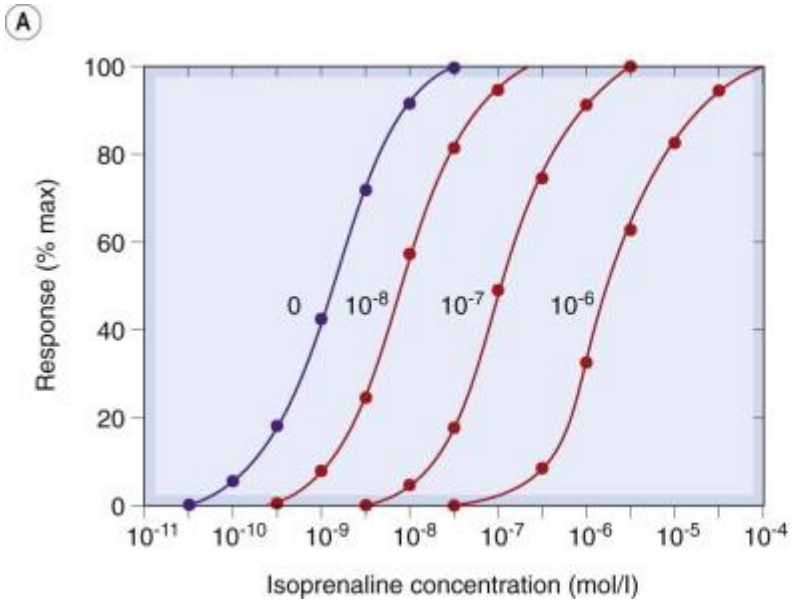
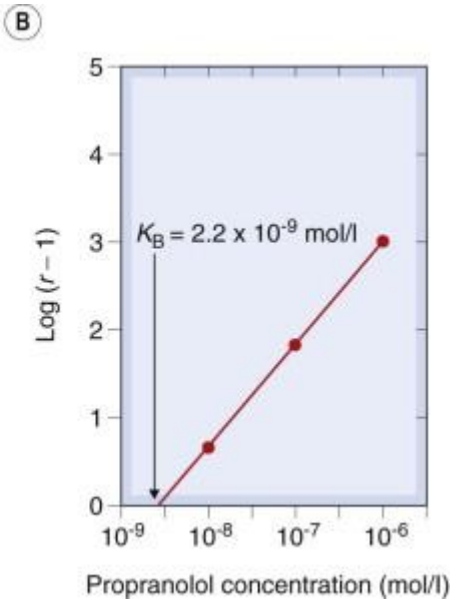
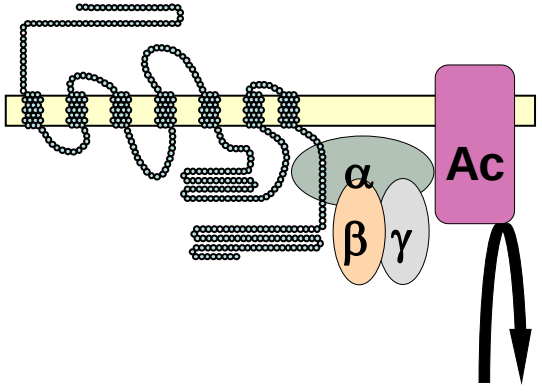
Principles of drug action





Prof Peter Molenaar



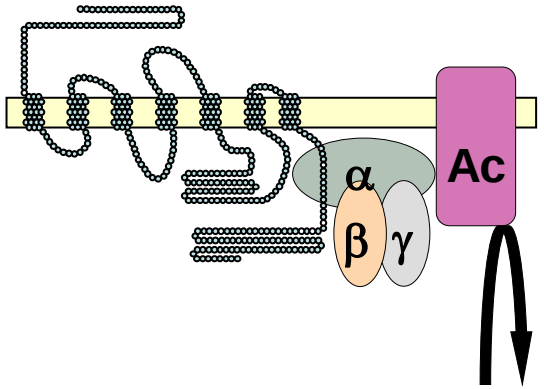
QUT
Queensland University of Technology

CARDIO-VASCULAR
MOLECULAR & THERAPEUTICS
Translation Research Group

Objectives and Aims

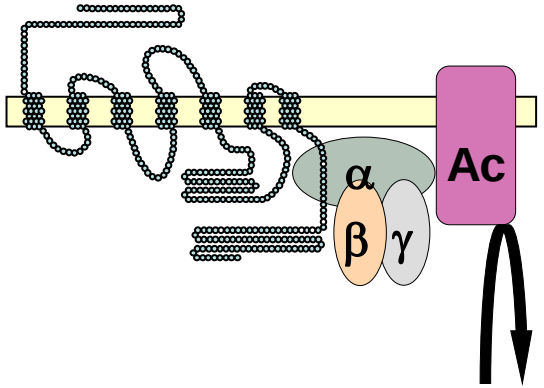
To obtain an understanding of:-

- what is Pharmacology
- what is a drug, a medicine
- protein targets for drug binding
- how drugs act
  - agonists
    - full agonists
    - partial agonists
    - efficacy
    - inverse agonists
    - biased agonists
  - antagonists
    - competitive
    - irreversible



Lecture Outline

1 What is Pharmacology?
2 What is a drug?
3 What is a medicine?
4 Protein targets for drug binding
5 How drugs act
- agonists
  - -full agonists
  - -partial agonists
  - -efficacy
  - -inverse agonists
  - -biased agonists
- antagonists
  - -competitive
  - -irreversible



What is pharmacology?

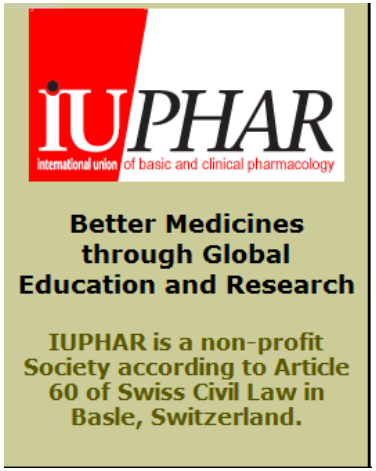
the study of the effects of drugs on the function of living systems.

