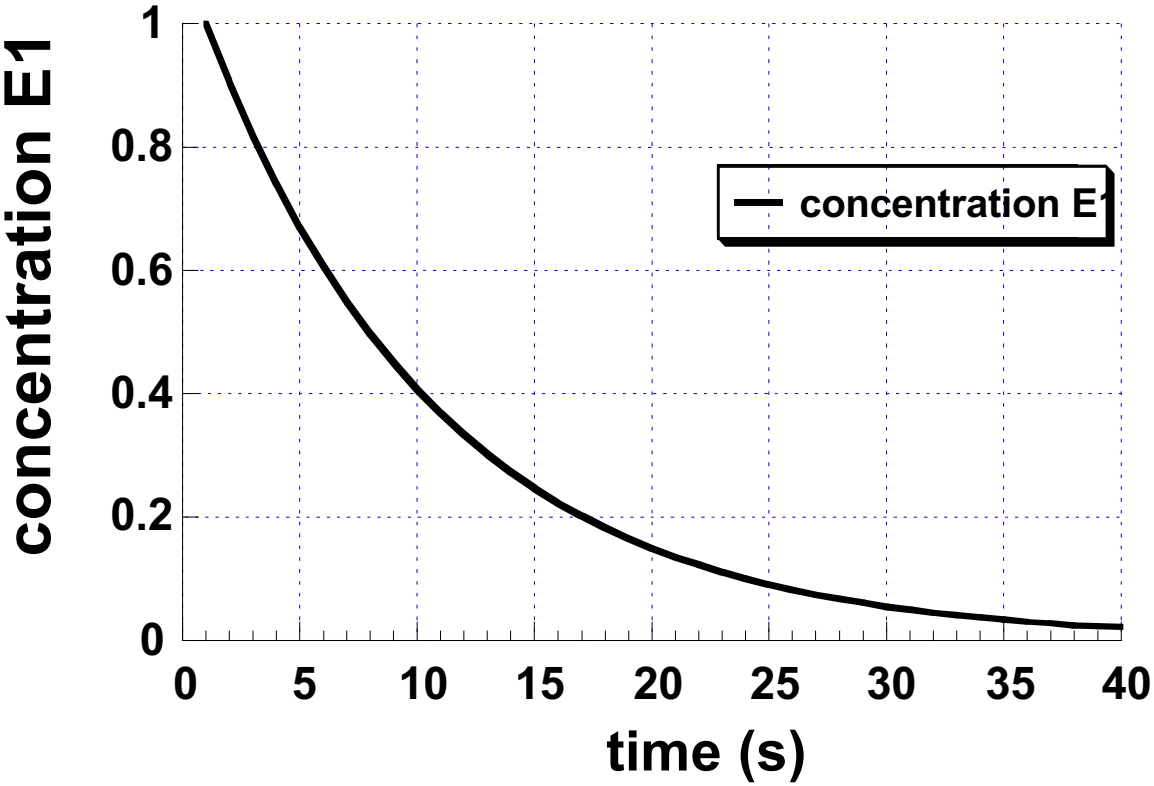
k_{off} : dissociation rate constant [s^{-1}]

Reversible binding

Irreversible binding : k_{off} = 0, the complex does not dissociate

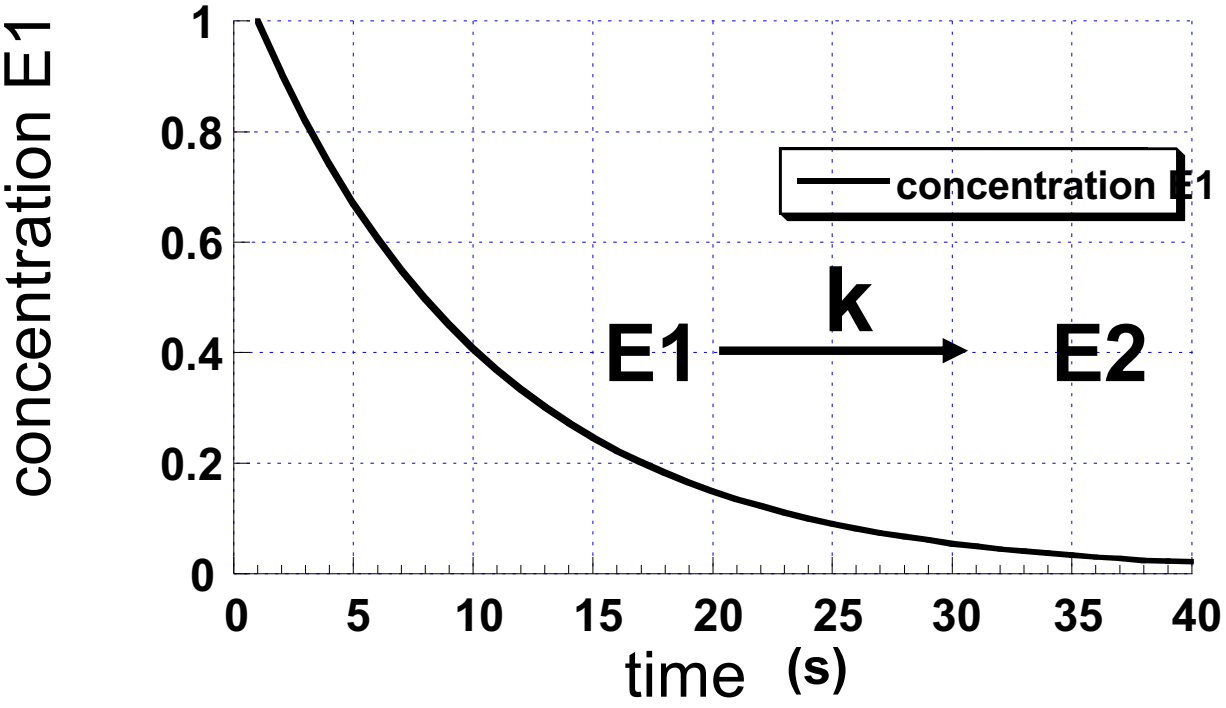
Reminder of chemical kinetics: First order reaction, rate constant (k) and half-life ($t_{1/2}$)





- First order reaction:
  - *For a first-order reaction, the rate of reaction is directly proportional to the concentration of one of the reactants.*
  - *The relaxation kinetics of a first-order reaction are exponential*

k, t1/2 and τ



Example: k = 0.1 s^{-1}

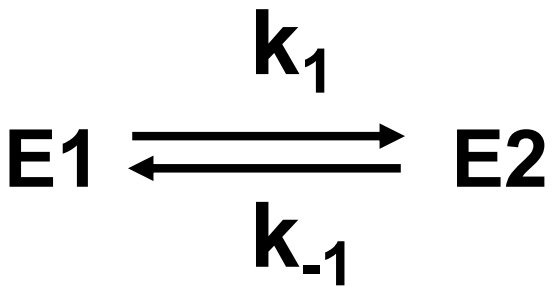
$t_{1/2}$ = 0.7/k =

τ = 1/k =

$[E1]_t/[E1]_0 = e^{-kt}$ (10)

- k : rate constant (constante de taux) unit : [1/s]
  = proportion of E1 going to E2 per time unit
- t1/2 : = half-life, is the time required for the concentration of E1 to drop from its initial value $[E1]_0$ to $[E1]_0$ /2, unit [s]
  with t1/2 = ln(2) / k or k = ln(2) / t1/2 ln(2) ~ 0.7 (11)
- τ := time constant (constante de temps), unit [s]
  with t = 1/k (12)

Reversible first-order reaction, applied to binding kinetics



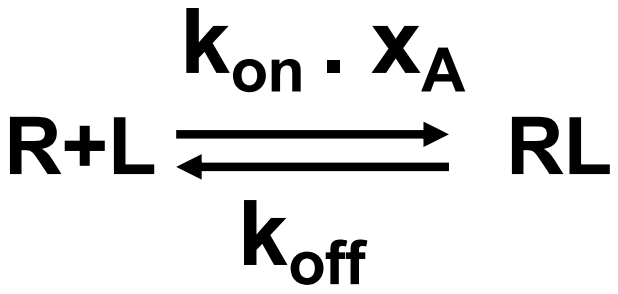
Relaxation kinetics towards an equilibrium state

$$k = k_1 + k_{-1}$$

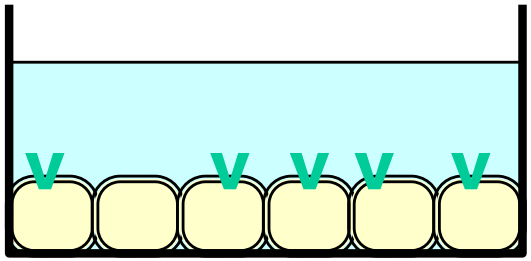
For example for binding kinetics with

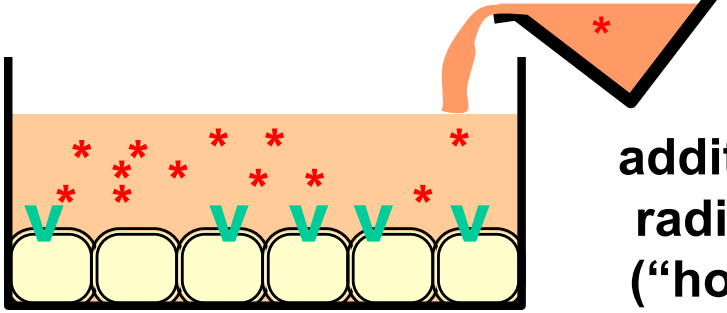
$$k_1 = k_{on} \cdot x_A \quad \text{and} \quad k_{-1} = k_{off}$$

- $$k = k_{on} \cdot x_A + k_{off} \qquad (13)$$



Measurement of the association rate constant k_{on}

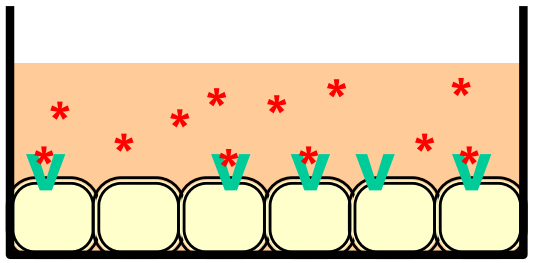




t = 0

addition of the radio-labeled ("hot") ligand

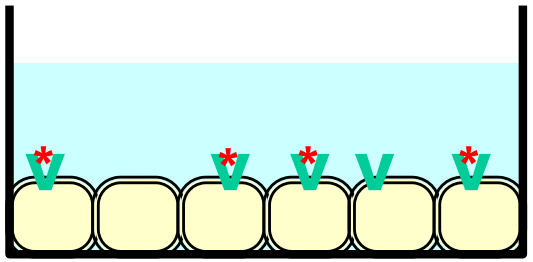
counting of the radioactivity associated with the cells



time t

Removal of the "hot" solution

for example by *rapid* rinsing



Measuring K_{on}

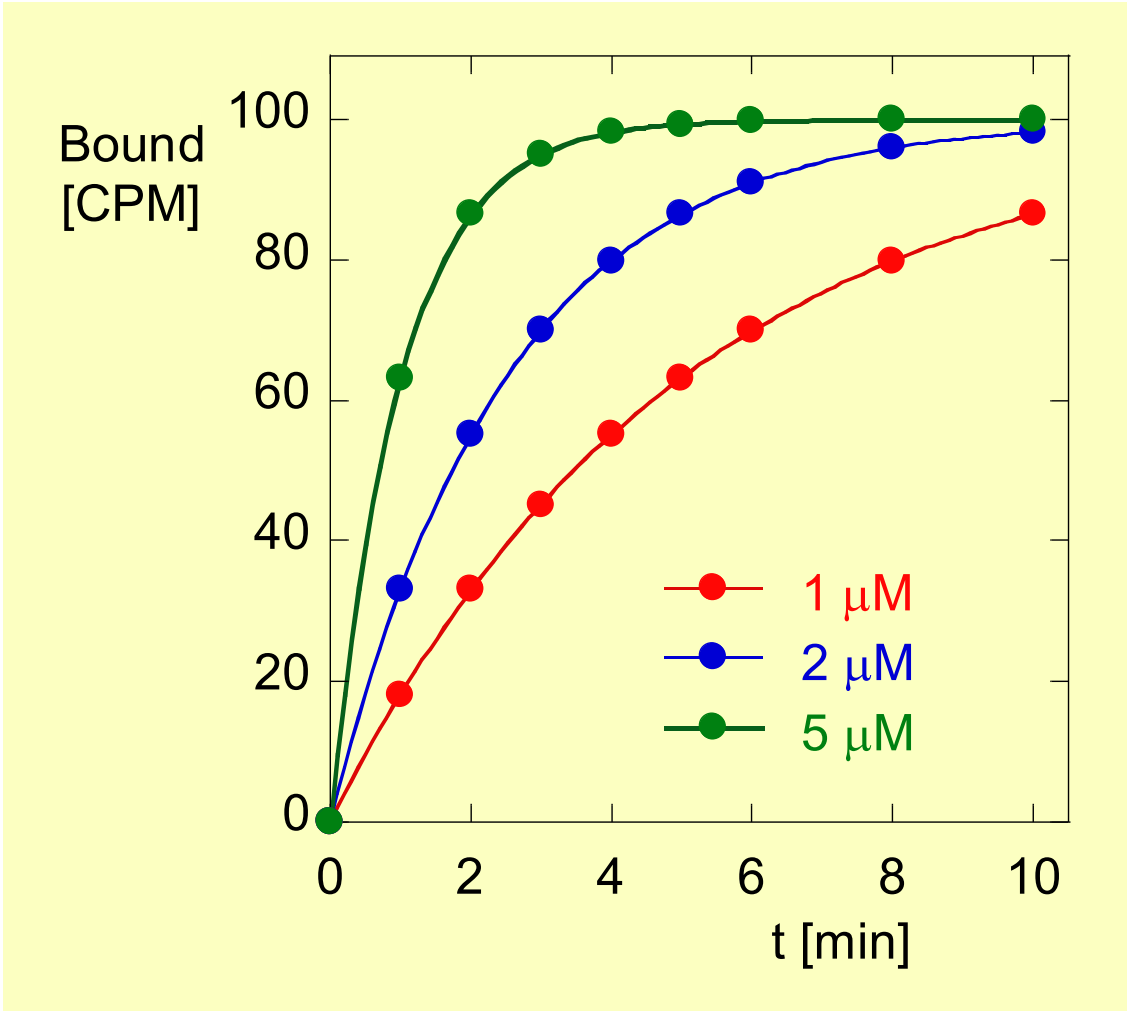
incubation time (min)	1	2	4	8
bound radioactivity for 5 μM	63	87	95	95
bound radioactivity for 1 μM	18	33	53	80

example with

Nmax = 100

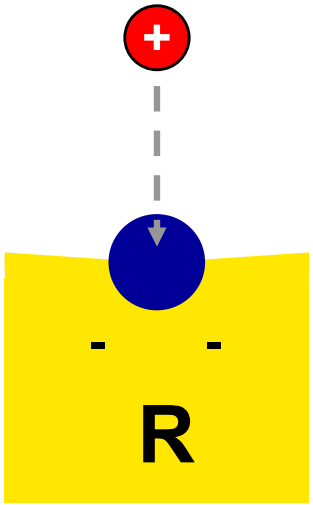
$k_{on} = 2.10^5\ M^{-1}\cdot min^{-1}$

$k_{off} = 0$

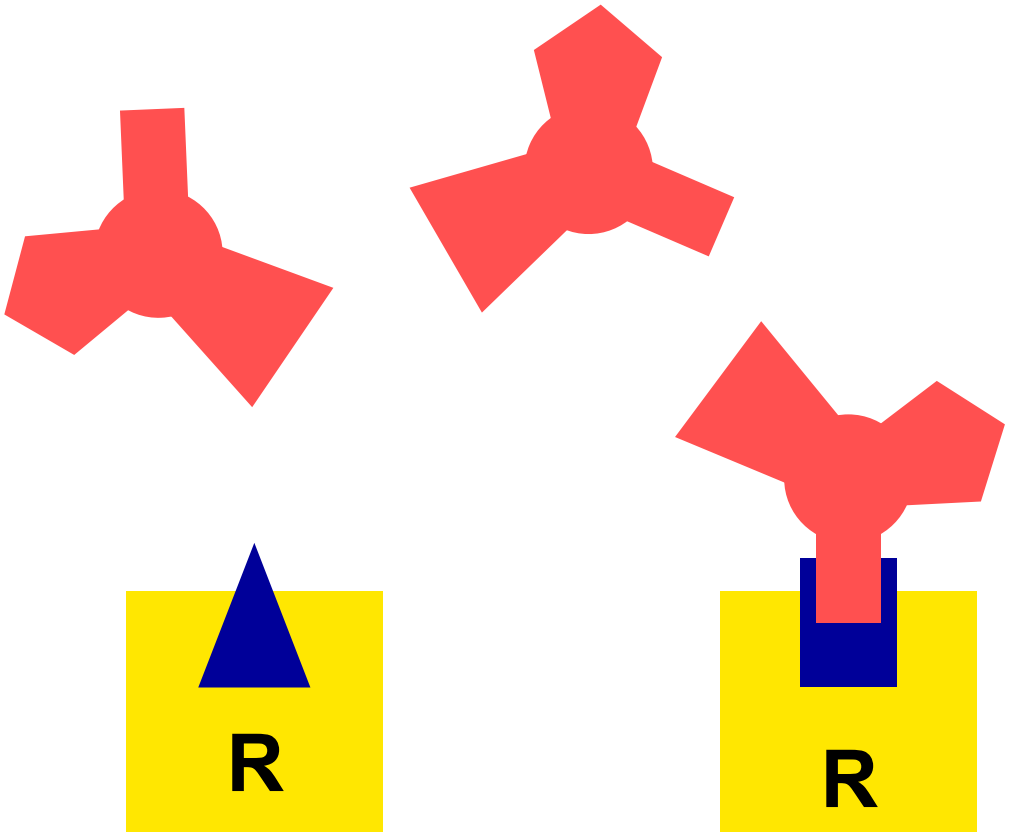


$$R + L \underset{k_{off}}{\overset{k_{on} \cdot x_A}{\rightleftharpoons}} RL$$

association kinetics



rapid association:
simple ligand,
accessible site



slow association:
complex, large ligand,
binding site only partially accessible

Association rate constant

k_{on} [$M^{-1} \cdot s^{-1}$]

Describes the rate of the complex formation for a ligand concentration of 1 M.

k_{on} depends on

- the size and the mobility of the ligand
- the complexity of the ligand
- the electrostatic interactions that can attract and/or orient the ligand
- the accessibility of the binding site, which may depend on the conformation of the receptor protein

Examples

- ~10^9 $M^{-1} \cdot s^{-1}$ for a very mobile ligand, as an ion of small diameter
- ~10^7 $M^{-1} \cdot s^{-1}$ for amiloride on ENaC
- ~10^4 $M^{-1} \cdot s^{-1}$ for ouabain on the Na,K-ATPase, whose binding site for ouabain is only accessible in a specific conformation

