Drug interactions

Receptor – A signal transducer, modifying cell function in response to an extracellular signal.

- Membrane proteins e.g. AChR,
- Cytoplasmic proteins e.g. steroid receptors

Drugs usually demonstrate ***Selectivity*** rather than absolute ***Specificity*** for their targets, and so there are often cross reactivities, which are commonly responsible for adverse reactions

Receptors interact with their ligands via various intermolecular forces including: covalent bonds, ionic bonds, hydrogen bonds, Van der Waals forces and hydrophobic interactions.

Agonist – Affinity and Efficacy
Antagonist – Affinity but no Efficacy
Partial agonist – Affinity and incomplete efficacy. They act as competitive antagonists when added to a full agonist, because they occupy receptors ineffectually preventing the full agonist from binding. They are unable to produce maximal response even when every receptor is occupied

(Efficacy is a measure of the size of response produced by receptor activation)

Differential antagonism = mechanism of differentiating between receptors, when a single agonist is able to produce the same response but using different receptors.

Stereoisomers - +/- isomers often have different potency. Inactive isomers may have affinity, resulting in different effects compared to those without affinity for receptor.

50% response vs 50% occupancy – Upon a ligand binding a receptor there are various downstream signalling events involving cascade effects, which means that 50% response usually doesn't require 50% occupancy – usually lower.

- **EC_{50} =** [D] producing 50% RESPONSE
- **Kdiss =** [D] producing 50% OCCUPANCY
- **These two values are not the same in most cases because in most biological systems you do not require 100% occupancy to achieve maximal response.**

Radioligand binding

- **SPECIFIC BINDING** – Saturable, binds to receptor, high affinity, low capacity
- **NON SPECIFIC BINDING** – Non saturable linear binding to non receptor material, low affinity, high capacity

Spare receptors – More receptors are usually present than is required to achieve maximum effect.

e.g. benzylcholine mustard (BCM) irreversibly alkylates muscarinic receptor. If add BCM, would predict decreased response. However, due to presence of spare receptors, up until a point, addition of BCM has no effect on maximum response, but a greater concentration is required to achieve it. (parallel shift in log conc. graph)

This is useful as most drugs have low affinity – to allow for termination of effect – and so spare receptors (or *receptor reserve*) allows an increased sensitivity

Spare receptors and efficacy – Different agonists at a receptor have a different % of spare receptors. I.e. different agonists must occupy different % total receptor population to produce maximum effect.

[an alternative model for efficacy considers VGIC where conductance through the ion channel is the same for all agonists, but rather the time the channel is open varies]

Cooperativity – occurs where the binding of the first agonist to a receptor that can bind multiple agonists, leads to some change such that the ability to bind consequent agonists is altered – usually increased (e.g. Hb and 4 O_2); GPCR's demonstrate negative cooperativity due to reduced affinity for subsequent agonist upon uncoupling of G protein. Leads to a Hill coefficient >1 or <1. Hill = 1 means either independent binding of individual ligands to receptors with >1 binding site, or 1:1 binding.

RECEPTOR TYPES

Ligand gated ion channels (LGIC)

- Agonist recognition and ion channel are intrinsic to a single protein complex.
- Mediate fast communication between cells
- Nicotinic Ach receptor is the best characterised
- Pentameric complexes typically e.g. nAChR = $\alpha_2\beta\delta\gamma$ or $\alpha_2\beta_3$ (depends on site)
- Ach binds to α-subunit. Hill coefficient ~2 – positive cooperativity
- Each subunit has 4 TMSD – M1-M4
- M2 forms a putative α-helix within membrane. - 5 x M2 line the pore
- LGIC are usually have a charged entrance which has a role in ion selectivity. E.g. nAChR – negatively charged – thus attracting Na and K
- Some receptors differ from this basic pentameric structure – e.g. P_2Y = trimeric; some glutamate receptors are trimeric

G protein coupled receptors (GPCR)

- ~ 7% genome encodes GPCR
- 7 TMSD. N terminus extracellular, C intracellular
- Interact with G protein via 3rd intracellular loop and C tail
- Usually generate 2nd messengers, but not always e.g. βγ unit mAChR directly activates a K^+ channel.

G_s – activates adenylate cyclase which activated PKA

$G_{i/o}$ – inhibits adenylate cyclase
$G_{q/11}$ – activates phospholipase C which clears PIP_2 into DAG (stimulates membrane bound PKC) and IP_3 (releasing ER calcium)

Activation leads G-protein α subunit to exchange its GDP for GTP, which leads to uncoupling from the receptor and disassociation from the βγ subunit. Intrinsic GTPase activity terminates the response, leading to recoupling with a GPCR.

Negative Cooperativity – The binding of agonist to a GPCR leading to GTP exchange, reduces the affinity of the receptor for the same agonist.