Rang and Dale 2012, Chapter 1

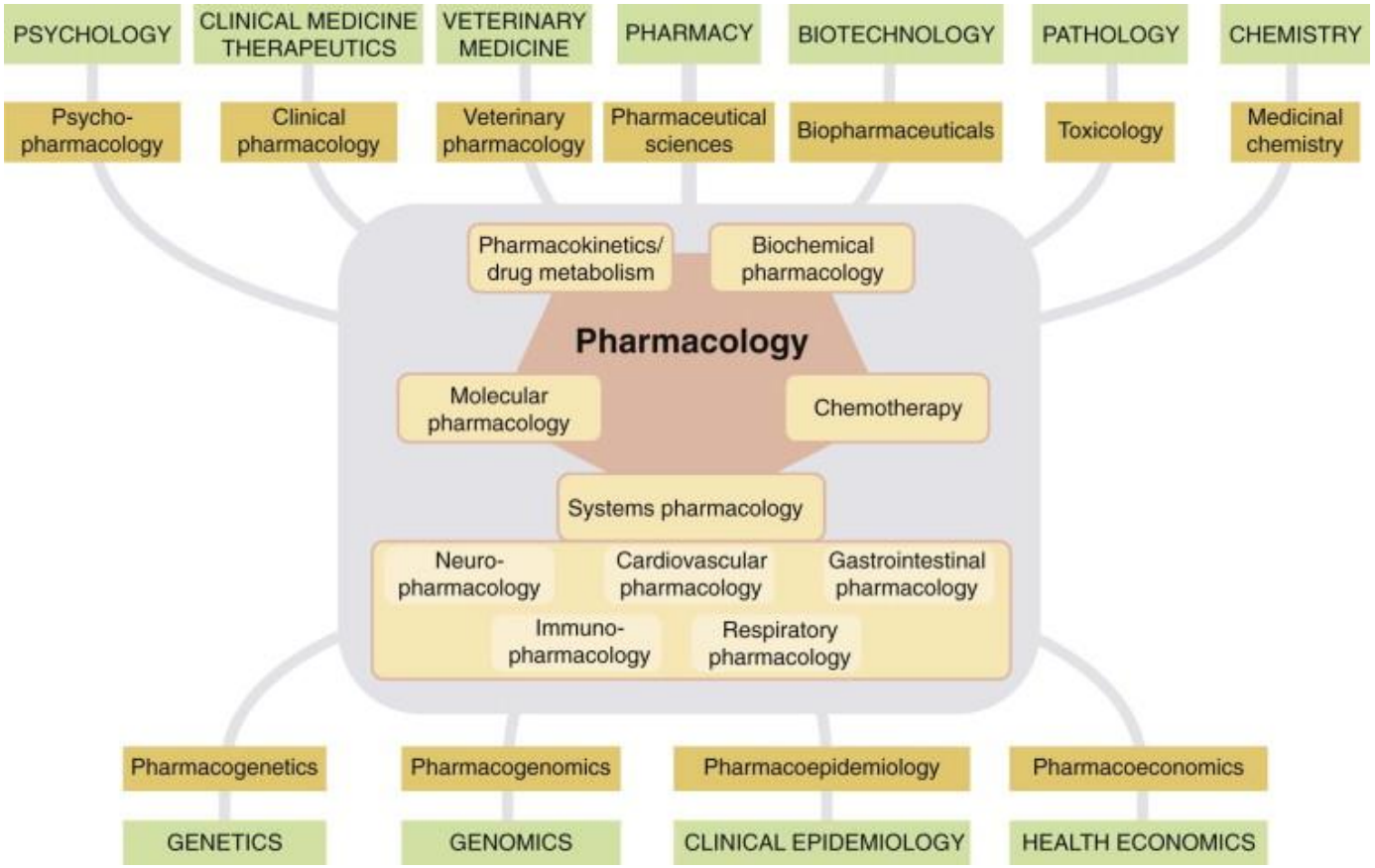
Organizations representing pharmacology and pharmacologists

Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists

~ 600 members
https://ascept.org/



The world of pharmacology



Rang and Dale 2012, Chapter 1

The world of pharmacology
employment

Research
- University
- Pharmaceutical Industry
- Biotech Industry

Academia

Medicine (Clinical Pharmacologists)

Sales Representatives
- equipment, medicines, drugs

Other
- ex TGA, Government Committees, Boards

What is a drug?

a chemical substance of known structure, other than a nutrient or an essential dietary ingredient, which, when administered to a living organism, produces a biological effect*

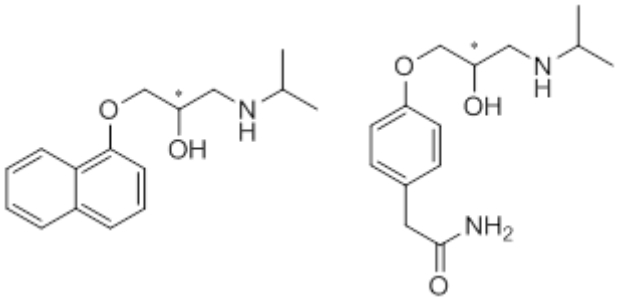
**The definition not perfect since* there are a number of essential dietary constituents, such as iron and various vitamins, that are used as medicines.

Rang and Dale 2012, Chapter 1

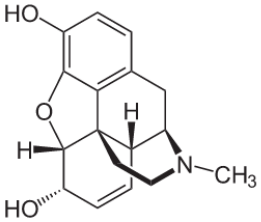
What is a drug?

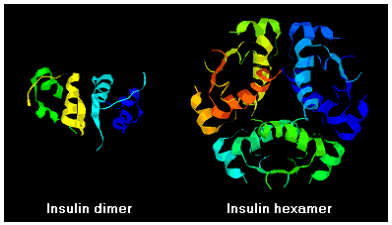
A chemical substance of known structure, other than a nutrient or an essential dietary ingredient, which, when administered to a living organism, produces a biological effect

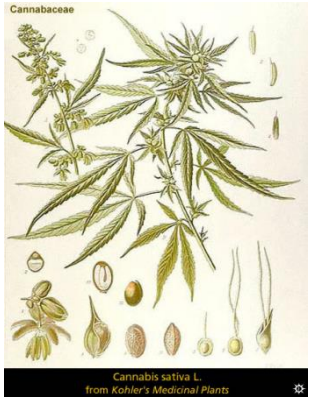
Examples:-



Production	**Example**
Synthetic chemicals	**β-blockers, AT_1 receptor blockers**
chemicals obtained from plants	**morphine, marijuana**
products of genetic engineering	**insulin**





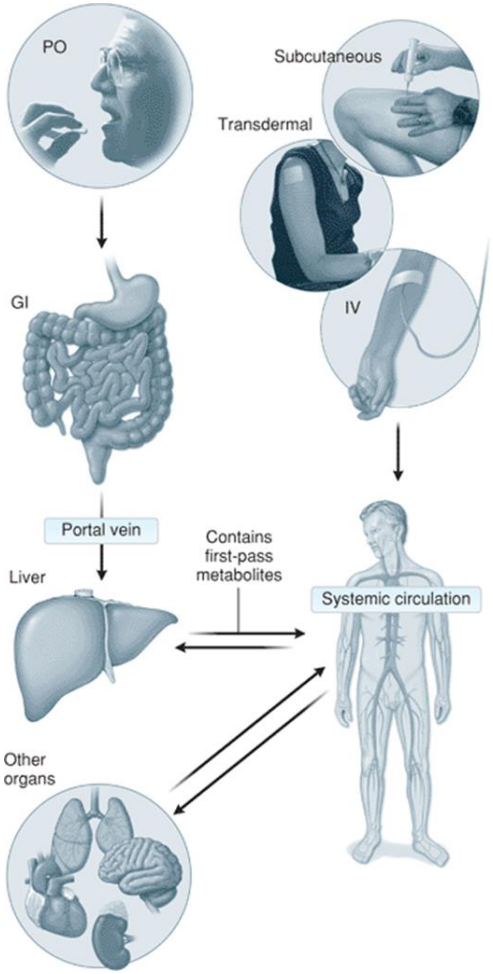


What is a medicine?

A *medicine* is a drug that is administered with the intention of producing a therapeutic effect.

Examples:-

Drug Class	Indication
β-blockers	coronary artery disease, heart failure, hypertension, arrhythmias
ACEI AT1 blockers	hypertension, heart failure
Statins	hypercholesterolaemia
NSAIDs	pain, inflammatory diseases, fever
Anti histamines	allergy



Administration
Orally, parenterally (i.v, i.m, i.p), rectally, inhalation, vaginally, transdermally, dermally, sublingually, …..