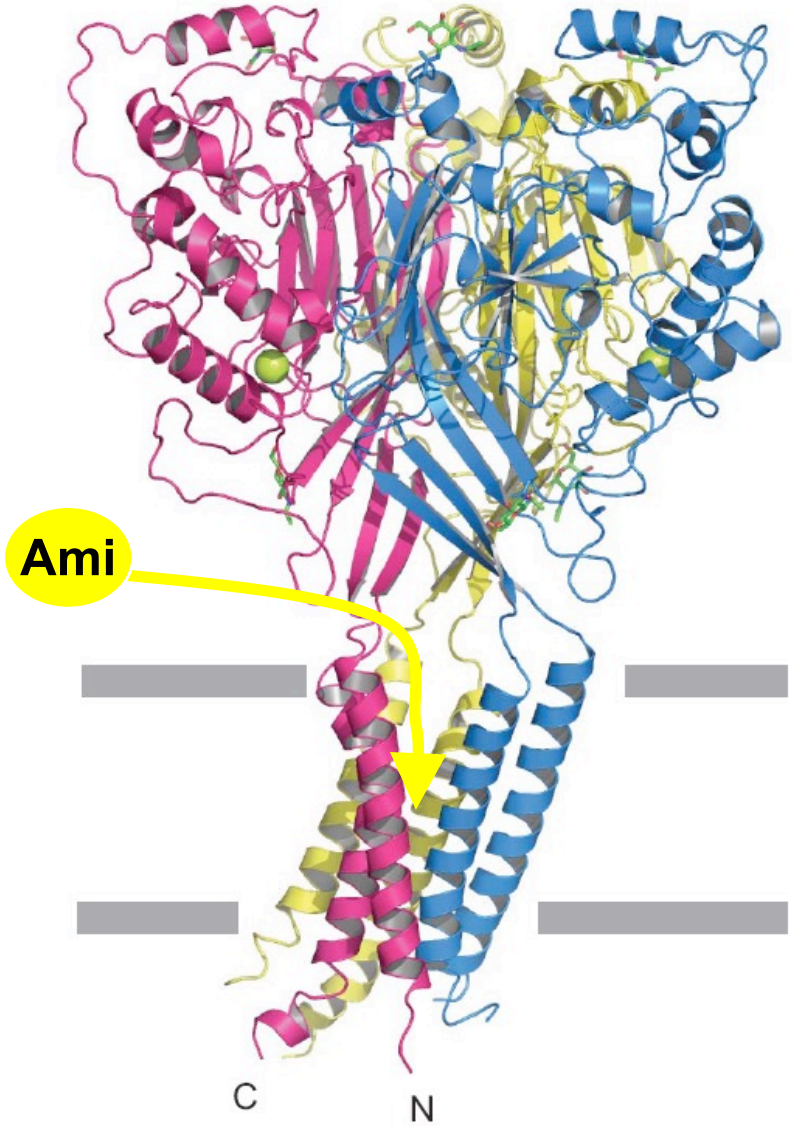
association kinetics: examples

C	1 nM		1 μM		1 mM	
k_{on}	k	$t_{1/2}$	k	$t_{1/2}$	k	$t_{1/2}$
10^9 $M^{-1}.s^{-1}$ (Ca^{++})	1 s^{-1}	0.7 s	1000 s^{-1}	0.7 ms	10^6 s-1	0.7 μs
10^7 $M^{-1}.s^{-1}$ (amiloride)	0.01 s^{-1}	70 s	10 s^{-1}	70 ms	10^4 s^{-1}	0.07 ms
10^4 $M^{-1}.s^{-1}$ (ouabain)	10^{-5} s^{-1}	20 h	0.01 s^{-1}	70 s	10 s^{-1}	70 ms

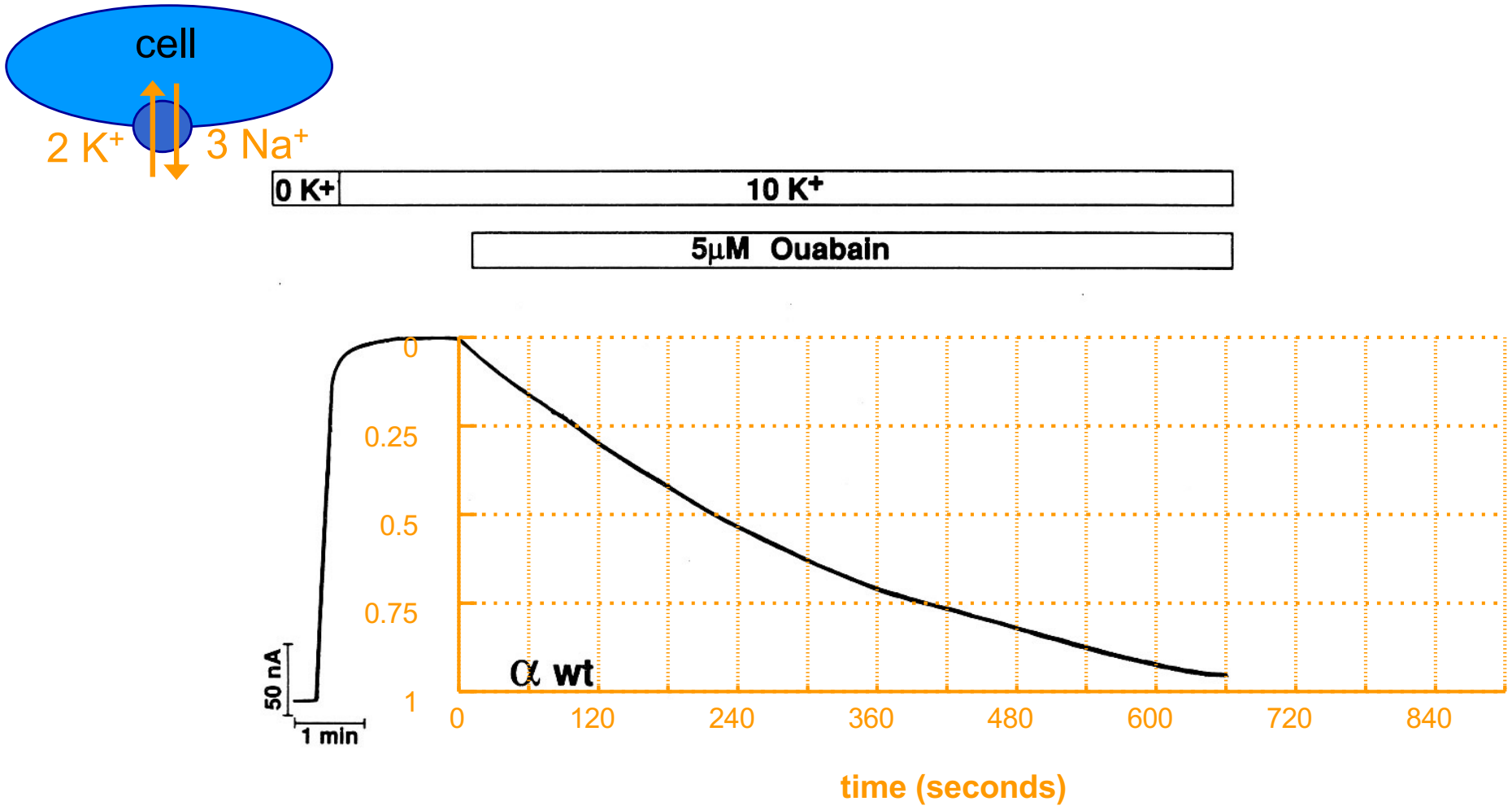
$$k = k_{on} . C$$

$$t_{1/2} = \ln(2) / k$$

At a concentration of 1 μM it takes amiloride 70 ms to occupy 50% of the epithelial Na channels ENaC, to which it binds deeply into the ion pore



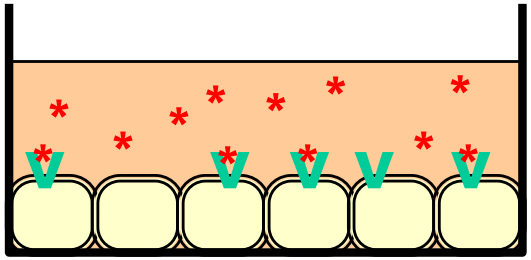
Examples of the kinetics of an effect: measurement of k_{on} by the appearance of an effect



Appearance of the inhibitory effect of 5 µM ouabain on the current generated by the Na,K-ATPase : determine the k_{on} *(Assumption: current inhibition occurs when ouabain binds; and $k_{off} << k_{on} * x_A$).*

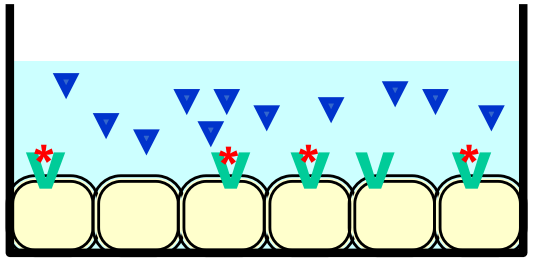
Measurement of dissociation kinetics: k_{off}

incubation in presence of "hot" (= radio-labeled) ligand *



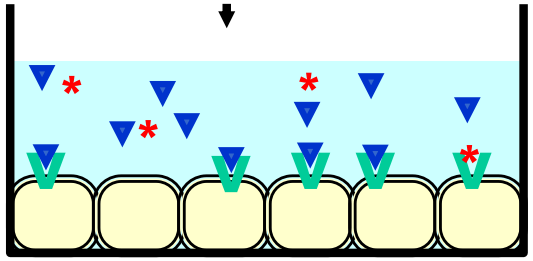
removal of "hot" solution by *rapid* rinsing

t = 0



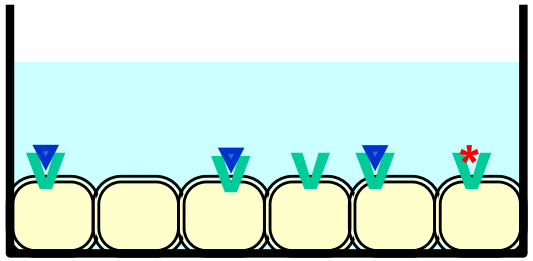
+ replacing of hot ligand * by cold ligand ▼

time t



rinsing

counting radioactivity associated with cells

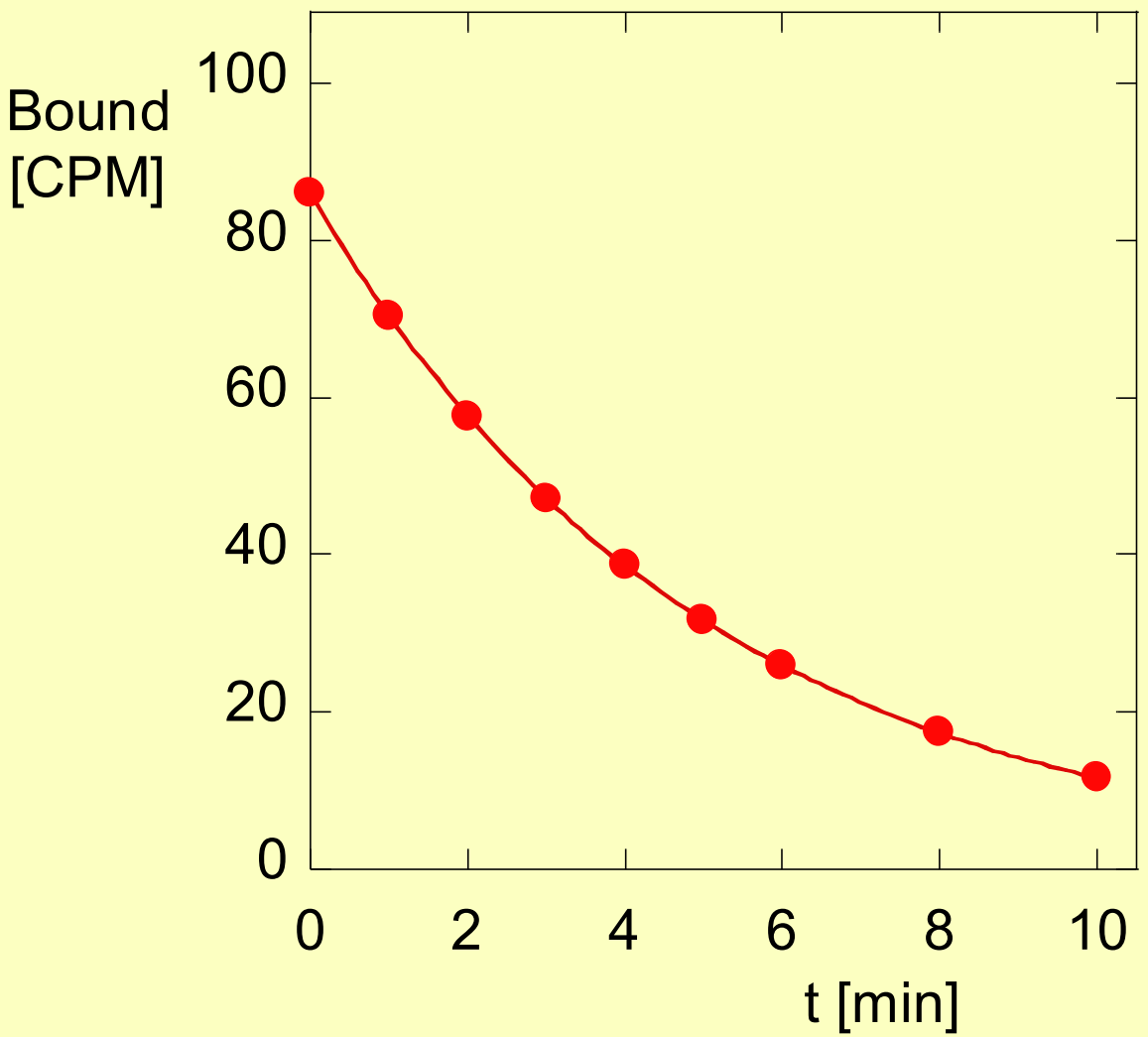


measuring k_{off}

example with

$k_{off} = 0.2\ min^{-1}$

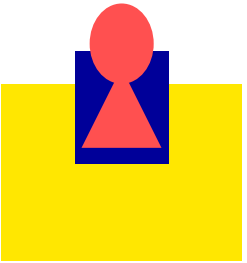
$k_{on} = 0$



k_{off} [s^{-1}]
dissociation rate constant

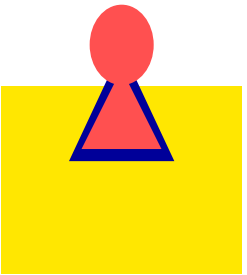
describes the rate of dissociation of the complex

- k_{off} depends essentially on the binding interactions at short distance between the ligand and the receptor
- a conformational change of the receptor protein may in some cases trap the ligand

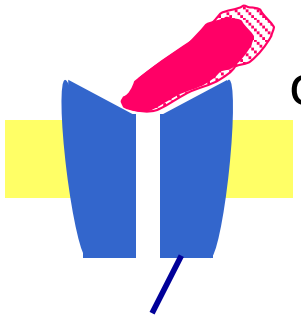


Examples

- ~2 s^{-1} ($t_{1/2}$ = 350 ms) for amiloride on ENaC
- ~2.10^{-4} s^{-1} ($t_{1/2}$ = 1 h) for ouabain on the Na,K-ATPase
- ~0 s^{-1} ($t_{1/2}$ = infinite) for a covalent bond

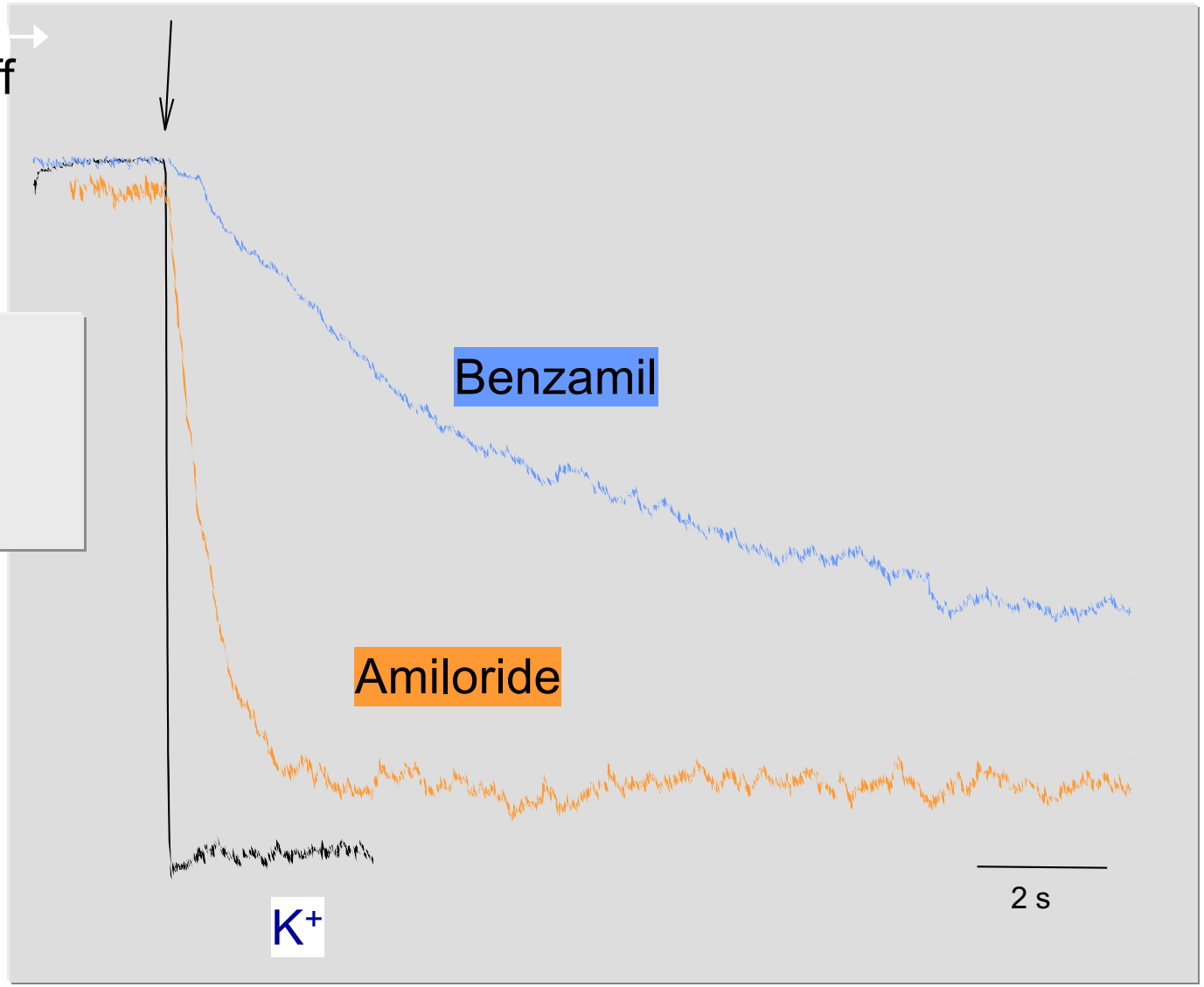


Example of binding the off-rate: The drug target is incubated with the inhibitor. At the beginning of the measurement, the solution around the target is rapidly and continuously changed, and it is measured how quickly the inhibitor comes off.



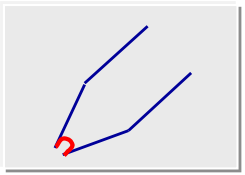
off

solution change from:



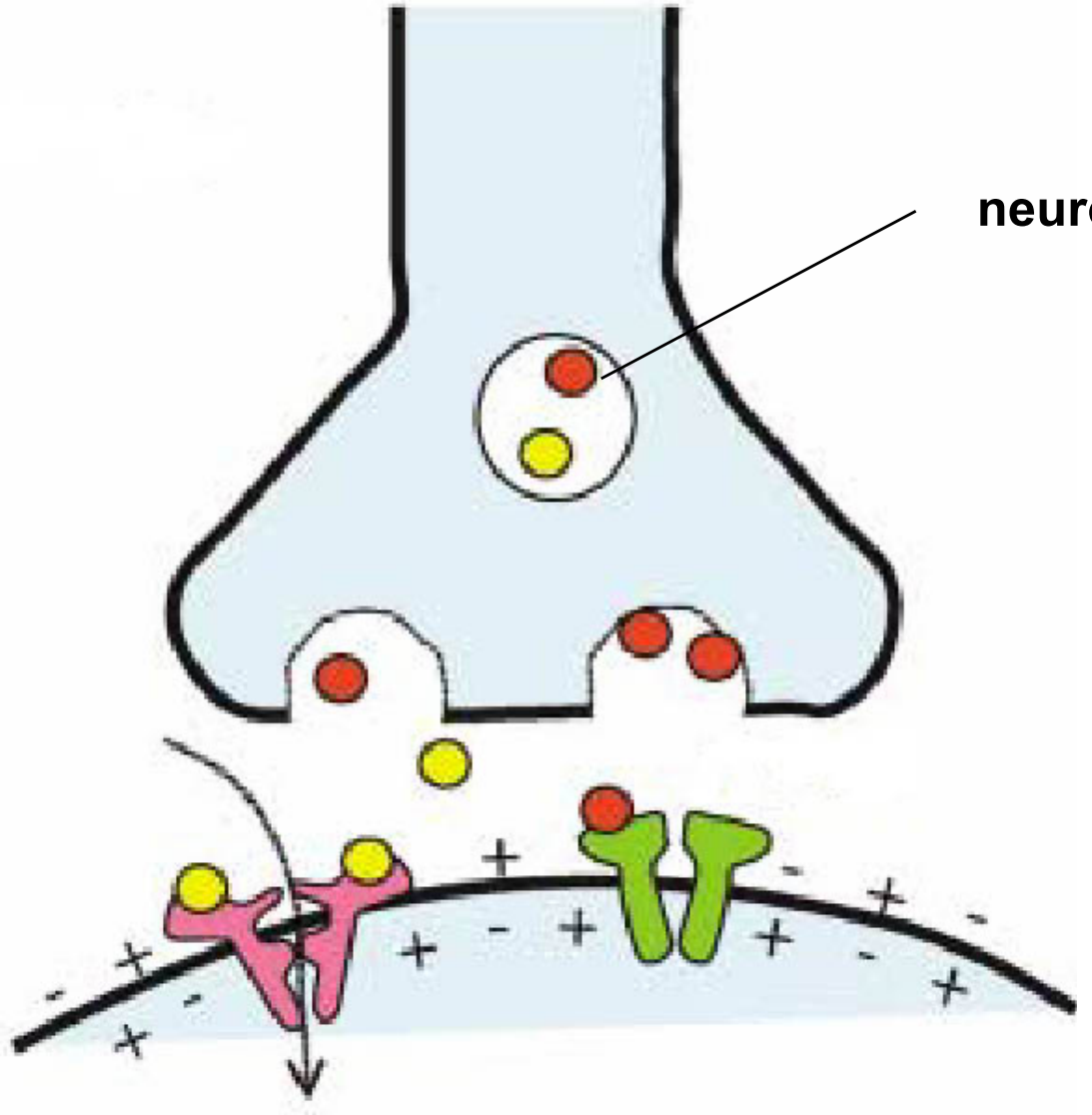
k_{off} = 0.1 s^{-1}

k_{off} = 2.1 s^{-1}



In endogenous ligand-receptor pairs, the k_{on} and k_{off} are adapted to the physiological needs.

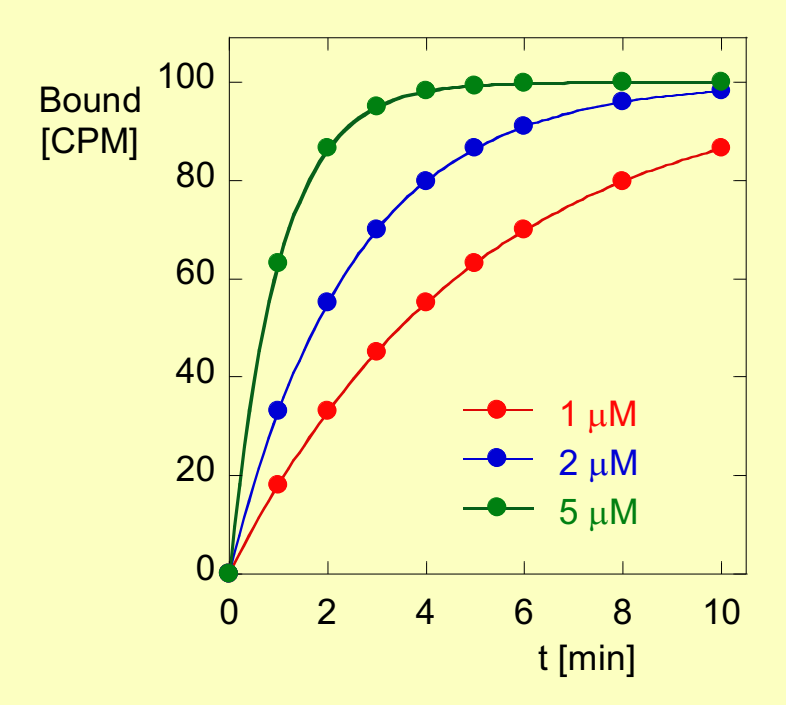
Example rapid synaptic transmission : rapid dissociation required

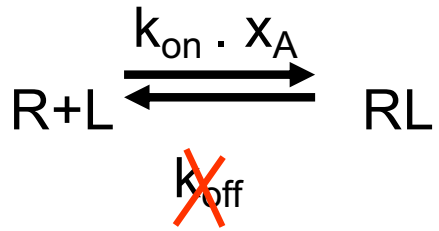


neurotransmitters

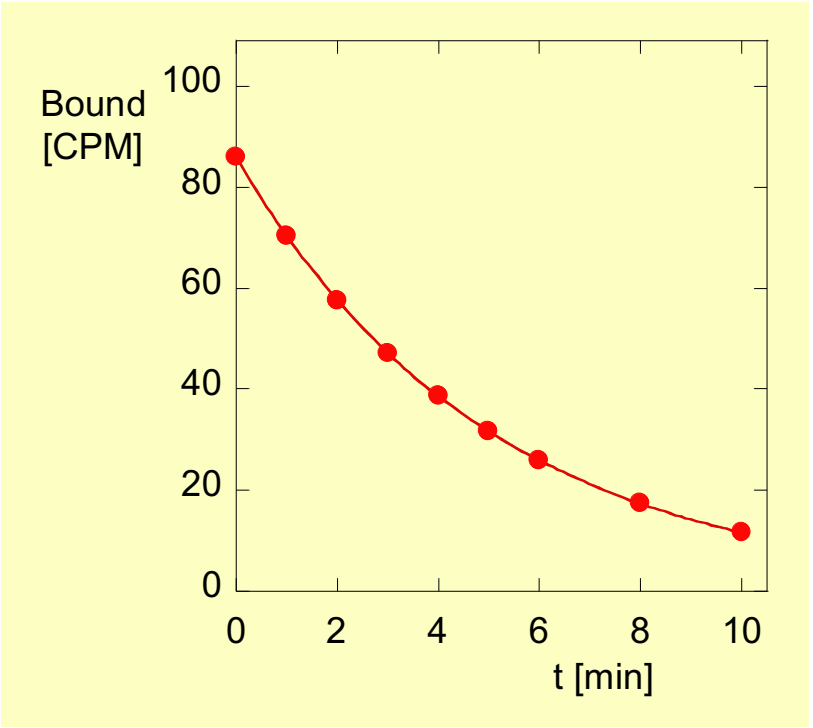
Receptors/ion channels in the the post-synaptic membrane

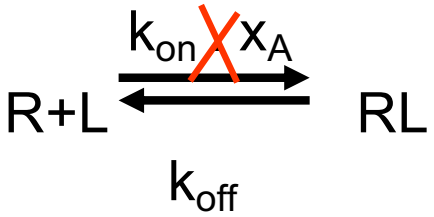
Kinetics of association, covalent bond





Kinetics of dissociation, drug washout





$$k = x_A \cdot k_{on} + k_{off}$$

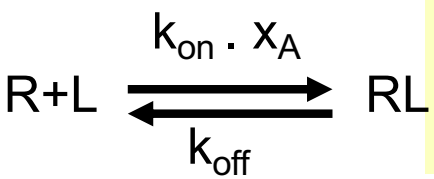
k_{on}, k_{off}, reversible binding, time to reach an equilibrium, starting from the ligand application

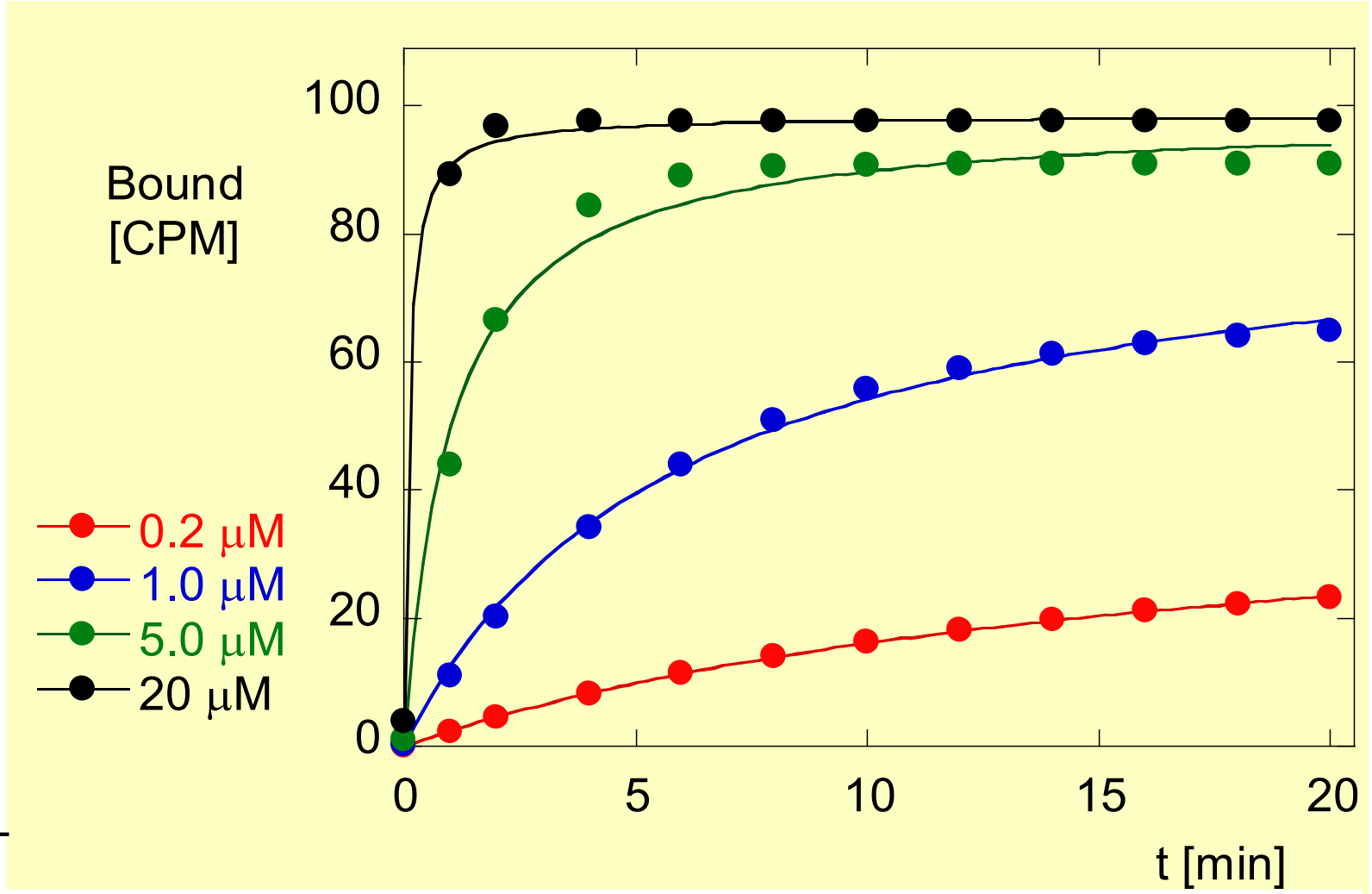
For any rapid change of the ligand concentration, the relaxation time course to a new equilibrium is exponential with $k = x_A \cdot k_{on} + k_{off}$ (12)

k_{on} 2000 $M^{-1}.s^{-1}$
k_{off} 0.001 s^{-1}

→Kd 0.5 μM

$k = C \cdot k_{on} + k_{off}$

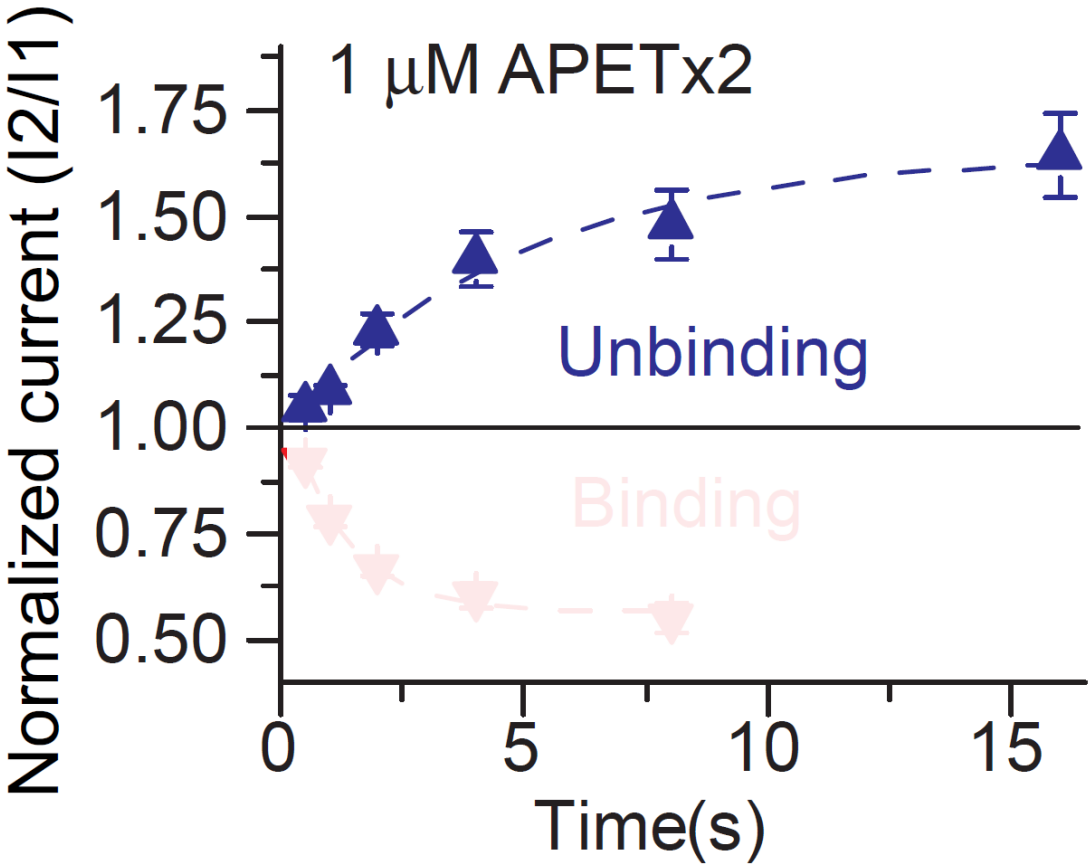


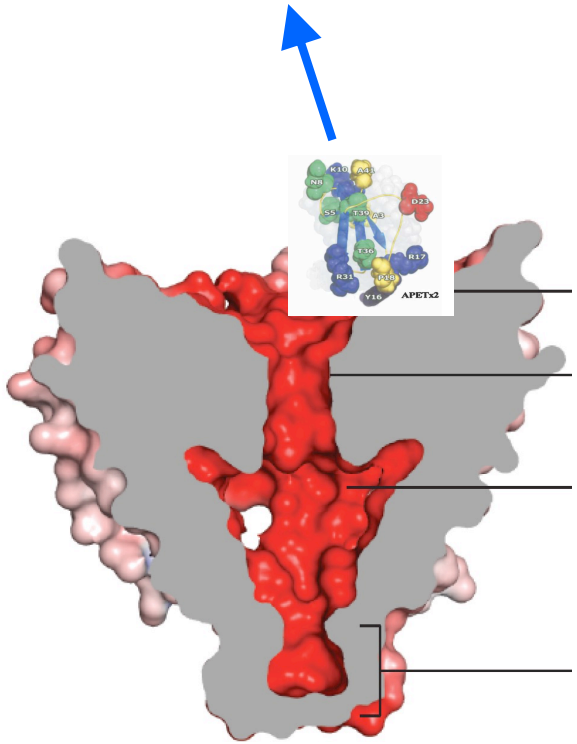


In the cases shown (i.e. changing the ligand concentration from 0 to the values indicated), the relaxation to the new equilibrium follows $pA_t = pA_{eq} \cdot (1\text{-}e^{-kt})$, with pAeq = occupancy at equilibrium (calculated with equations 5 and 8)

Off rate k_{off}

APETx2, a 40 aa toxin, and Nav1.8



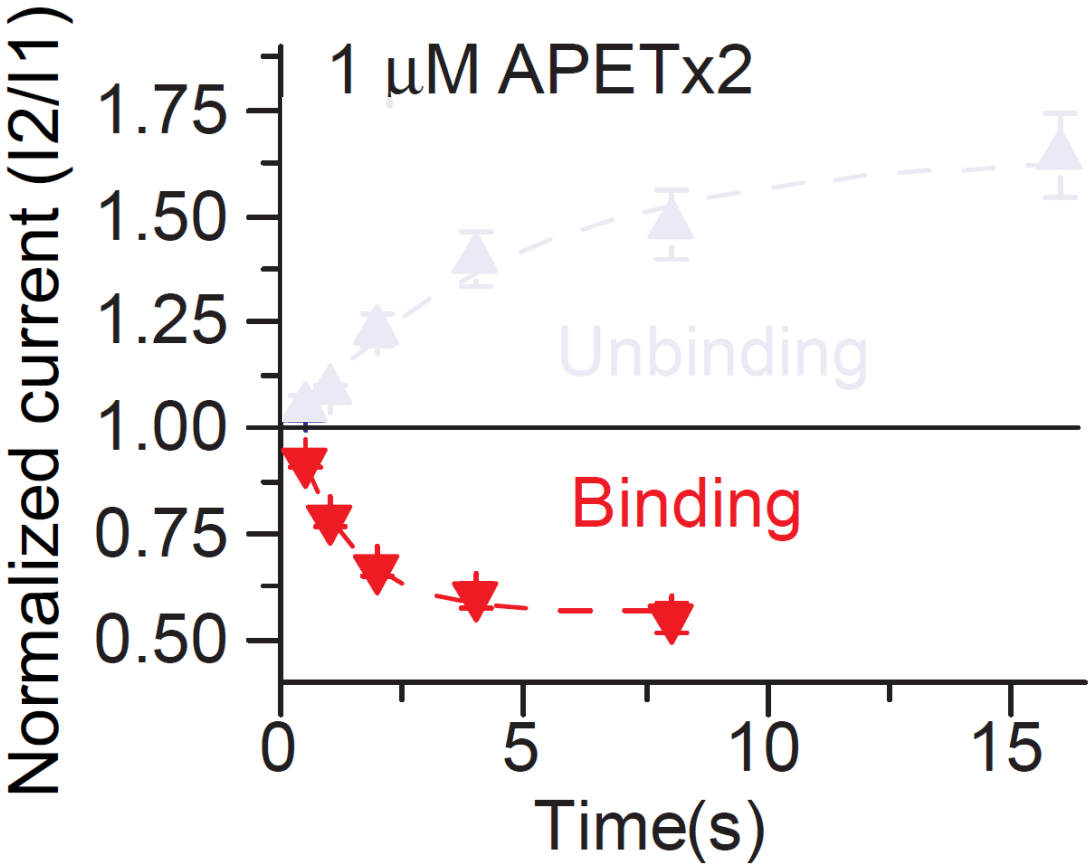


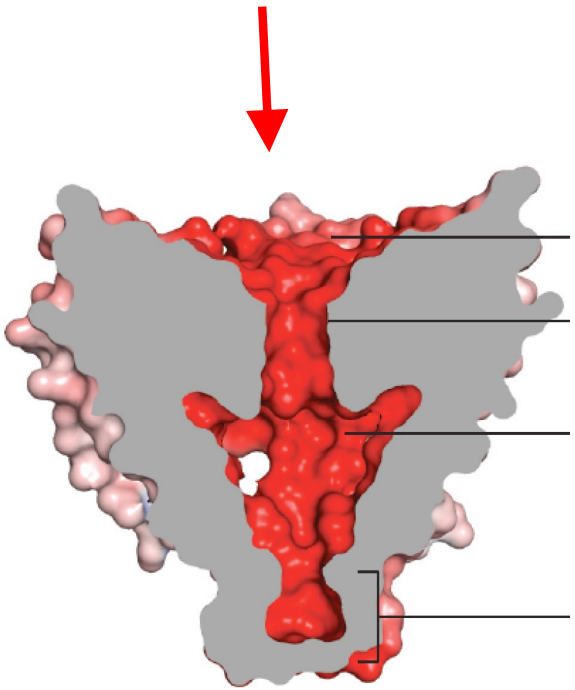
t1/2 = ?

k_{off} (dissociation rate constant) = ? s^{-1}

On rate k_{on}

APETx2, a 40 aa toxin, to Nav1.8





k_{obs} (at 1 µM) = 0.67 s^{-1}

$k_{obs} = x_A * k_{on} + k_{off}$

k_{on} = ?