Protein targets for drug binding

Four main kinds of regulatory protein are commonly involved as primary drug targets:-

-Receptors ex β-adrenoceptors, AT_1 receptors
-Enzymes ex ACE
-Carrier molecules (transporters) ex SLC6A2, SLC6A4
-Ion Channels ex L-type Ca^{2+} channels

Generally, drugs bind to drug targets to activate them or block/inhibit them. Drug effect is terminated by dissociation away from the drug target, metabolism and excretion

Rang and Dale 2012, Chapter 2,3
IUPHAR/BPS Guide to Pharmacology http://www.guidetopharmacology.org/

Protein targets for drug binding

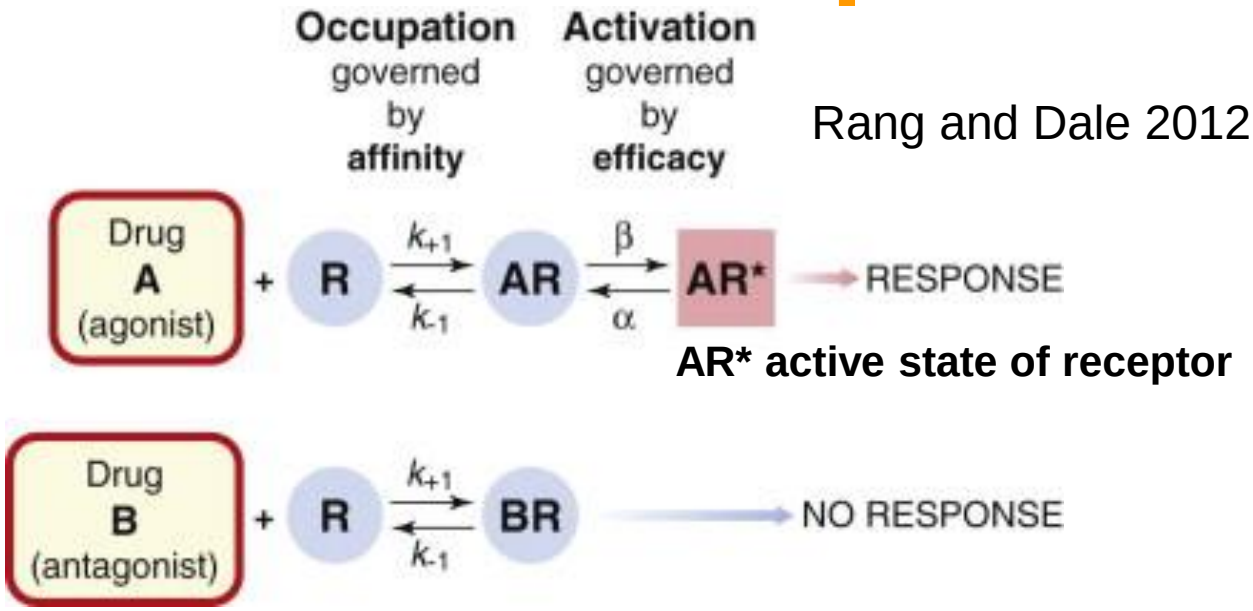
Receptors

β_1-Adrenoceptors in the heart

- (-)-Noradrenaline released from sympathetic nerve terminals
  - activates β_1-adrenoceptors
    - ↑ heart rate
    - ↑ force of contraction
    - ↓ duration of contraction
    - ↓ conduction time

- β-Blockers, ex atenolol, metoprolol, carvedilol, others
  - block β_1- (± β_2) - adrenoceptors
    - ↓ reduce heart rate, reduce force of contraction
  - -indicated for:-
    - -hypertension (atenolol, metoprolol)
    - -heart failure (metoprolol, carvedilol)
    - -arrhythmias (atenolol, metoprolol, carvedilol)

How drugs act
General Principles



Rang and Dale 2012, Chapter 2

<u>Agonist</u>

Ligand A is an agonist, because when it is bound, the receptor (R) tends to become activated

<u>Antagonist (blocker)</u>

Ligand B is an antagonist, because binding does not lead to activation.

The rate constants k_{+1} , k_{-1} , α and β for the binding and activation steps vary between drugs. For an antagonist, which does not activate the receptor, β = 0.

How drugs act
General Principles

Affinity

The ability of a drug to bind to a receptor is determined by the affinity of the drug for the receptor

- -The affinity of the drug is a property of the drug receptor interaction
- -The occupancy of the receptor is determined by concentration of the drug and affinity
- -Mass action equation describes the simple bi molecular interaction between drug and receptor

$$f = [D]/([D] + K_D)$$

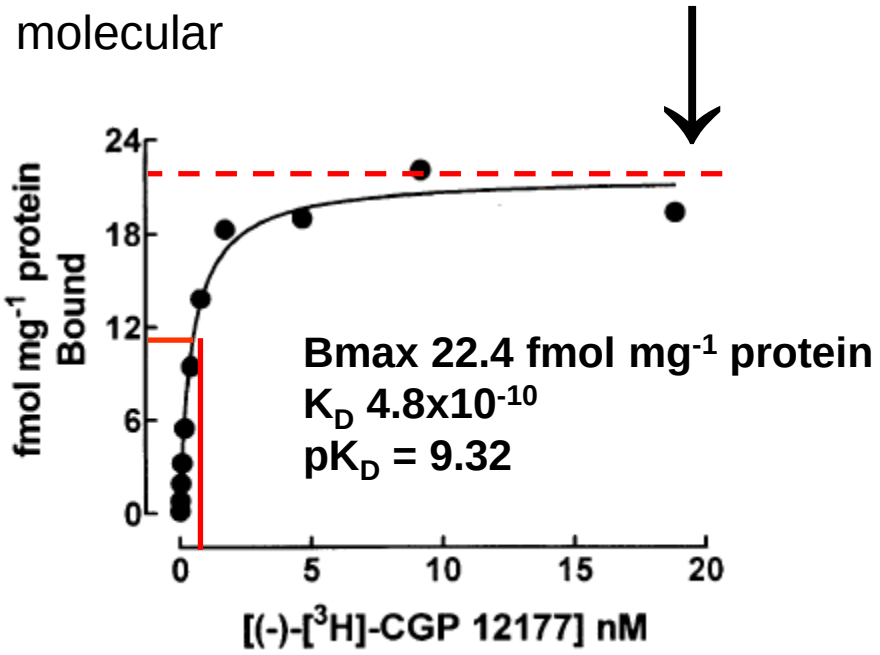
f is the fraction of receptors occupied
[D] is the concentration of drug (Molar)
K_D is the equilibrium dissociation constant of the drug for the receptor

How drugs act
General Principles

Affinity

Mass action equation describes the simple bi molecular interaction between drug and receptor

$$f = [D]/([D] + K_D)$$



Sarsero et al 1999, Br J Pharmacol 128, 1445-1460

f is the fraction of receptors occupied
[D] is the concentration of drug (Molar)
K_D is the equilibrium dissociation constant of the drug for the receptor
- the concentration of drug that occupies 50% of receptors

How drugs act
General Principles

Affinity

Does not provide information about the ability of the drug to produce a response

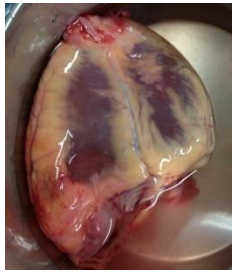
Bioassay system

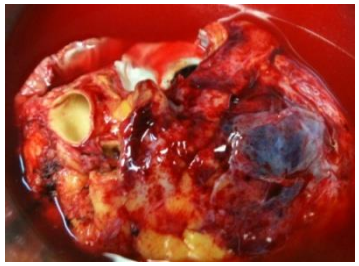
A bioassay system is required to determine the effect of a drug

- in vivo preparations
- isolated tissues
-cell lines

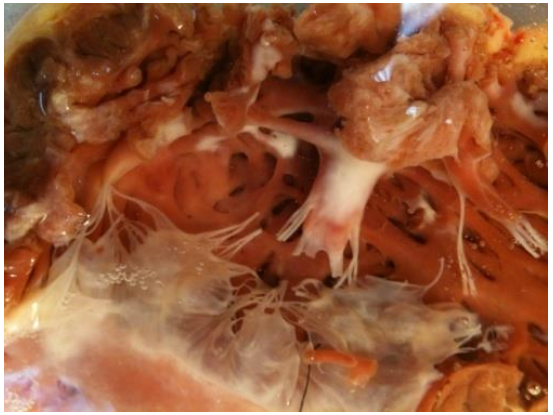
Human Heart

Heart

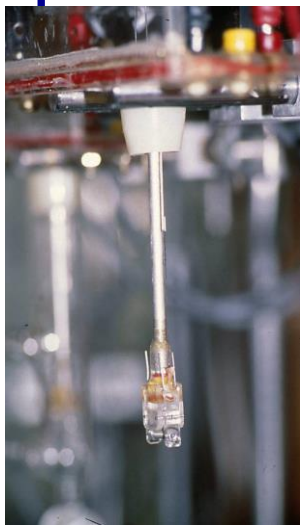




Dissection

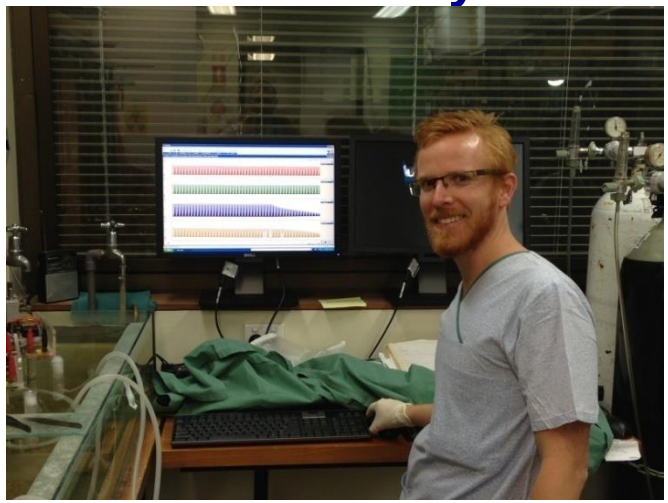


Set-Up and Optimization



Addition of drugs

Digital Data Recording Contractility

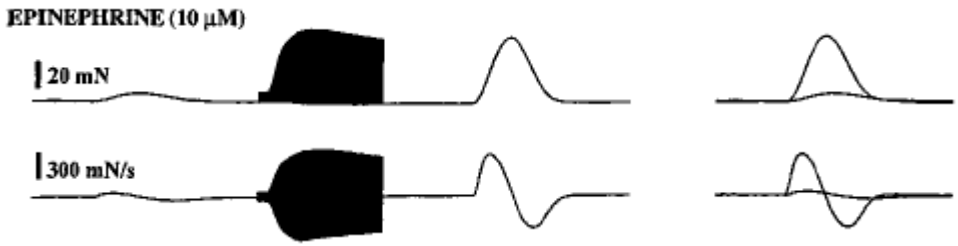


Human heart β-adrenoceptors

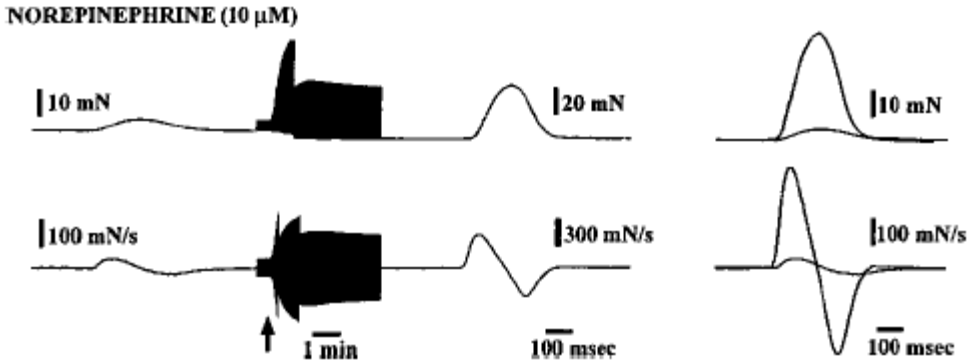
β_2- and β_1-adrenoceptors

Right Ventricular Trabeculae

β_2AR



β_1AR



Both β_2- and β_1-adrenoceptors mediate powerful increases in contractile force and hastening of relaxation

Kaumann et al 1999 Circulation 99, 65-72
Molenaar et al 2000 Circulation 102, 1814-1821

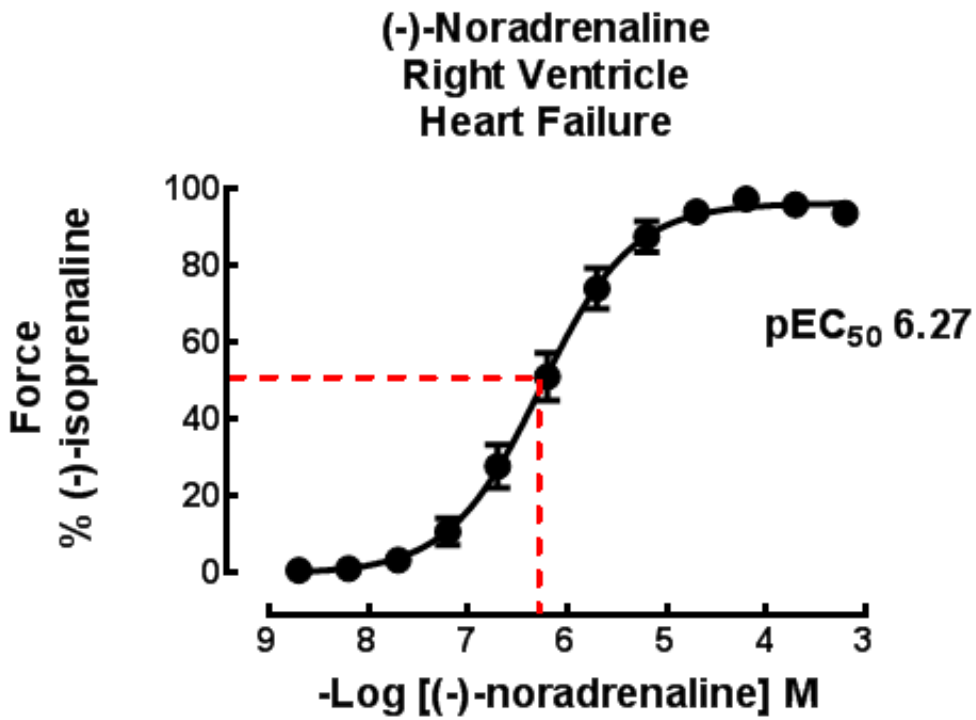
How drugs act
General Principles

Potency

-EC_{50} is the concentration of drug that causes 50% of the maximum effect of the drug

pEC_{50} is $-\log EC_{50}$

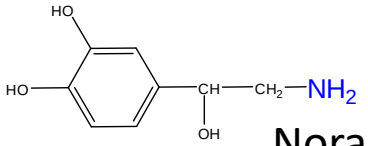
<u>Experiment</u>
pEC_{50} = 6.27
EC_{50} = 540 nM



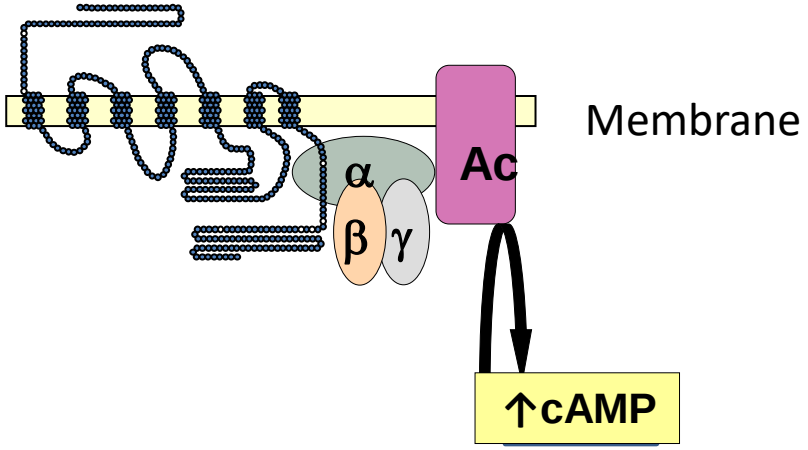
Molenaar lab data

Human heart

$\beta_{1,2}$ AR→Gsα → Ac → cAMP pathway

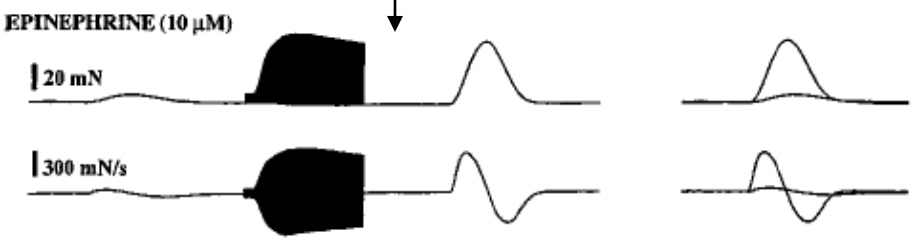


Noradrenaline
Adrenaline



The ability of (-)-noradrenaline and (-)-adrenaline to cause cardistimulant effects is determined by:-