The neurotransmitter glutamate

In synapses of the CNS, high concentrations of the neurotransmitter glutamate are reached in the synaptic cleft after presynaptic stimulation. Glutamate acts on post-synaptic receptors, as e.g. NMDA receptors. Glutamate is rapidly cleared from the synaptic cleft, and it is estimated that glutamate stays less than 1 ms at these high concentrations in the synaptic cleft. The removal of glutamate from the synaptic cleft is faster than its unbinding from the postsynaptic receptors, therefore the unbinding (koff) of glutamate determines the current decrease. On high affinity NMDA receptors glutamate has a Kd of 1 μM, and its k_{on} is $2 \cdot 10^8$ M^{-1} s^{-1}.

→ Calculate the time course ($t_{1/2}$) of glutamate unbinding from the NMDA receptor.

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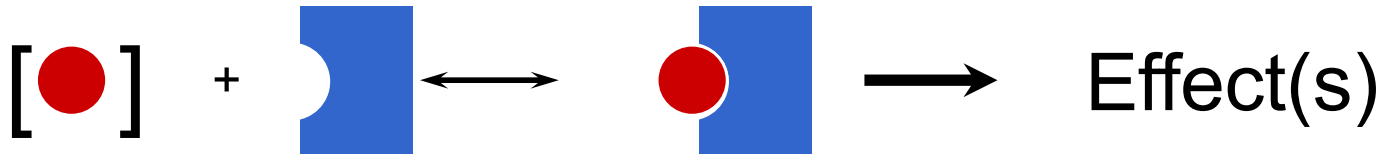

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Relationship between binding and effect (2): examples of more or less complex signaling between binding and effect

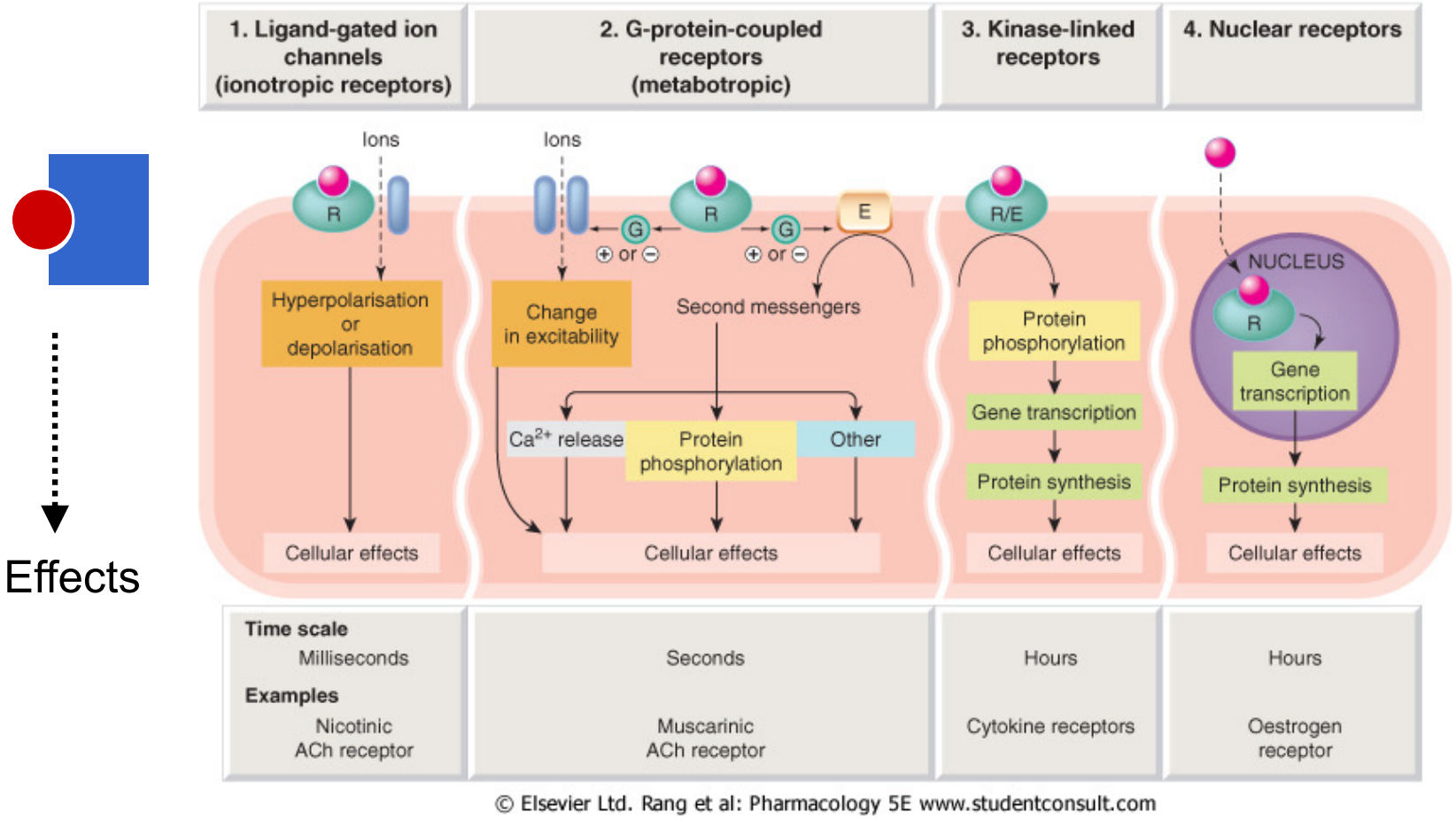


Figure 3.2 Types of receptor-effector linkage. (R, receptor; G, G-protein; E, enzyme; ACh, acetylcholine.)

drug to organism

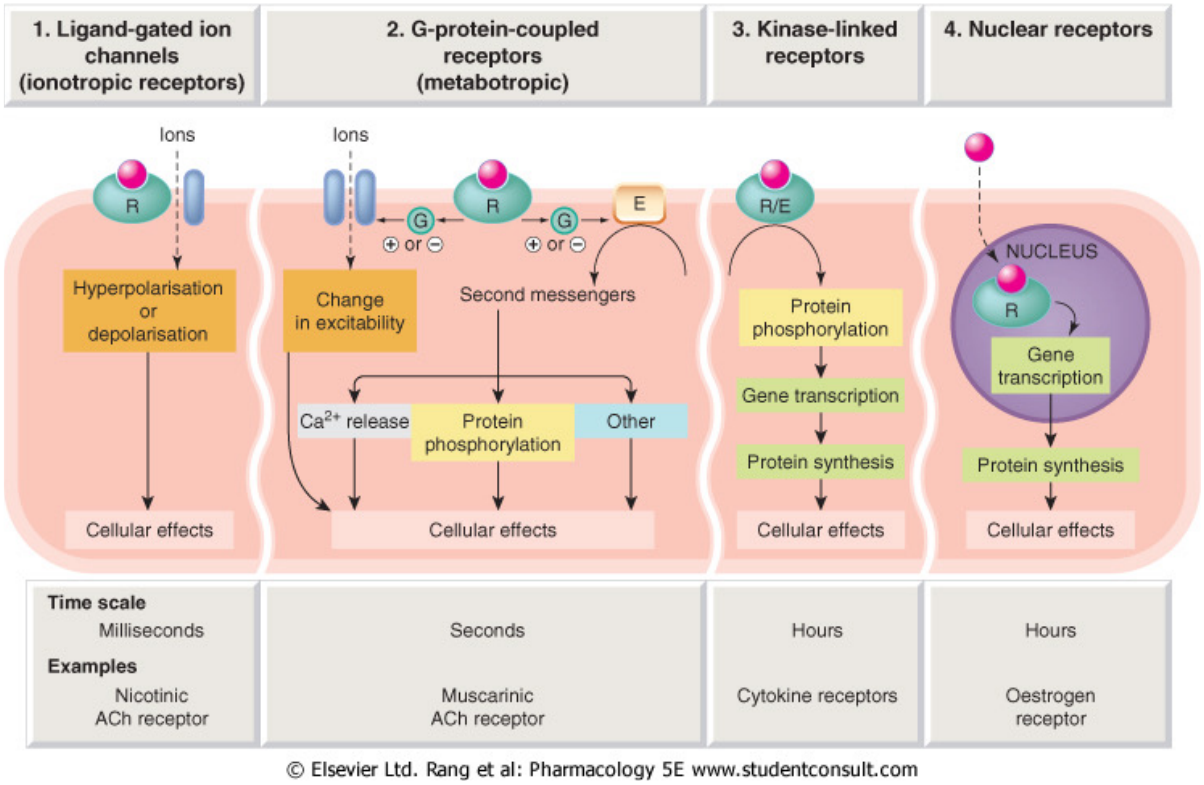
drug to tissue

R + L → LR

Effects

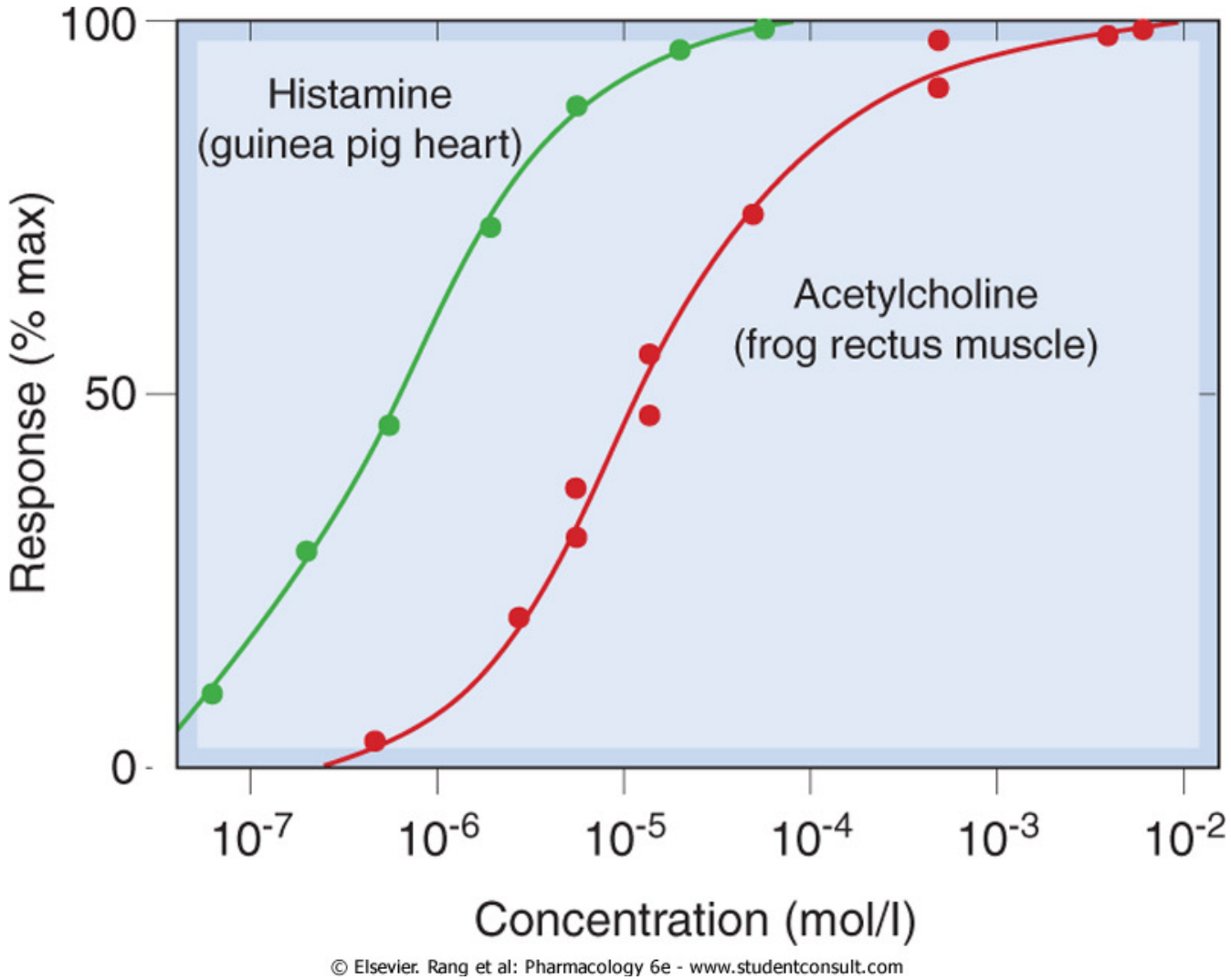
effect, e.g. release of factors

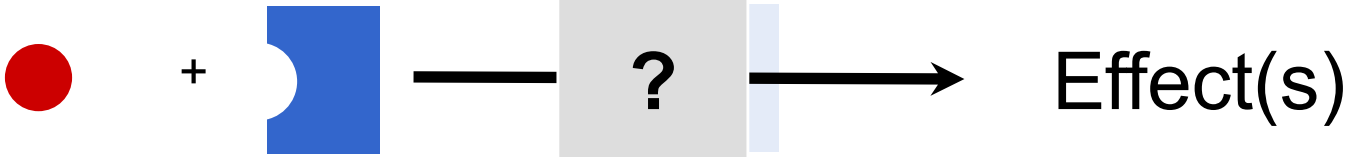
effect, e.g. change in blood pressure



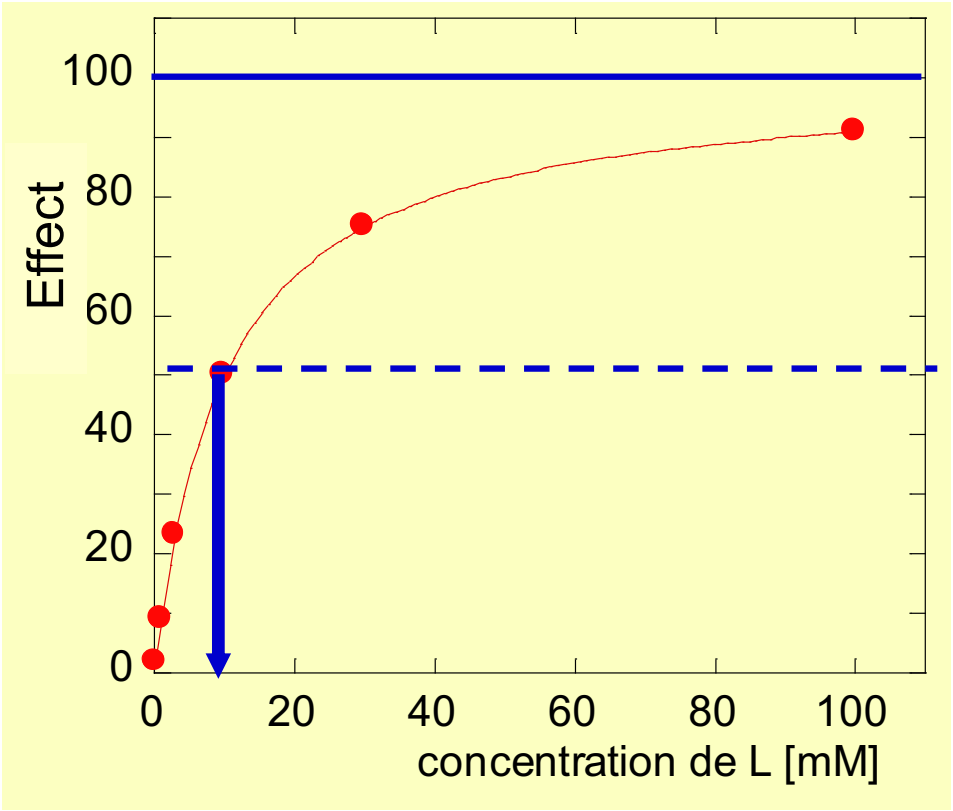
..and even a seemingly direct coupling, for example the opening of a ligand-gated ion channel after binding of the ligand, is complex, involving many conformational changes across the channel protein

Experimentally observed concentration-effect curves: an example

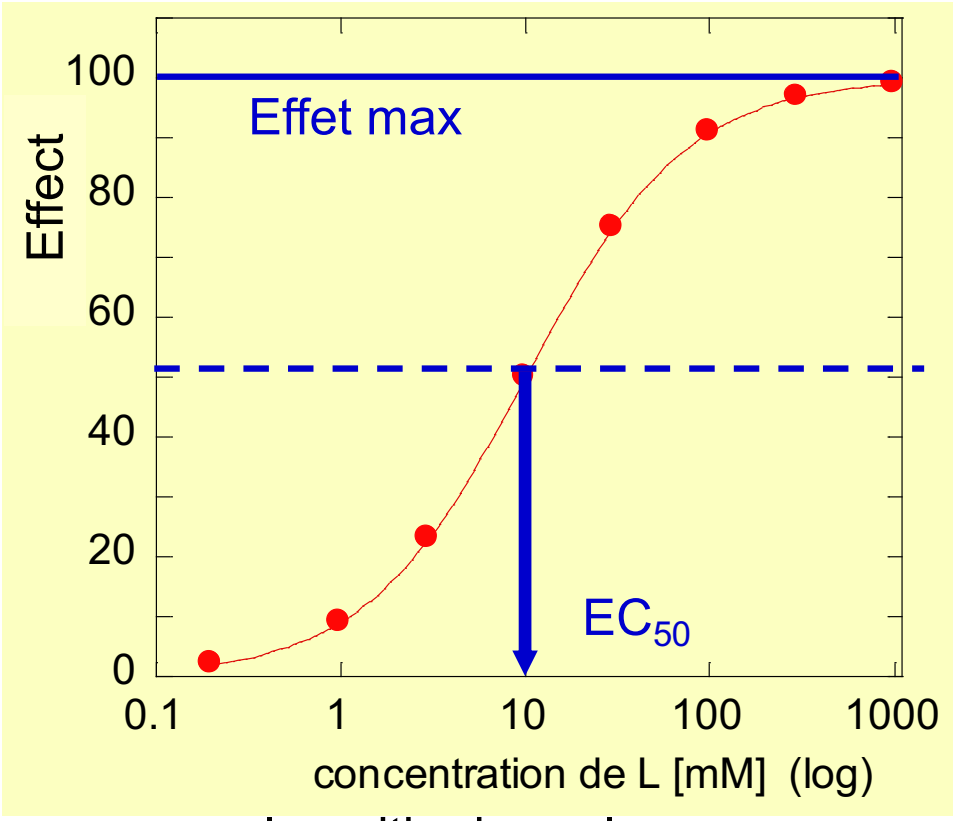




Concentration / effect curves



linear scale



logarithmic scale

EC50 = concentration for half-maximal effect, ≠ Kd !

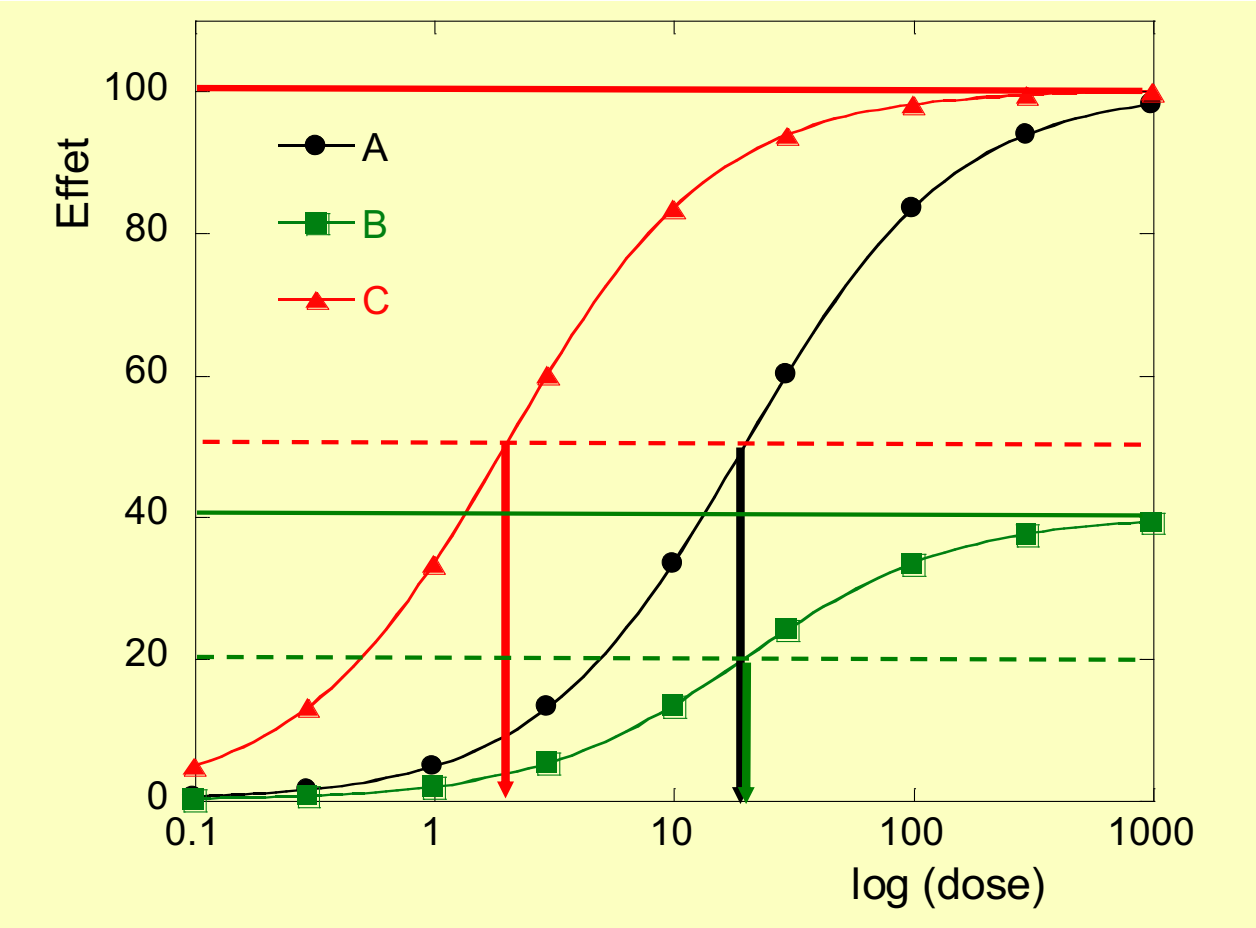
Example with max. effect = 100 and EC_{50} = 10 mM

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

Definitions : efficacy and potency

The **efficacy** of a drug (« efficacité » in French) is defined by the amplitude of the maximal effect.

The **potency** (“puissance”) of a drug is defined by the EC_{50}



C is more potent than A

B has lower efficacy than A

Illustration difference in efficacy, definition of a partial agonist

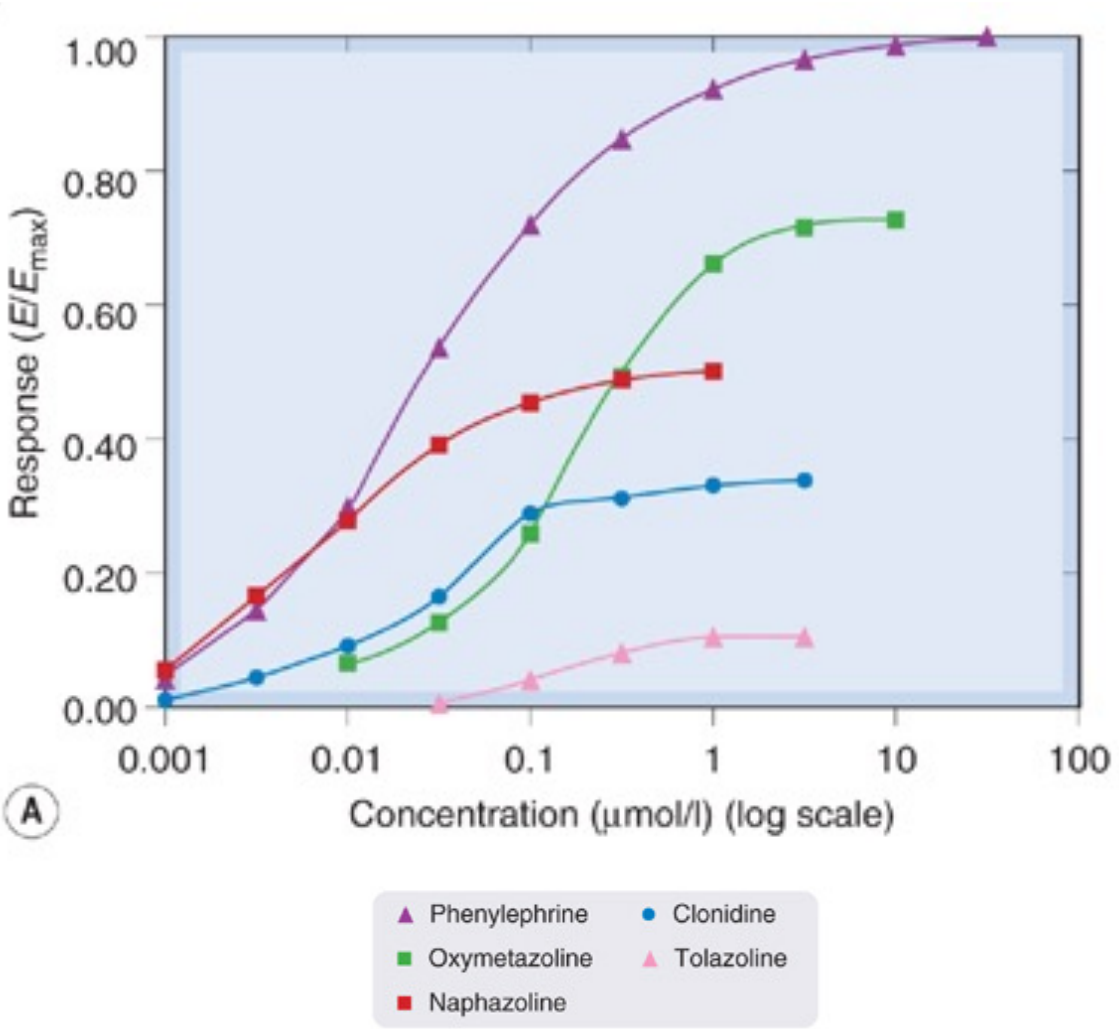


Figure 2.7 Partial agonists. [A] Log concentration-effect curves for a series of α-adrenoceptor agonists causing contraction of an isolated strip of rabbit aorta. Phenylephrine is a full agonist. The others are partial agonists with different efficacies. (Data from Ruffolo et al. 1979 J Pharmacol Exp Ther 209: 429-436.)

*Ramsey, Minnesota (CNN)*Toxicology tests for Prince concluded that the entertainer died from an accidental overdose of the opioid fentanyl, according to a report on his death by the Midwest Medical Examiner's Office. Fentanyl is 50 to 100 times more potent than morphine.
Prince, whose full name was Prince Rogers Nelson, died April 21 2016 at age 57, after being found unresponsive in an elevator at Paisley Park, his home and recording studio in Chanhassen, Minnesota. (Source: CNN)

case

Pittsburgh, PA; November 1988: A 34-year-old man is dropped off by a private car at the ambulance entry of an emergency department. He is disheveled and unresponsive and is rushed by security guards into the department. He is quickly placed on a cardiac monitor, and intravenous access is established. His vital signs reveal a heart rate of 26 bpm and he is apneic. He has no palpable blood pressure but has a palpable slow pulse at his femoral artery. Fresh needle track marks, consistent with recent injections, are present in his left antecubital fossa. Physicians suspect he is a victim of the current epidemic of “superpotent” heroin, “China White,” which is sweeping Allegheny County. Despite oral intubation, mechanical ventilation, advanced cardiac life support measures, and large intravenous doses of an **antidote**, the patient dies.
In 1988, the Pittsburgh, PA area experienced an epidemic of heroin abusers dying from accidental overdoses of a short-acting synthetic opioid agonist, 3-methyl fentanyl, known on the street as China White. These synthetic analogues of fentanyl were estimated to have 6000 times the **potency** of morphine. Pharmaceutical fentanyl has 75 to 100 times the potency of morphine.

→ what is the difference of the action of china white and morphine on the opioid receptors ?
→ what is the action of the antidote ?

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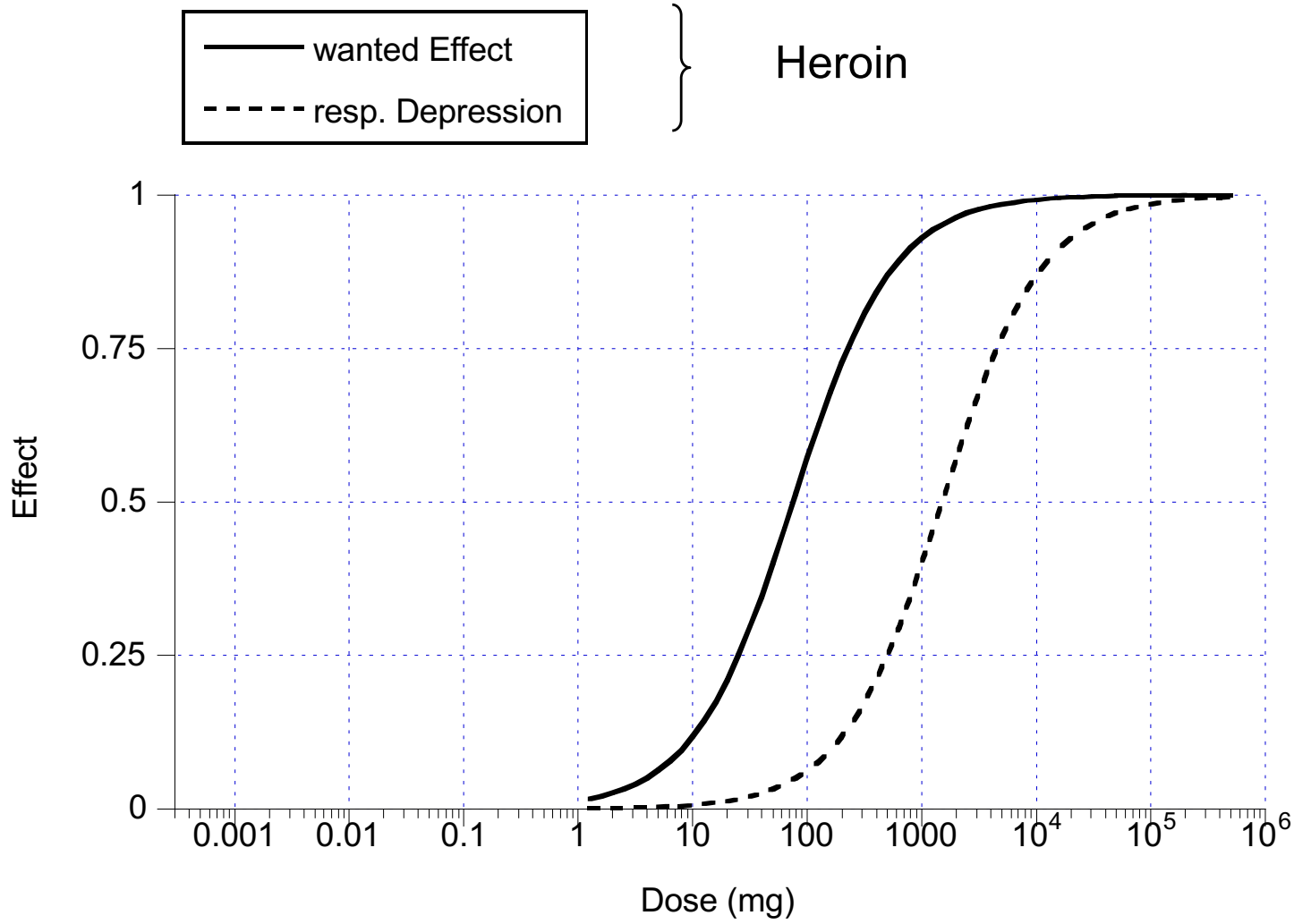
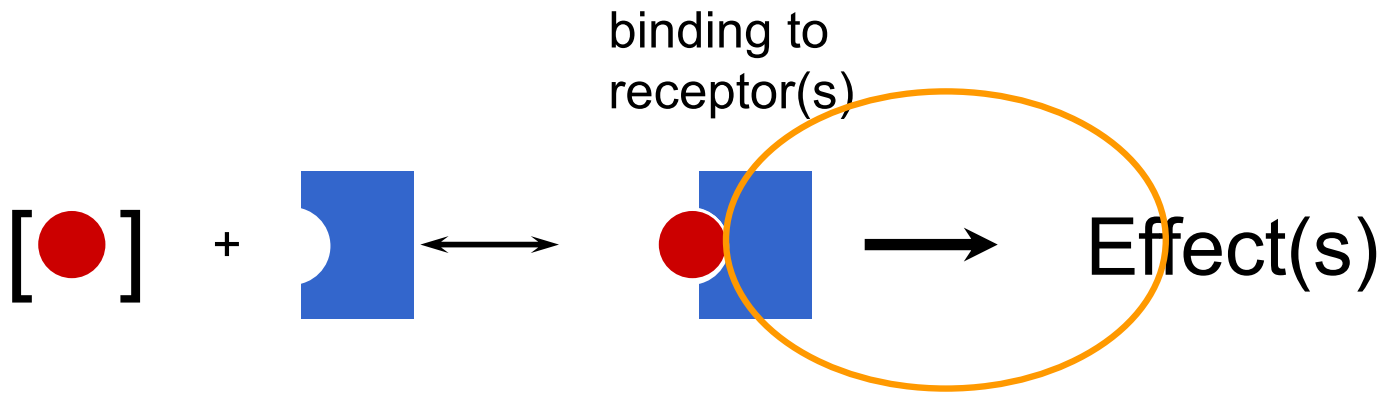
Département de pharmacologie et de toxicologie

Exercise Heroin and 3-methyl fentanyl

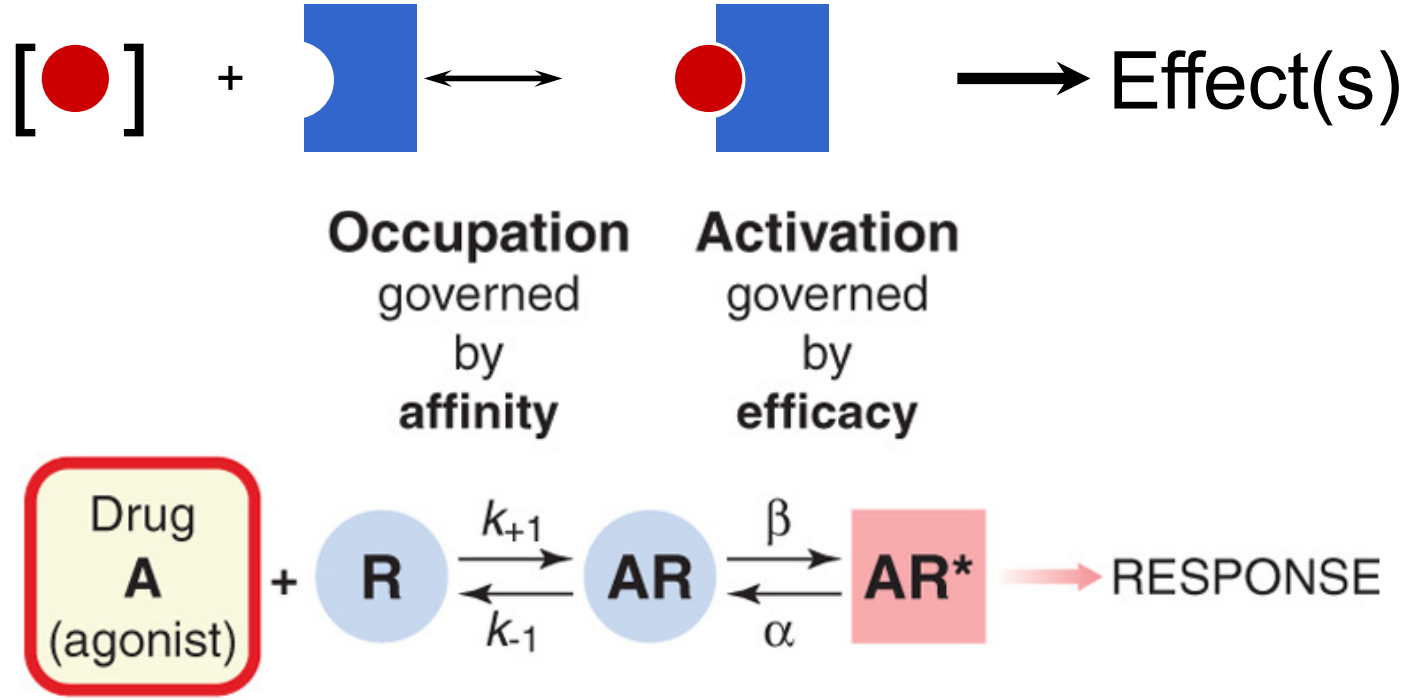


Heroin is usually measured in doses of 25-mg bags. Unsuspecting users of China White, who might have achieved a desired “high” with three bags of heroin, died after injecting one bag of 3-methyl fentanyl. Assuming that 3-methyl fentanyl has a 3000x higher potency than heroin, for the wanted and unwanted effects,