-concentration
-affinity
-efficacy



Kaumann et al 1999 Circulation 99, 65-72
Molenaar et al 2000 Circulation 102, 1814-1821
Molenaar et al 2007 Naunyn-Schmiedeberg's Arch Pharmacol 2007, 375, 11-28

How drugs act
General Principles

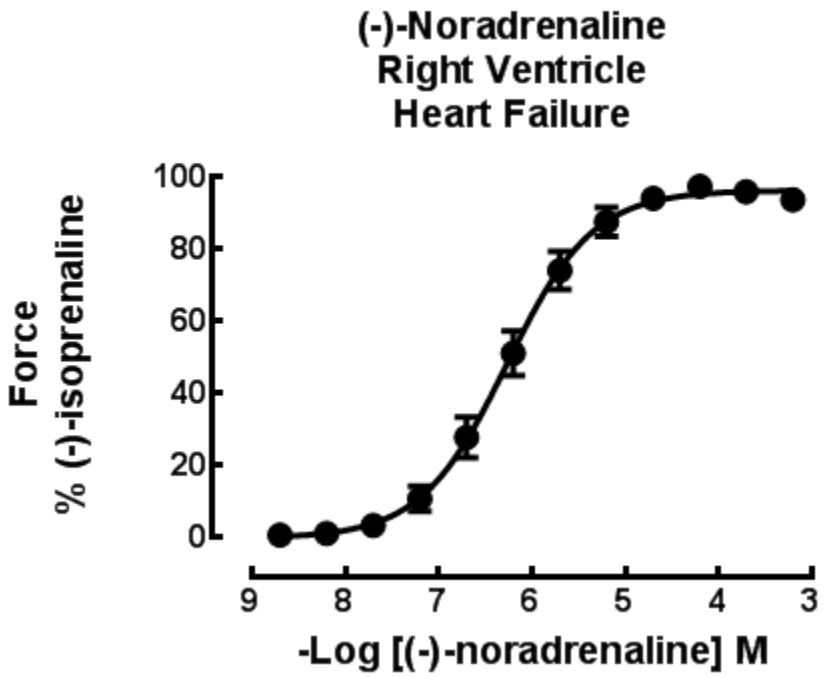
Full Agonist

A ‘full agonist’ is an agonist that produces a ‘maximal’ effect with respect to that receptor

A full agonist can produce a maximal effect by occupying less than 100 % receptors

(-)-Isoprenaline is a full agonist with respect to β_1- , β_2- and β_3-adrenoceptors

(-)-Noradrenaline is a full agonist at β_1-adrenoceptors in human right ventricle



How drugs act
General Principles

Full Agonist

A ‘full agonist’ is an agonist that produces a ‘maximal’ effect with respect to that receptor

A full agonist can produce a maximal effect by occupying less than 100 % receptors

- the mechanism linking the response to receptor occupancy has a substantial receptor capacity
-the system has ‘spare receptors’ or a ‘receptor reserve’

How drugs act
General Principles

Partial Agonist

A ‘partial agonist’ produces a ‘sub-maximal’ effect with respect to that receptor

Submaximal effect produced while occupying 100 % receptors

For a partial agonist, affinity ~ pEC_{50}

(-)-Isoprenaline is a full agonist with respect to β_1- and β_2-adrenoceptors

(-)-CGP 12177 and cyanopindolol are ‘partial agonists’ at β_3-adrenoceptors in rat colon

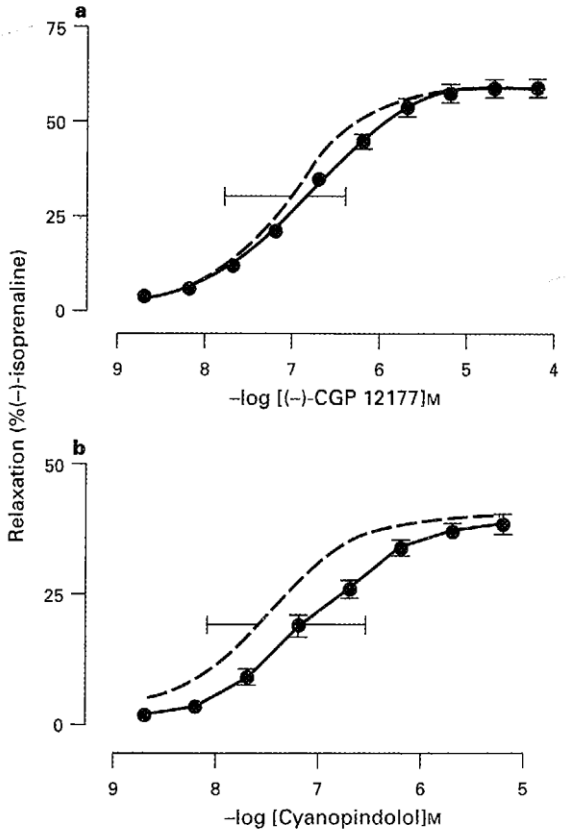


Figure 7 Relaxant responses in colon of (−)-CGP 12177 (a) and cyanopindolol (b) in the presence of 200 nM (−)-propranolol (●). Responses are expressed as a percentage of relaxation caused by a maximal concentration of (−)-isoprenaline. Also shown is receptor occupancy (broken lines) for each partial agonist. Horizontal error bars are standard deviation at the pK_p (receptor occupancy) and pD_2, vertical error bars are ±s.e.mean, $n = 12$ (cyanopindolol) and 36 ((−)-CGP 12177) tissues.