- draw the two corresponding curves for 3-methyl fentanyl
- explain why the drug users died



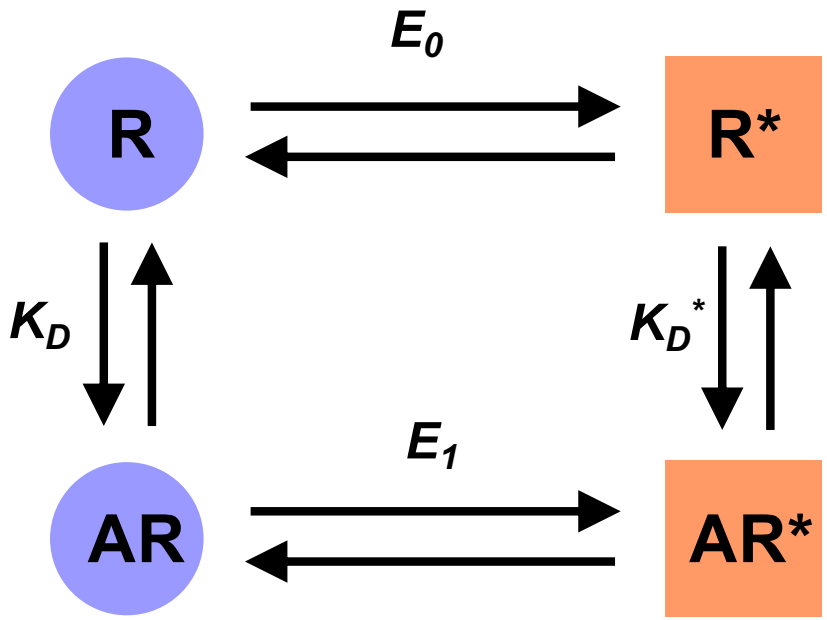
Model for the action of agonists



(sequential model, as proposed by Katz)

"induced fit"

The two-state model (required to explain the constitutive receptor activity, R*)



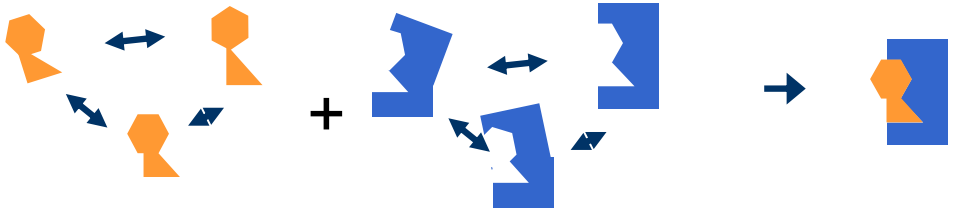
Two conformational states, 'resting' (R, AR) and 'activated' (R*, AR*), in equilibrium.
AR, receptor has a ligand (for example an agonist) bound.
In absence of ligands, the equilibrium lies far to the left, and few receptors are found in the R* state.
Activation in the absence of any ligand is called **constitutive activation**. For constitutively active receptors, an appreciable proportion of receptors adopt the R* conformation in the absence of any ligand.
Agonists have higher affinity for R* than for R, so agonist-bound receptors are mostly in the AR* conformation. The greater the relative affinity for R* with respect to R, the greater the efficacy of the agonist.

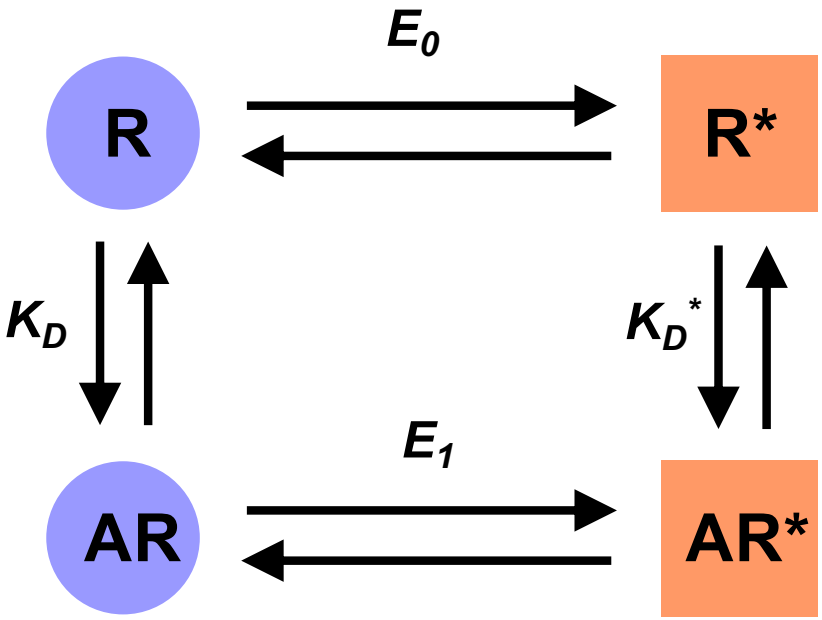
Differences to sequential model:
-Conformational change does not follow binding of the ligand
-Allows constitutive activity

Models of ligand – receptor interaction

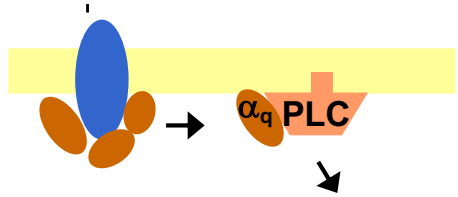
- induced fit (or “conformational induction”): R ⟶ AR ⟶ AR*

- Conformation selection: R* ⟶ AR*

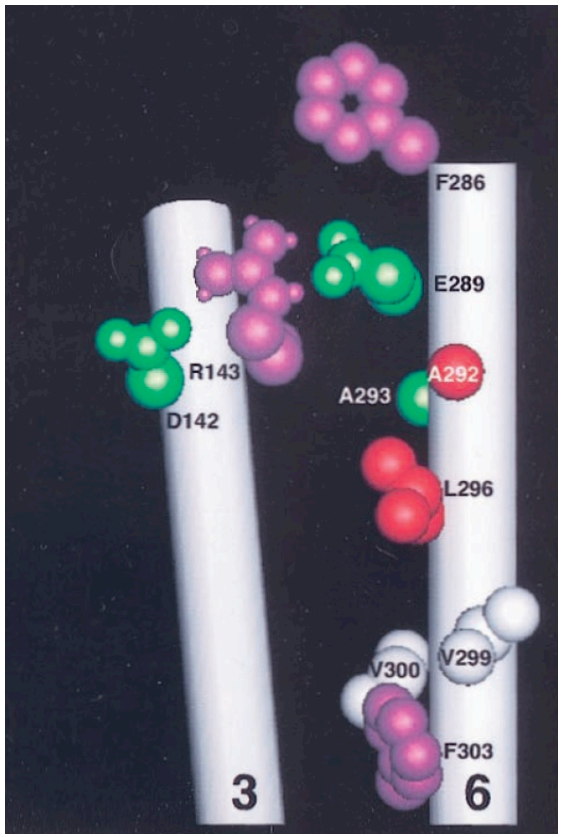


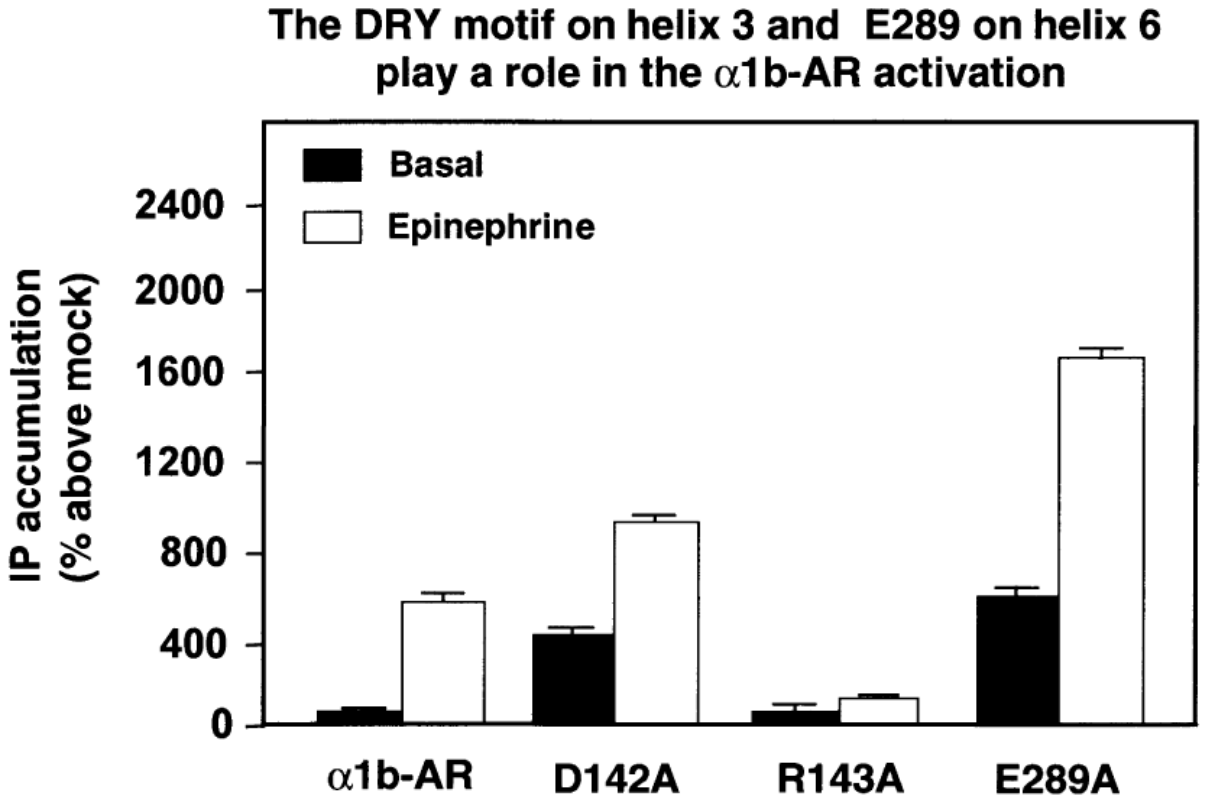


Constitutive activation in the α1b adrenergic receptor



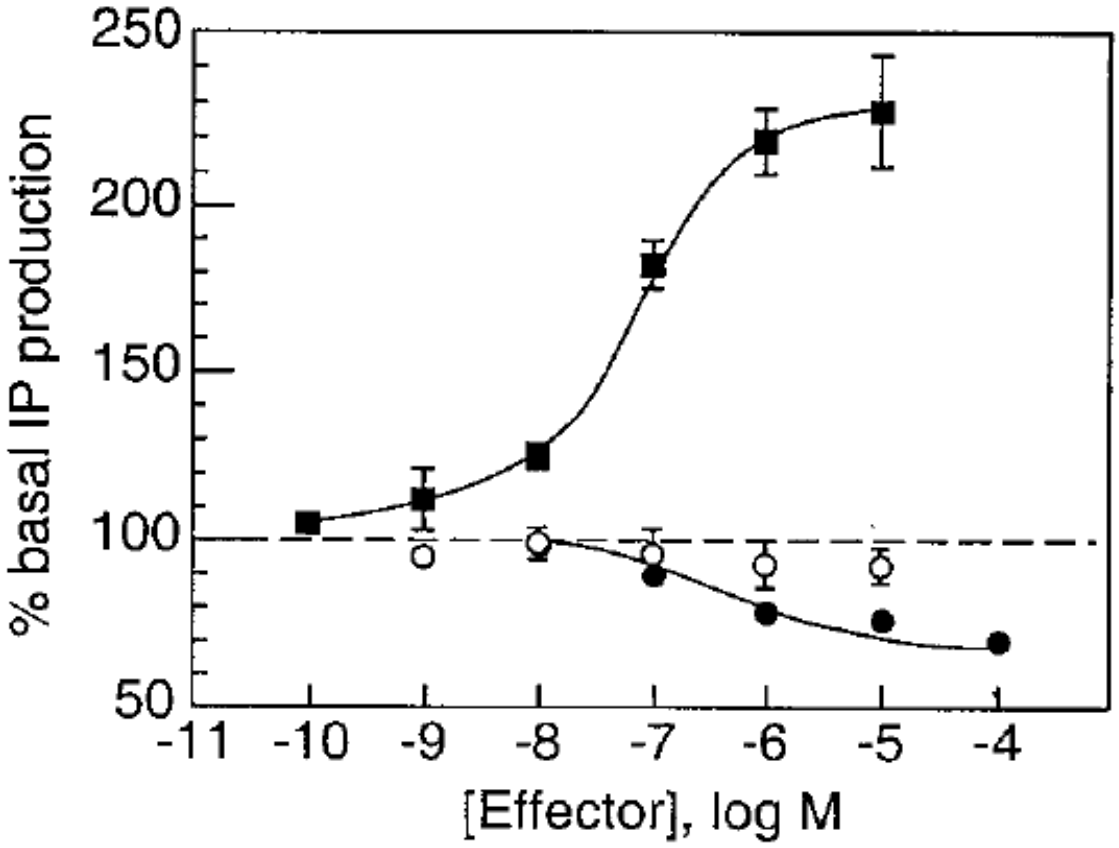
Inositol phosphate (IP)

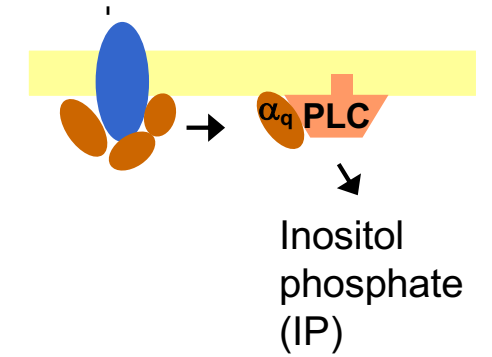




J Recept Signal Transd 22, 1-16

Pharmacology at the Constitutively Active Human Neurotensin Receptor 2





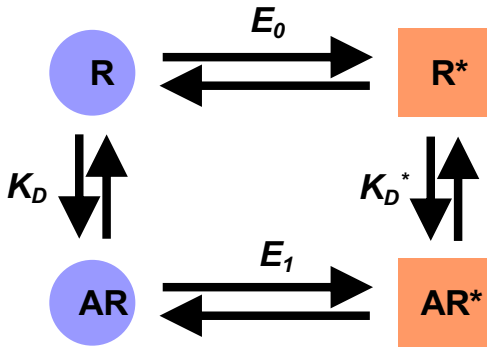


Fig. 2. Effect of SR 48692, NT, and levocabastine on IP production. Concentration-response curves for the effect of SR 48692 (■), NT (○) and levocabastine (●) on basal IP production in hNTR2-transfected cells. IP levels measured in the absence of Li^+ were subtracted from the data that are expressed as the percentage of IP production without added drugs (1595 ± 85 dpm/3 × 10^5 cells). Values are the means ± S.E. from three independent experiments. S.E. values smaller than symbol size are not represented.

Mol Pharmacol 60:1392–1398, 2001

Transfected into cos cells

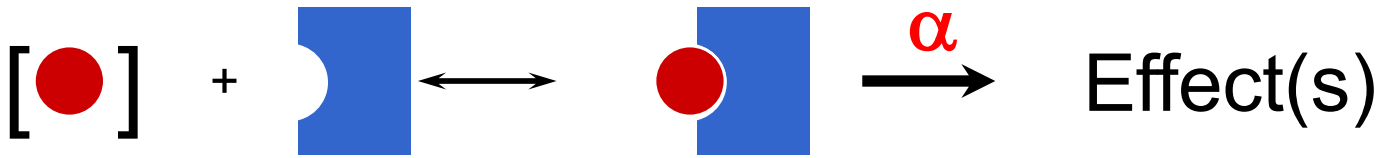
Relating concentration, binding and effect: the concept of efficacy

Initial model that described the action of drugs based on the law of mass action (Ariens, Clark):

$$\frac{E_A}{E_M} = \alpha \cdot p_A = \alpha \cdot \frac{1}{1 + K_A / x_A} \quad (15)$$

$$pA = \frac{1}{1 + K_d/x_A} \quad (8)$$

where E_A represents the effect produced by agonist A, E_M is the maximal response, N_A is the concentration of the agonist-receptor complex, N_{tot} is the total density of receptors, x_A is the agonist concentration and α is a proportionality term called "intrinsic activity" which defines the ability of the drug to produce an effect in a tissue and which can assume values between **0 and 1** (antagonist: 0; full agonist: 1).



→ This was a first approach for explaining the relationship between binding and effect
→ Ok for partial agonists
→ Limit of the approach: assumes that the size of the effect is proportional to the occupancy. This is however not always the case!

Theoretical occupancy and response curves for full and partial agonists

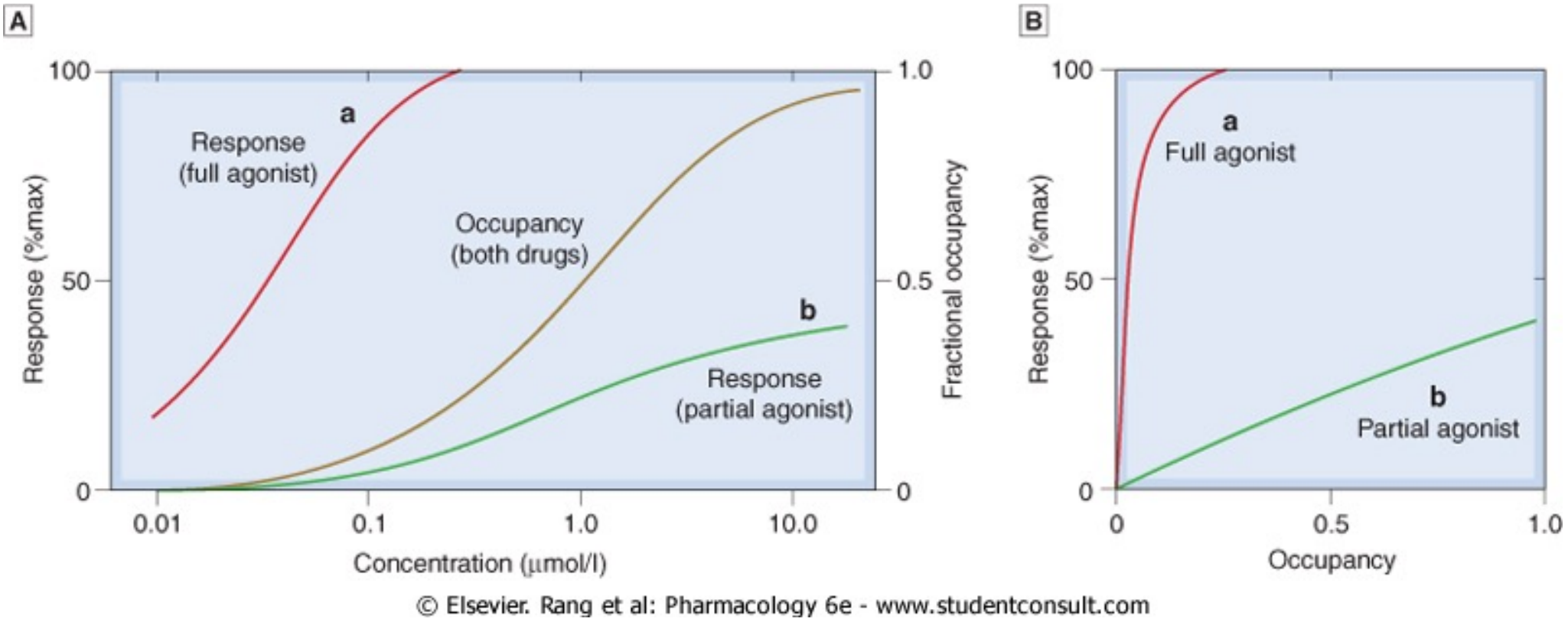


Figure 2-5 The occupancy curve is for both drugs, the response curves a and b are for full and partial agonist, respectively. The relationship between response and occupancy for full and partial agonist, corresponding to the response curves in A. Note that curve a produces maximal response at about 20% occupancy, while curve b produces only a submaximal response even at 100% occupancy. In some tissues, some full agonists are capable of eliciting maximal responses at low occupancies, as illustrated here for agonist a. This means that the mechanism linking the response to receptor occupancy has a substantial reserve capacity. Such systems may be said to possess spare receptors, or a receptor reserve.