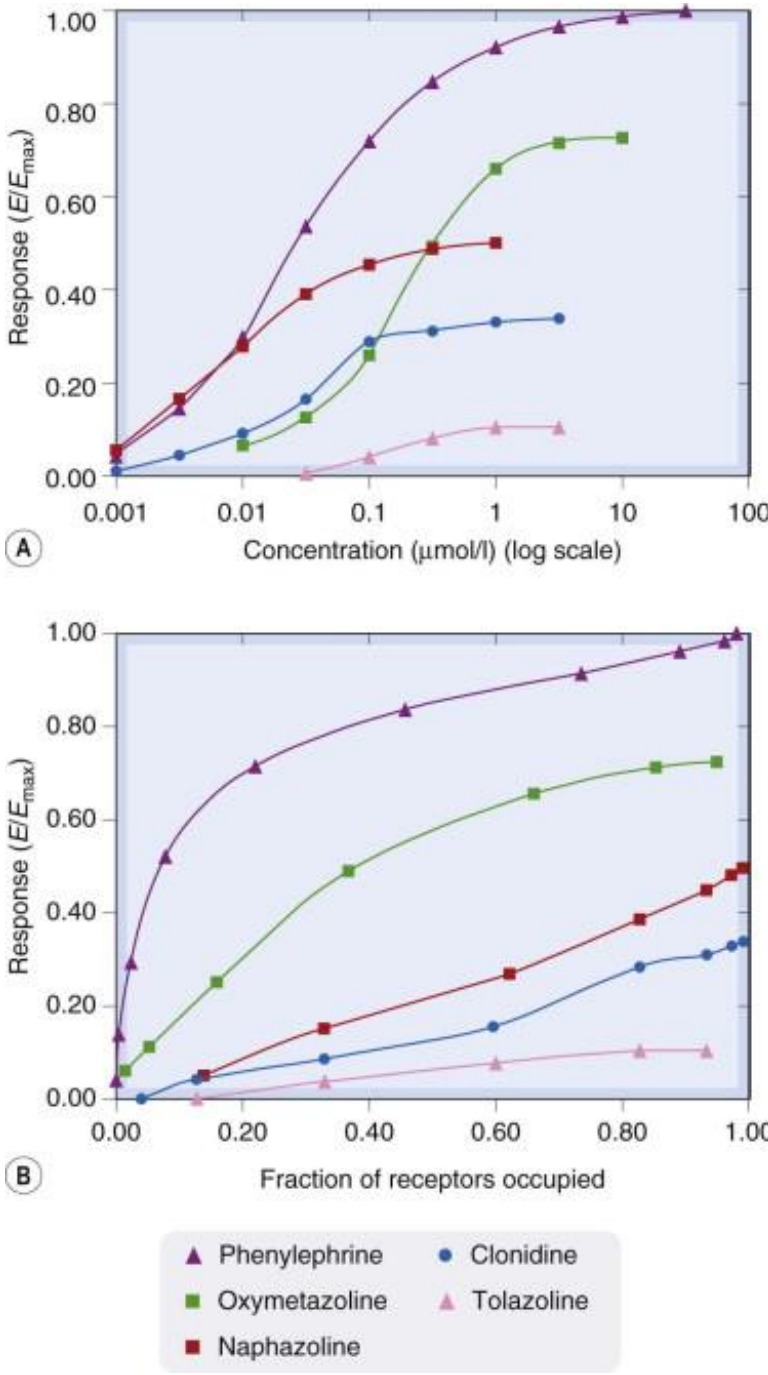
Kaumann and Molenaar 1996, 118, 2085-2098

How drugs act

General Principles

Partial Agonist and efficacy (1)

[**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 (see Ch. 14). In these experiments, receptor occupancy was not measured directly, but was calculated from pharmacological estimates of the equilibrium constants of the drugs.



How drugs act
General Principles

Efficacy

Efficacy is the ‘strength’ of the agonist-receptor complex to evoke a response

Efficacy describes the tendency of the drug-receptor complex to adopt the active (AR*) rather than the resting (AR) state)

Full Agonist Efficacy = 1 (100 %)
Partial Agonist Efficacy > 0, < 1 (> 0 %, < 100 %)
Antagonist Efficacy = 0 (0 %)

Rand and Dale 2012

How drugs act
General Principles

Constitutive Receptor Activation and Inverse Agonists

Constitutive receptors are ‘active’ in the absence of agonist

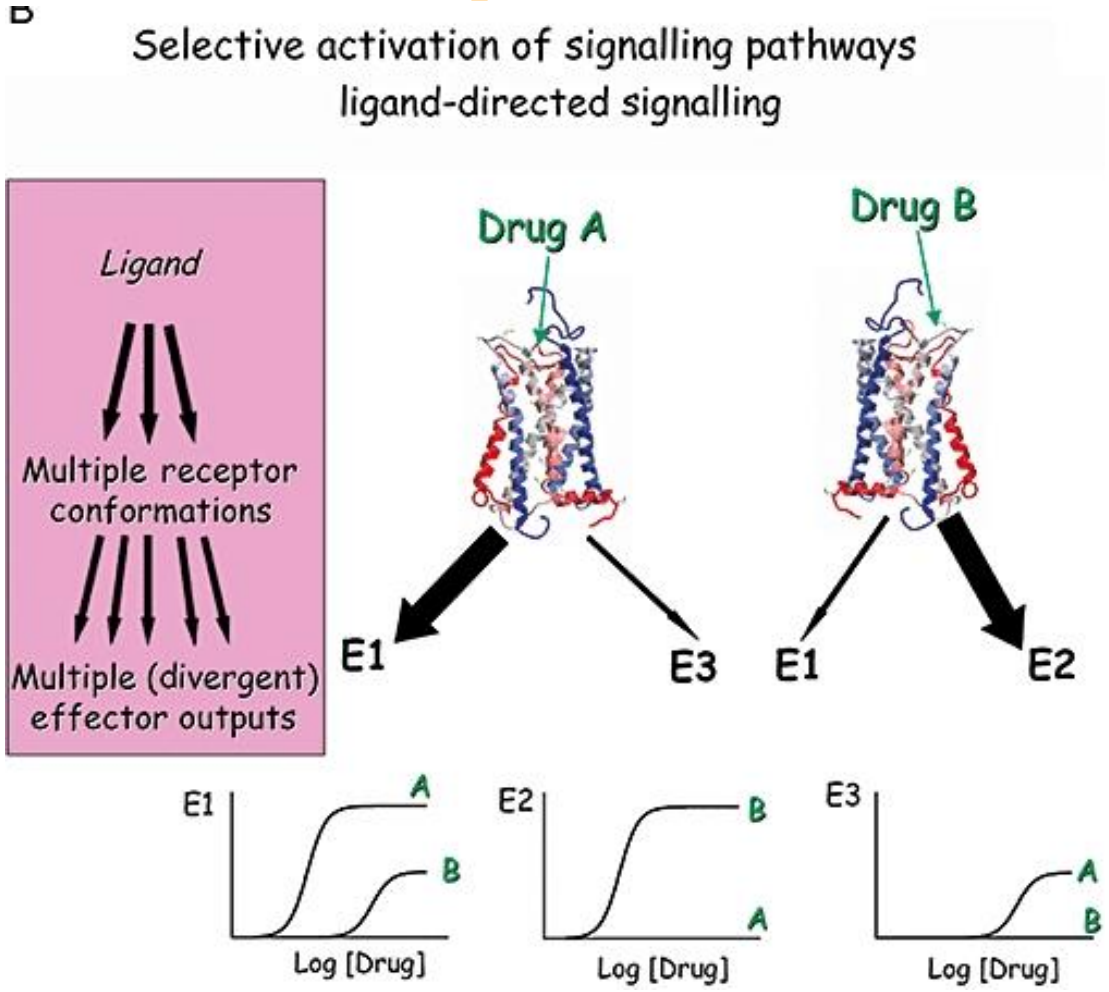
Constitutive receptors are more easily demonstrated when ‘over expressed’

- artificial receptor expression systems
  - -transgenic mice ex Bond et al (1995) Nature 374, 272-276
  - -cell lines

How drugs act
General Principles

Biased Agonism
(Ligand directed signalling)

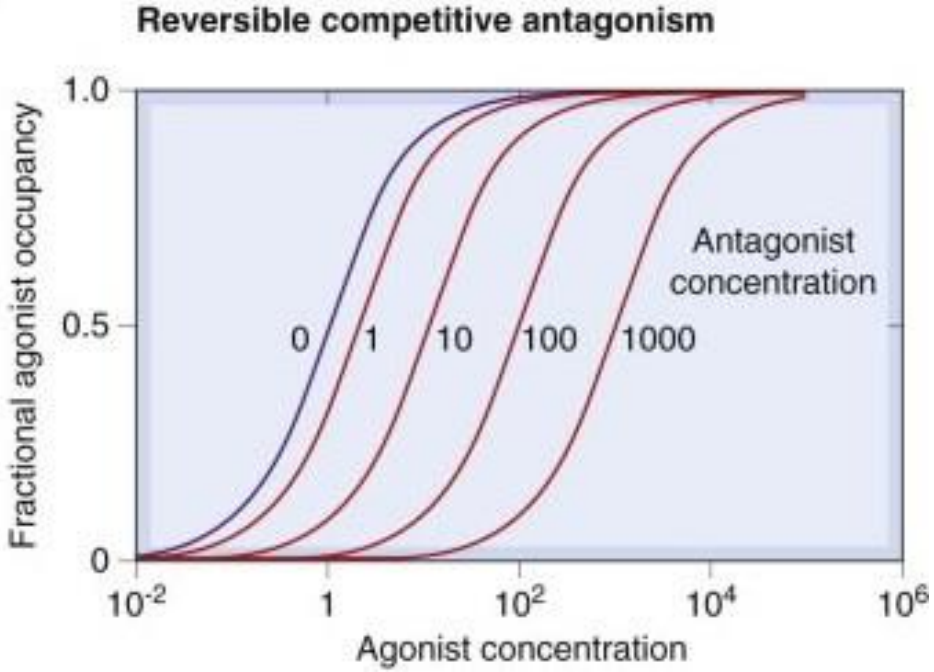
Two drugs, A and B,display reversal of efficacy. For effectors E1 and E3, drug A acts as an agonist, whereas B is a low-efficacy agonist or has no effect. However, for effector E2, drug B is a full agonist, whereas A has no effect. The reversal of efficacy seen for E1 versus E2, and E2 versus E3 strongly suggests ligand-directed signalling