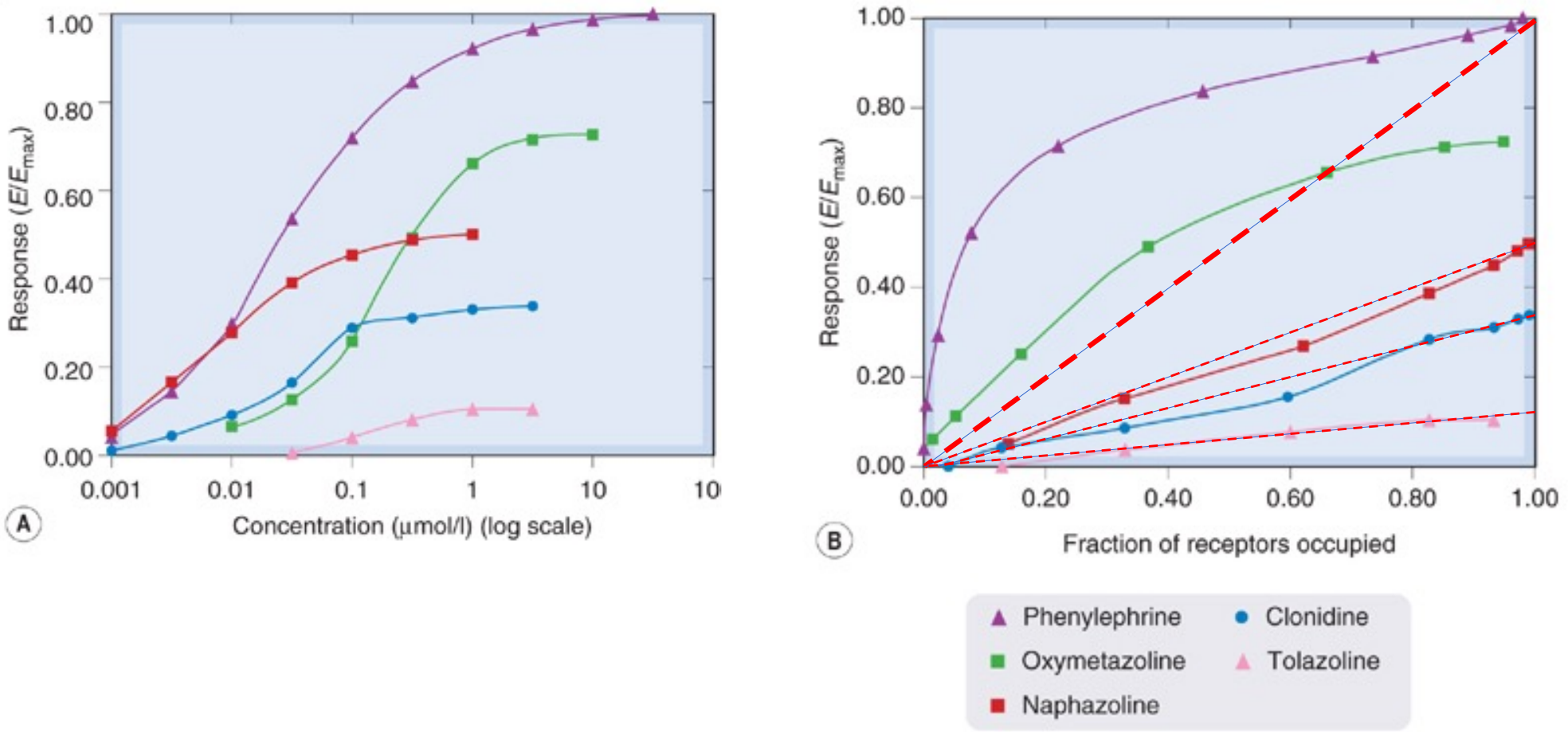


Figure 2.7 Partial agonists. [A] Log concentration-effect curves for a series of α-adrenoceptor agonists causing contraction of an isolated strip of rabbit aorta. Phenylephrine is a full agonist. The others are partial agonists with different efficacies. [B] The relationship between response and receptor occupancy for the series. Note that the full agonist, phenylephrine, produces a near-maximal response when only about half the receptors are occupied, whereas partial agonists produce submaximal responses even when occupying all of the receptors. The efficacy of tolazoline is so low that it is classified as an α–adrenoceptor antagonist. (Data from Ruffolo et al. 1979 J Pharmacol Exp Ther 209: 429-436.)

A more complete approach for relating concentration and effect: contributions of ligand-receptor pair and tissue

The following equation, proposed by Stephenson and refined by Furchgott, contains the concept of receptor stimulus (S). Response is a function of S; the undefined function f describes the ability of the tissue to convert the receptor stimulus into a response. The meaning of the symbols is the same as in equation 15; ε_a is the intrinsic efficacy, defined as the ability of a drug to produce a stimulus from a single receptor. ε_a is not the same as the term "efficacy" defined above!

$$E = f(S) = f\left(\frac{\varepsilon_a \cdot N_{tot}}{1 + K_A/[x_A]}\right) \quad (16)$$

$$pA = \frac{1}{1 + K_d/x_A} \quad (8)$$

Characteristics of the tissue:

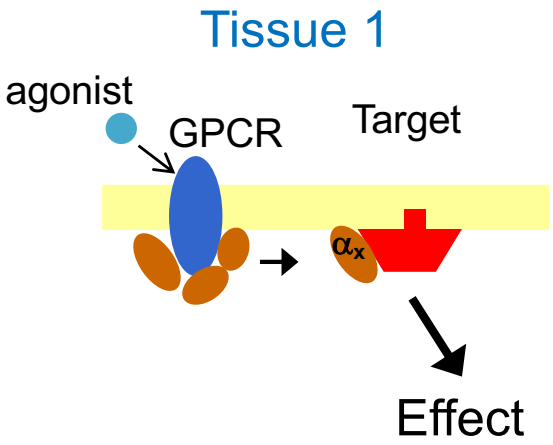
- *f* (the transducer function, *i.e. not a linear relationship*)
- N_{tot} (the total number of receptors)

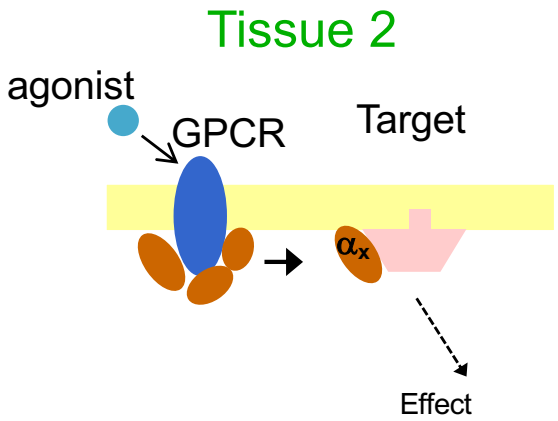
Characteristics of the agonist-receptor pair:

- ε_a (the intrinsic efficacy)
- K_A (the equilibrium constant)

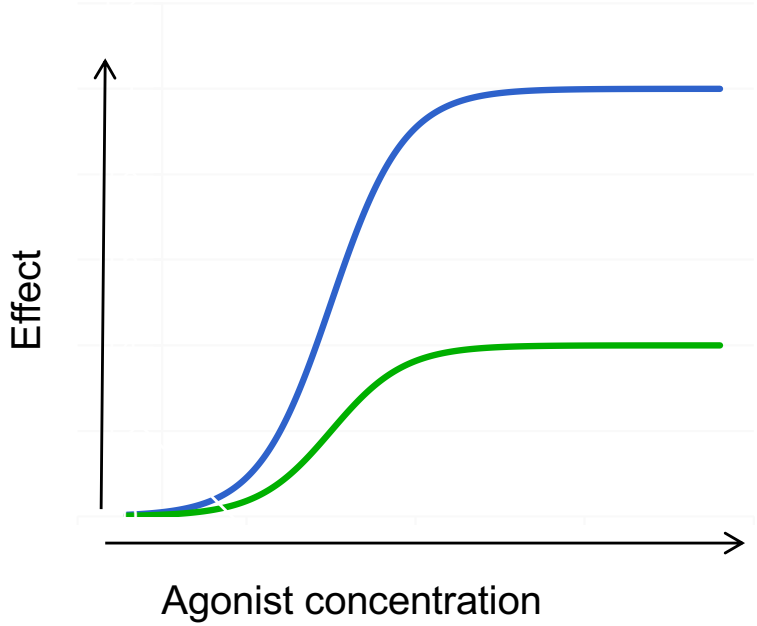
→ the same agonist can be full agonist in one tissue and partial agonist in another tissue
→ the relative potencies of two agonists may be different in different tissues or with regard to different downstream signaling pathways, even though the receptor is the same (→ ex).

Same receptor in different tissues





$$E = f(S) = f\left(\frac{\varepsilon_a \cdot N_{tot}}{1 + \frac{K_A}{[x_A]}}\right) \quad (16)$$



Other parameters could be different between tissues and would also affect the concentration-effect curve

Revisiting the term "efficacy": ligand-dependent coupling to different signaling pathways (= biased agonism or ligand-biased efficacy)

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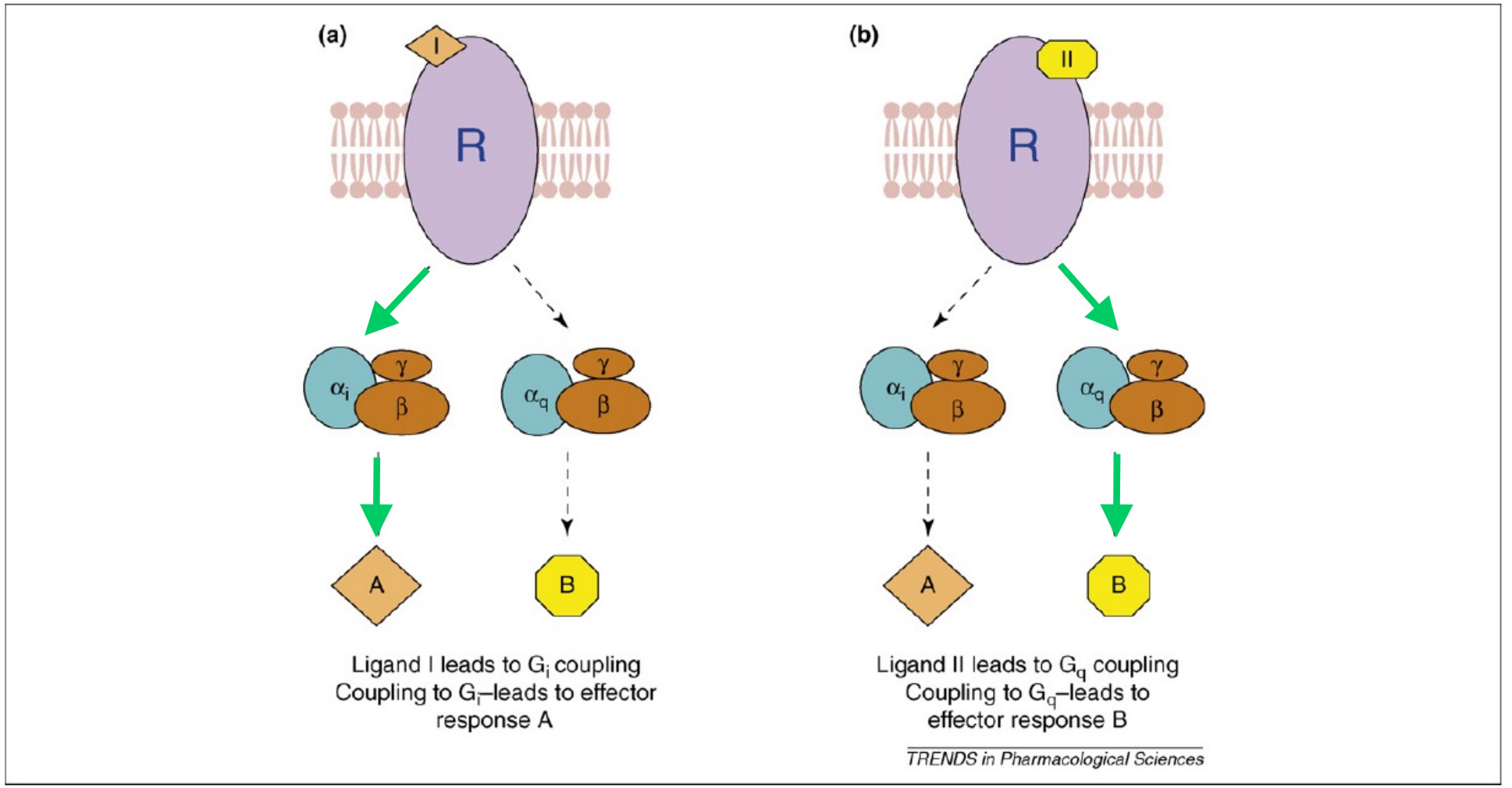


Figure 2. Functional selectivity. GPCRs often couple to multiple G proteins. Functional selectivity is seen when ligand binding influences which G protein associates with the receptor by promoting distinct coupling efficiencies. For example, binding of a distinct ligand (I) to the GPCR leads to activation of G_i and effector responses initiated through this G protein (**a**), whereas binding of a different ligand (II) to the same GPCR leads to activation of G_q and to an alternative set of effector responses driven through this G protein (**b**).

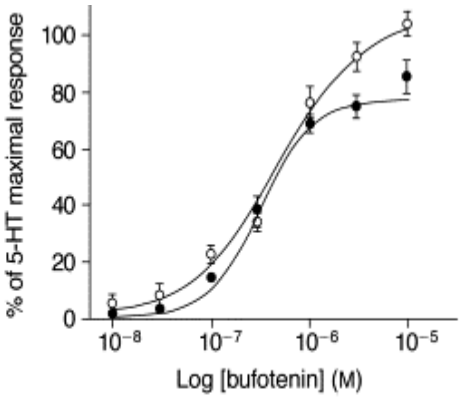
The serotonin (5-HT)2C receptor

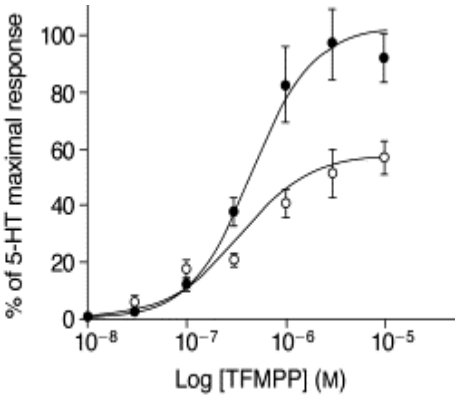
-densely expressed in regions implicated in anxiety, mood, drug-induced hallucinogenesis, reward, neuroendocrine regulation, and appetite.

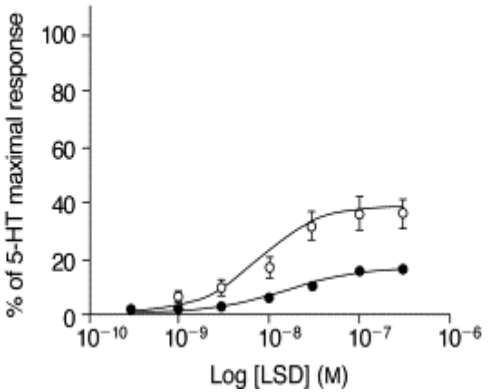
- 5-HT2C receptor knockout mice become overweight as a result of abnormal control of feeding behavior and are prone to spontaneous death from seizure

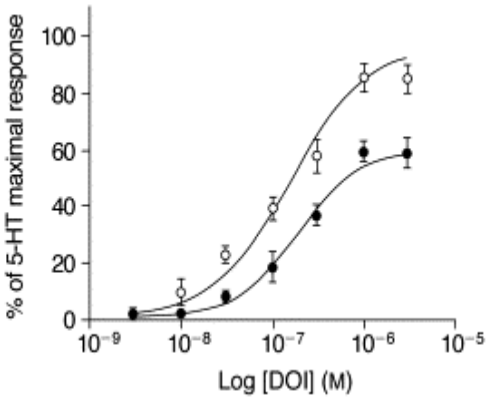
- 5-HT2C receptor mutant mice exhibit abnormal spatial learning and a reduced aversion to a novel environment and consume more food.