Evans et al (2010) Br J Pharmacol 159:1022-1038

How drugs act
General Principles

Competitive Antagonism

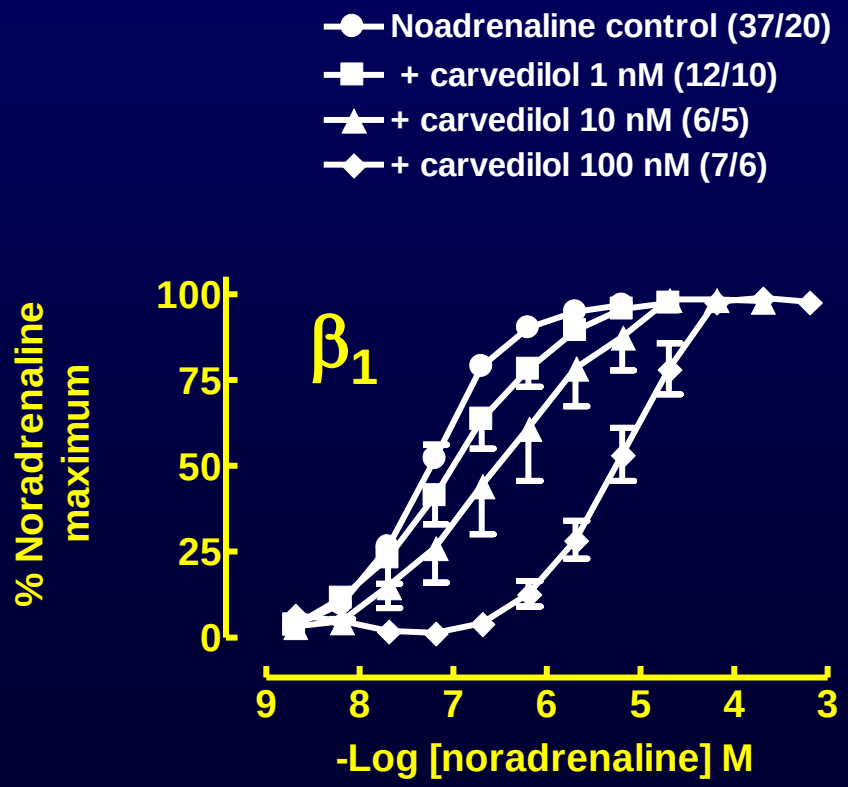


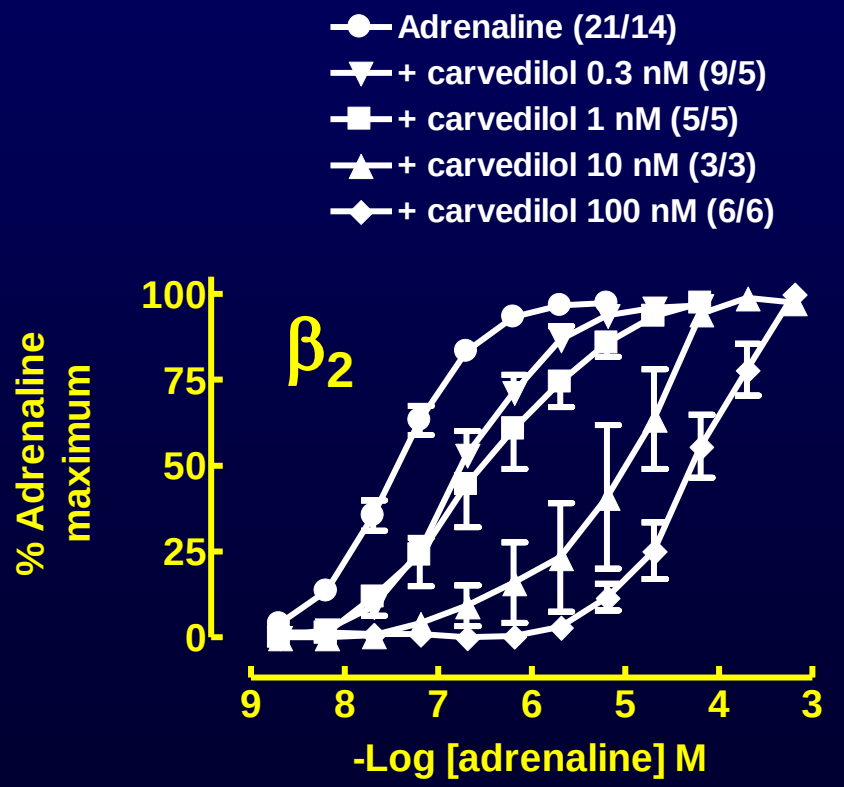
Hypothetical agonist concentration–occupancy curves in the presence of a reversible competitive antagonists. The concentrations are normalised with respect to the equilibrium constants, *K* (i.e. 1.0 corresponds to a concentration equal to *K* and results in 50% occupancy). Note that increasing the agonist concentration overcomes the effect of a reversible antagonist (i.e. the block is surmountable), so that the maximal response is unchanged

Rang and Dale 2012

Competitive blockade of the positive inotropic effects of noradrenaline at β_1-adrenoceptors and adrenaline at β_2-adrenoceptors by carvedilol

Human right atrial trabeculae



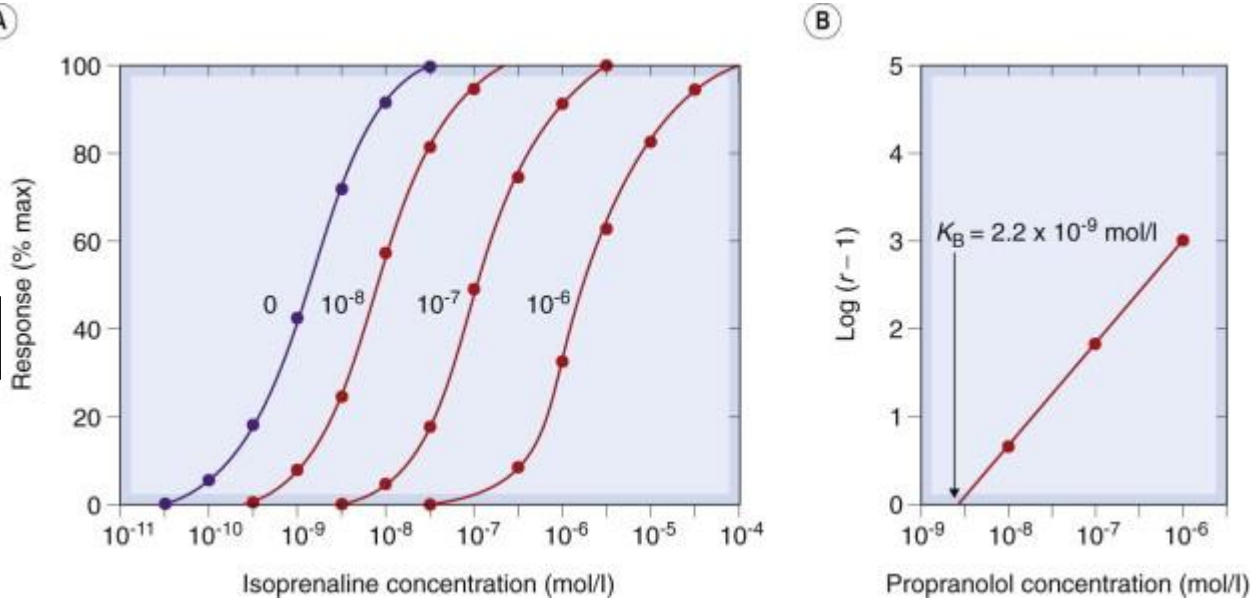


Molenaar et al 2006 Cardiovasc Res 69, 128-139

How drugs act
General Principles

Competitive Antagonism
Schild plot

$$pK_B = \log_{10}(DR-1) - \log_{10}[B]$$



Competitive antagonism of isoprenaline by propranolol measured on isolated guinea pig atria.
[**A**] Concentration–effect curves at various propranolol concentrations (indicated on the curves). Note the progressive shift to the right without a change of slope or maximum. [**B**] Schild plot The equilibrium constant (*K*) for propranolol is given by the abscissal intercept, 2.2×10^{-9} mol/l.

Rang and Dale 2012

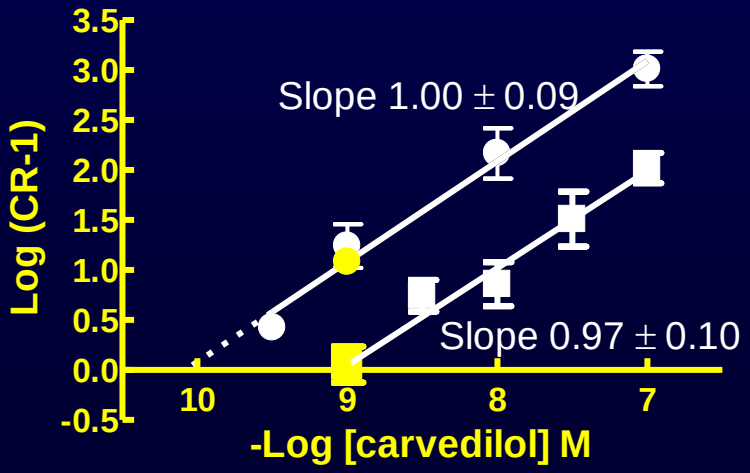
Schild-plots for carvedilol at β_1- and β_2-adrenoceptors

Human right atrial trabeculae

-logK_B vs adrenaline 10.13 ± 0.08 (n = 19 concentration-ratios)

vs noradrenaline 9.02 ± 0.07 (n = 32 concentration-ratios)

Incubation time 240 min unless otherwise indicated



Adrenaline 4 hr

- **atenolol/metoprolol treated patients (n = 14)**
- **non-β-blocker treated patients (n = 4)**

Noradrenaline

- all atenolol/metoprolol treated patients

- **90 min (n = 20)**
- **4 hr (n = 5)**

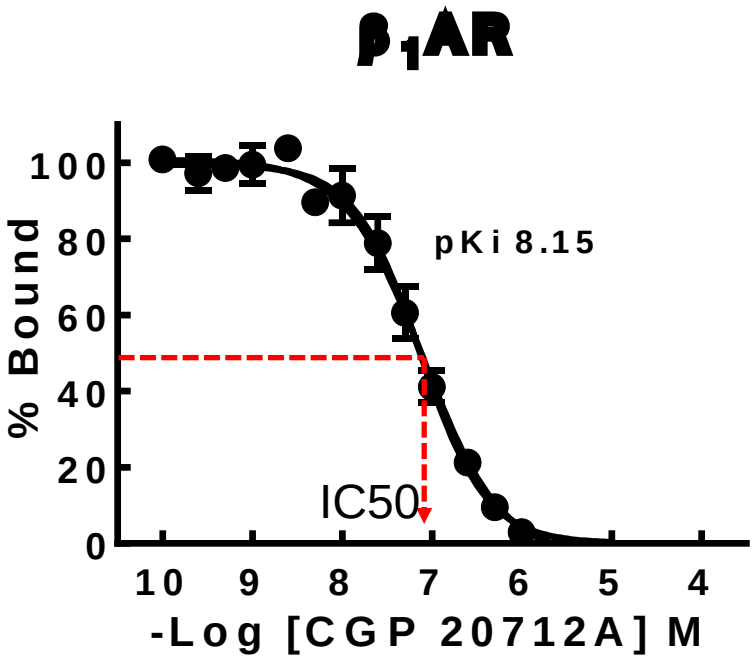
Molenaar et al 2006 Cardiovasc Res 69, 128-139

How drugs act
General Principles

Competition binding experiments

Competition binding experiment between (-)-[^{3}H]CGP 12177 and CGP 20712A

Fixed concentration of radiolabel (-)-[^{3}H]CGP 12177 used. Increasing concentrations of competitor CGP 20712A used



Affinity determined by Cheng and Prussoff Equation

-relates IC_{50} to Ki

$$Ki\ (CGP20712A) = IC_{50}/(1 + [^3HCGP\ 12177]/K_D\ [^3H]CGP\ 12177)$$

Molenaar lab

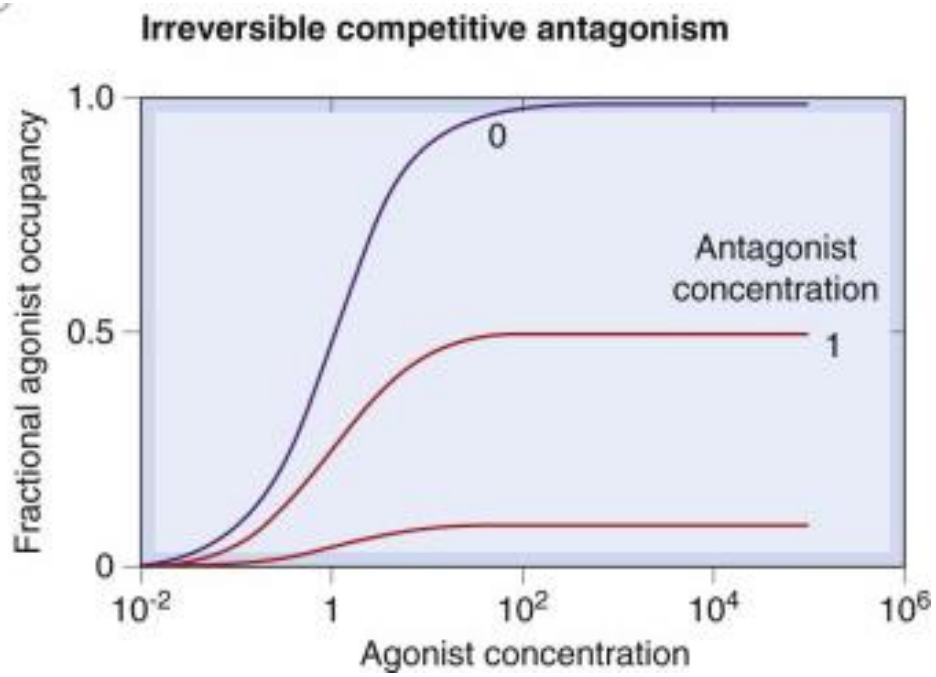
How drugs act
General Principles

Irreversible Antagonism

Can form a covalent bond with the receptor to reduce the number of 'available' receptors.

In the presence of increasing concentrations of 'irreversible antagonist':-

- the effect of the agonist is reduced
- the pEC_{50} ~ affinity of the agonist



Rang and Dale 2012
Furchgott and Bursztyn, 1967