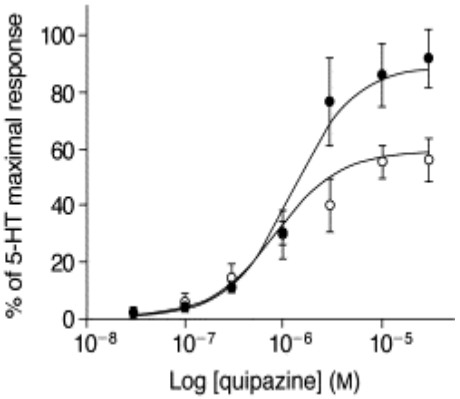
Effector pathway-dependent relative efficacy at the 5-HT_{2C} receptor

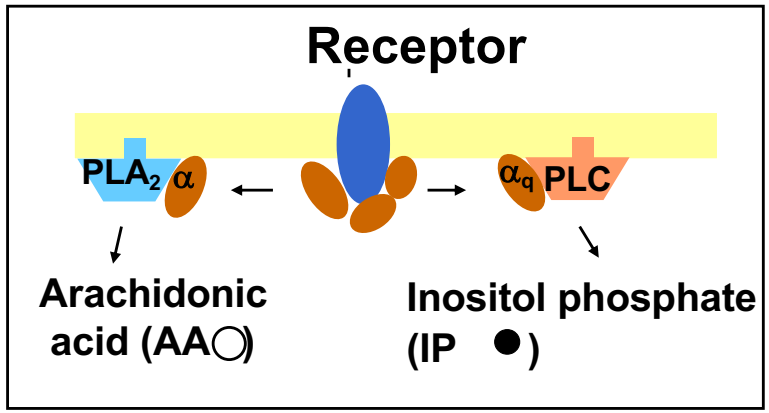










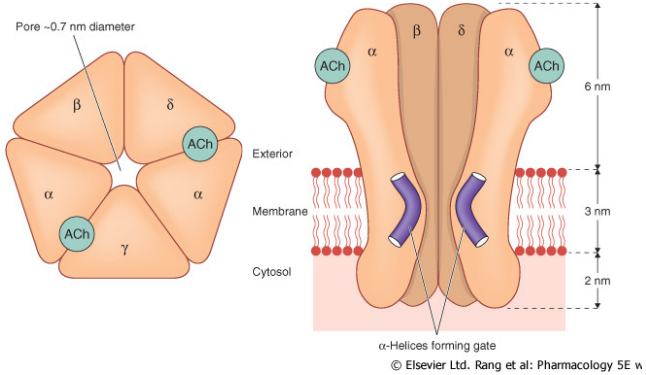


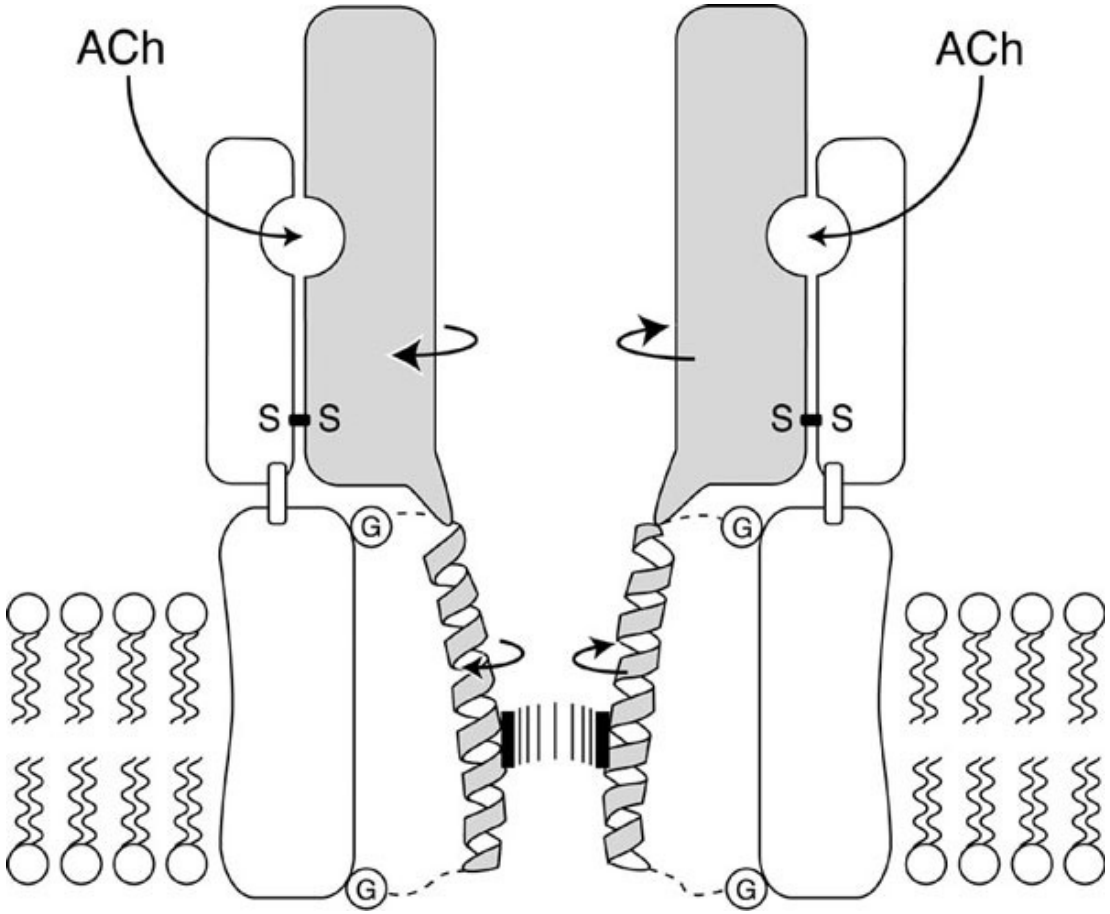
The graphs show concentration–response curves for the effect of 5-HT_{2C} agonists on the release of arachidonic acid (AA) and the accumulation of inositol phosphates (IP) in CHO-1C19 cells expressing the human 5-HT_{2C} receptor. IP accumulation mediated by phospholipase C (PLC) and AA release mediated by phospholipase A_2 (PLA_2) were measured. Data are expressed as a percentage of the maximal response to 5-HT, concentration–response curves for which were run in each experiment. Data were fitted to a three -parameter logistic equation using nonlinear regression analysis to obtain estimates of E_{max}, EC_{50} and slope parameters. Note that the drugs do not differ in potency between responses (AA release or IP accumulation), as expected for drugs acting at a single receptor in the absence of receptor reserve. Data points shown represent the mean ± SEM of 5–9 experiments. **Open circles, AA release; solid circles, IP accumulation**. DOI, ±-1- (2,5-dimethoxy-4-iodophenyl)-2-aminopropane; LSD, lysergic acid diethylamide; TFMPP ,trifluoromethylphenyl piperazine.

Conclusions for receptor activation and efficacy

- Binding of a given ligand to a receptor induces conformational changes that affect downstream signaling pathways in a ligand-specific manner
- The activation mechanisms of receptors require a description that goes beyond the inactive and active (R, R*) form of the receptor
- Efficacy for a ligand-receptor pair needs to be defined with regard to a specified downstream signaling pathway

Model of the activation mechanism of the nicotinic Acetylcholine receptor





The ACh-induced rotations in the α-subunits[8] are transmitted to the gate—a hydrophobic barrier to ion permeation—through the M2 helices. The rotations destabilize the gate, causing the helices to adopt an alternative configuration which is permeable to the ions. The helices move freely during gating because they are mainly separated from the outer protein wall and connected to it by flexible loops, containing glycine residues (G). S-S is the disulphide-bridge pivot in the ligand-binding domain, which is anchored to the fixed outer shell of the pore. The relevant moving parts are shaded. (Atsuo Miyazawa, Yoshinori Fujiyoshi and Nigel Unwin, Nature 423, 949-955 (26 June 2003))

Conformation change in GPCRs

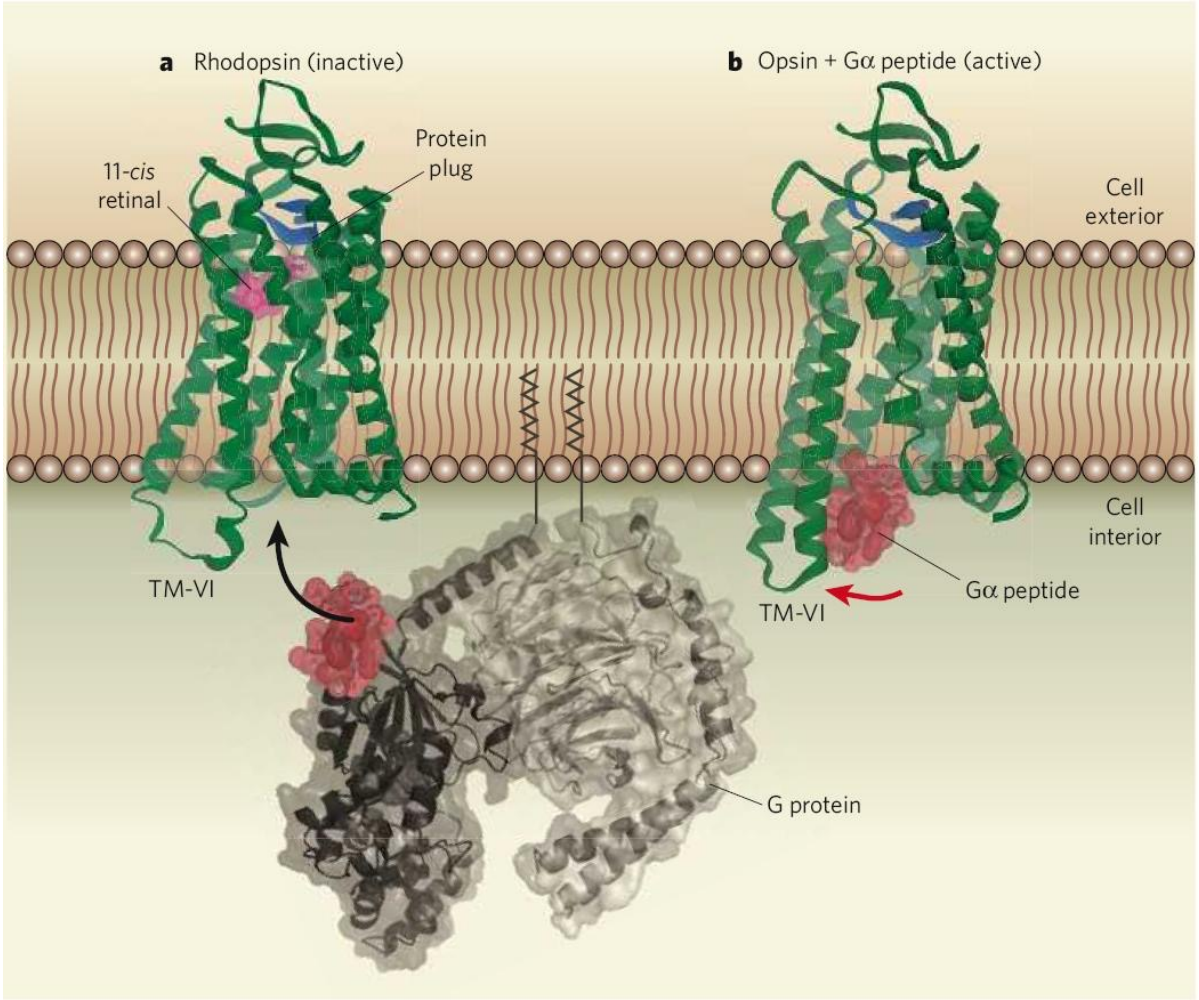
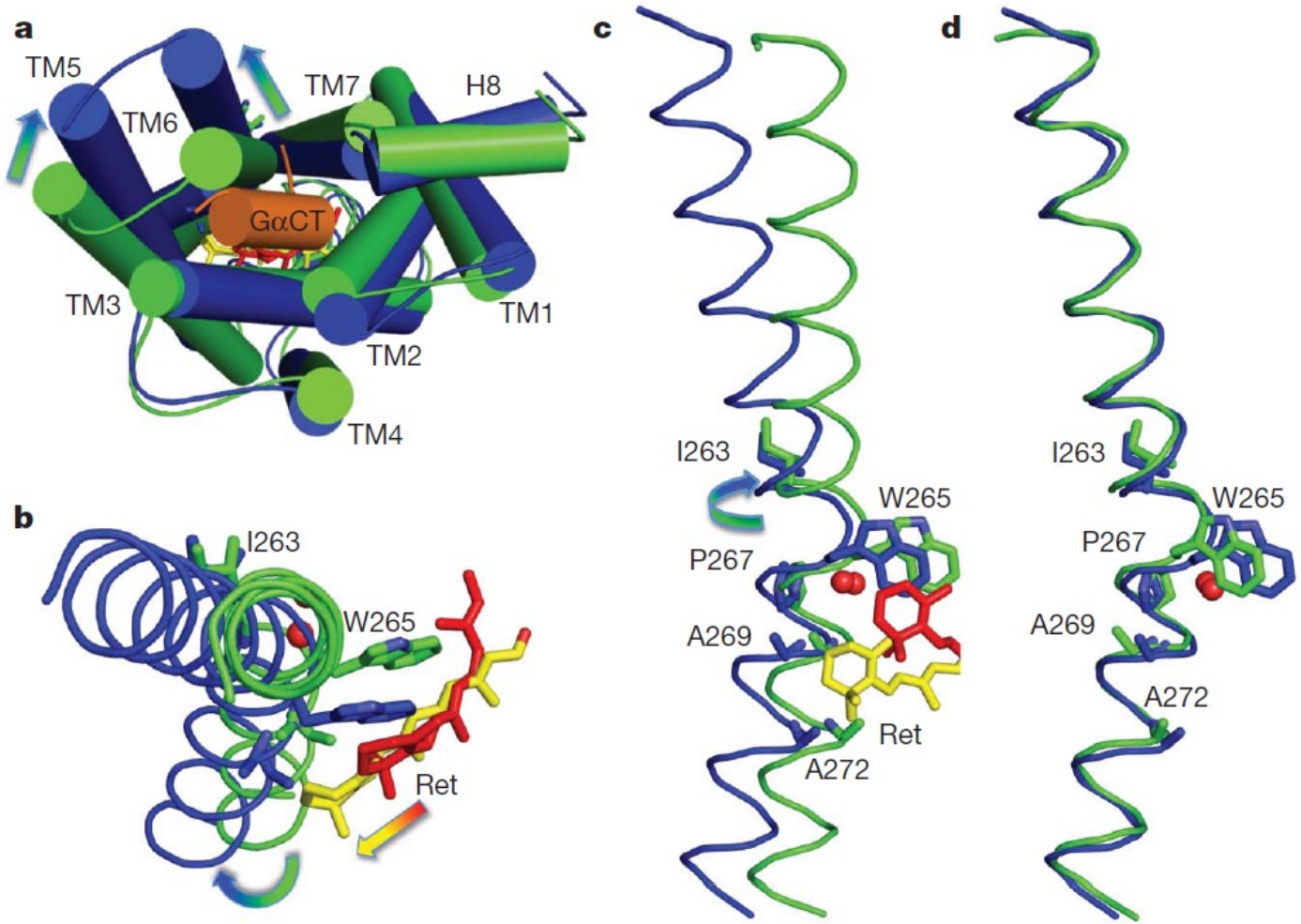


Figure 1 | Activation of a G-protein-coupled receptor. a, Rhodopsin, shown here in its inactivated conformation, is a light-sensing receptor found in cell membranes. It consists of a protein (opsin, green) and a ligand (retinal, pink, also shown in its inactivated conformation). When activated by light, rhodopsin binds to part of an adjacent G protein (binding region in red), triggering a cascade of biological responses. The protein plug (blue) is part of the extracellular domain of opsin, and immobilizes the extracellular transmembrane segments of the receptor. b, Scheerer *et al.*2 have determined the activated structure of opsin in complex with the receptor-binding peptide fragment of the G protein (the Gα peptide). The most notable difference when compared with the inactivated receptor is that transmembrane helix 6 (TM-VI) has moved substantially outward (indicated by the red arrow), thereby creating the binding pocket for the G-protein peptide. (article: Scheerer P. et al., Nature 455, pp497 (2008)).

Structural changes in rhodopsin



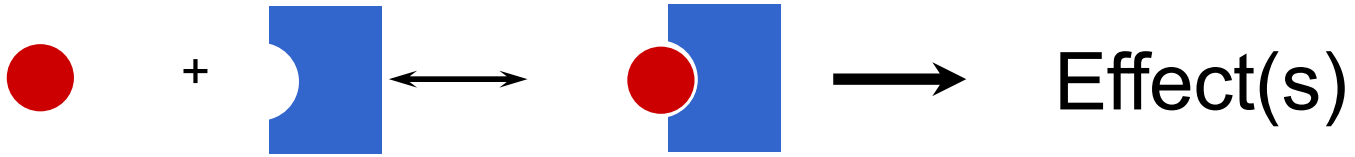
Rearrangement of the heptahelix bundle and rotation of TM6

11-cis retinal (red): inverse agonist, all-trans retinal (yellow): agonist

Green: ground state rhodopsin, blue: active; G-Prot C-term peptide: orange

Standfuss,…Schertler, Nature 471, 656 (2011)

conclusion/summary receptor activation



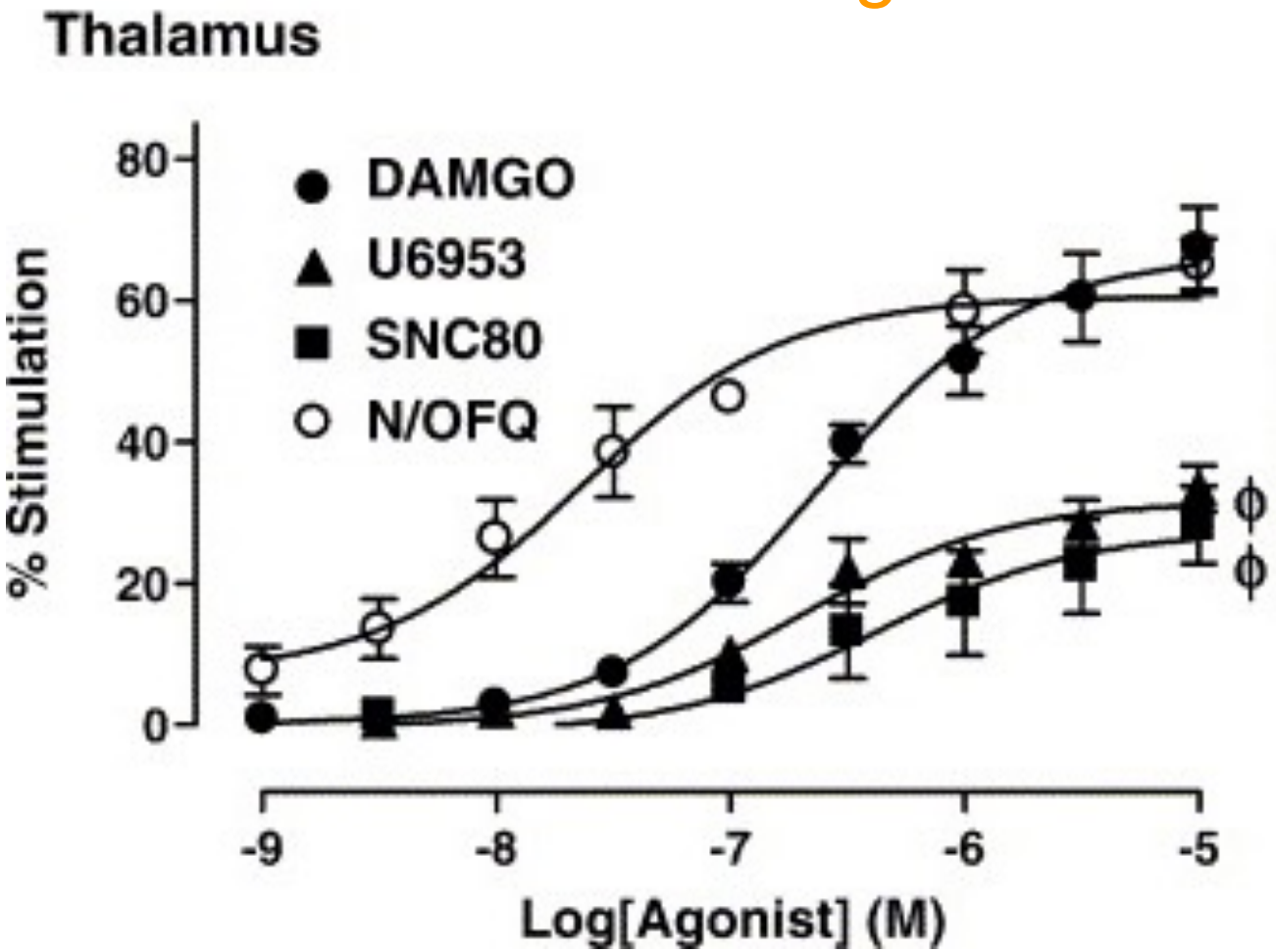
Description relating agonist concentration to binding and effect, indicating the important factors:

$$E = f(S) = f\left(\frac{\varepsilon_a \cdot N_{tot}}{1 + K_A / [x_A]}\right) \quad (16)$$

Most practical approach, relating agonist concentration to effect:

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

Compare the efficiency and the potency of these agonists



	EC 50	Max stimulation (%)
DAMGO		
U6953		
SNC80		
N/OFQ		

Fig. 1. Stimulation of [^{35}S]GTPγS binding by selective μ (DAMGO), δ (SNC80), κ (U69593) or ORL_1 (N/OFQ) agonists in frontal cortical membrane homogenates or thalamic membrane homogenates of the dog. Concentration–effect curves were generated using 15–20 μg membrane protein and of 0.1 nM [^{35}S]GTPγS as described in Experimental procedures. Data are expressed as mean values ± SEM from experiments performed in tissues from two female dogs and one male dog repeated twice in duplicate. *P < 0.001 versus U69595, P < 0.01 versus N/OFQ, P < 0.05 versus SNC80; ϕP < 0.001 versus DAMGO and N/OFQ.
(Brain Res. 1073-1074, 290)