- ***Cholera toxin*** – ADP ribosylates α_s inhibiting its GTPase activity and thus leading to sustained activity
- ***Pertussis toxin*** – ADP ribosylates α_i preventing its activation
- ***AlF_4^-*** - mimics the γ-phosphate of GTP and causes persistent activation of G-proteins
- ***Lithium*** – blocks recycling of inositol by blocking phosphatase responsible for converting IP_1 to inositol

β_2 receptor desensitisation

Prolonged exposure to agonist, receptors are prevented from producing their normal effect because their transduction mechanism is uncoupled

ROLE? – prevent excessive stimulation
- recovery after overstimulation
- ability to respond to wide range of stimulus intensities (adaptation)

1- ***Uncoupling GPCR from α-subunit of G protein***

- HETEROLOGOUS: PKA activated as a consequence of increase AC activity, can phosphorylate serines on 3rd cytosolic loop which prevents binding of the alpha subunit. Ability to affect receptors that are not ligand bound, AND non β_2 receptors. This process occurs with low agonist concentrations

- HOMOLOGOUS: upon ligand binding receptor is able to recruit and phosphorylate βARK which recruits β-arrestin that phosphorylates a series or residues preventing G protein coupling. There is agonist dependence for this process, so is important only at high agonist concentration.

2- ***Sequestration*** – internalisation of receptors from surface membrane possibly with increased degradation in lysosomes.

3- ***Downregulation*** – enhanced degradation vs. reduced synthesis, takes place over longer period

Receptor tyrosine kinases

- E.g. Insulin, EGF, PDGF
- 4 domains: ligand binding, transmembrane, catalytic, autophosphorylation
- Agonist binding – conformational change – autophosphorylation
- Dimerisation usually involved – insulin receptor constitutively dimerised, EGF dimerises upon ligand binding.
- Phosphorylated tyrosine (Y) recruits a protein containing an SH2 (src homology type 2) domain and initiates a phosphorylation cascade
- Many responses involve activation/down-regulation of transcription factors and hence effects occur over long periods, but they can also produce more rapid effects

Cytoplasmic (steroid) receptors

- 3 main domains: ligand binding, DNA binding [zinc finger], transcription activating
- usually resides in cytoplasm and is translocated to nucleus following ligand binding
- Oestrogen is exception with receptor constitutively found in nucleus
- Prolonged period of time before effects of receptor binding occur, compared with others e.g. ligand gated – this is because response depends on transcription/translation which takes time

Voltage gated ion channels (VGIC)

- Many have similar basic structure: 4 x 6 TMSD proteins (S1 – S6) [Additional subunits are often associated with this core structure and act as regulatory units]
- S4 is putative voltage sensor (every other residue is charged)
- S4-5 linker in responsible for selectivity of channel
- The channel of Ca and Na VGIC is contributed by a single peptide, whereas K receptor channel comprises 4 separate peptides

Na VGIC and Local anaesthetic

- Na VGIC are the target of LA, e.g Procaine (ester – rapidly degraded) and Lignocaine (amide). Benzocaine is uncharged
- Sensory (small diameter, low myelination and low conduction velocity C and Aδ fibres are most susceptible.
- Large motor neurones Aβ are least affected
- Na channel 3 states: closed ---- transiently open ---- inactivated
- Lignocaine binds preferentially to inactivated state and stabilises this
- Lignocaine is a weak base, hence exists as protonated and un-protonated forms at physiological pH

High pH ←

$$B + H^+ \leftrightarrow BH^+$$

→ low pH

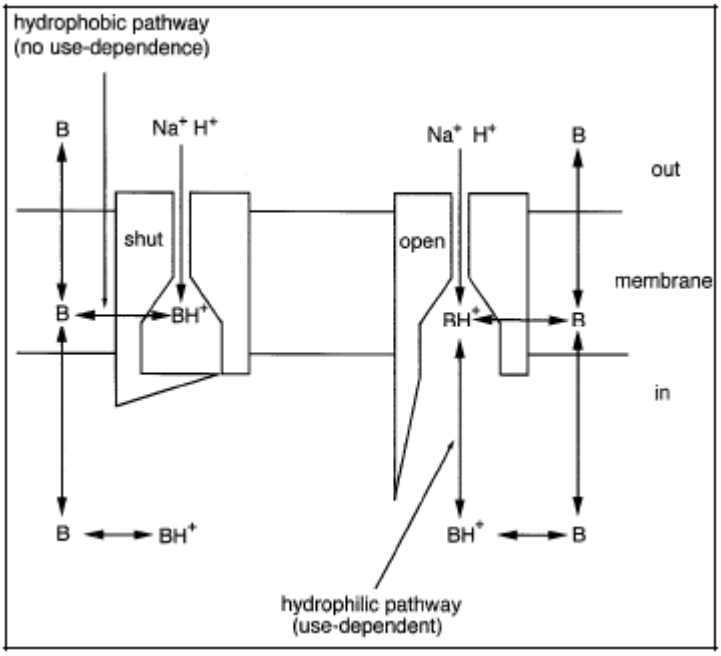
- This helps to explain: pH, voltage and use dependence.

Use dependence

The more frequently the channels are activated the greater the degree of block produced. BUT the frequency at which this use dependence occurs depends on the kinetics of the LA action – e.g. lidocaine is fast on fast off and so only demonstrates use dependence at high frequencies

LA can ONLY enter the cell by crossing the cell membrane, which only the uncharged form can achieve. (Its presence for charged LA's is due to being in equilibrium). LA needs to enter the channel as its binding site is within the channel. Lipophilic LA can enter the channel within the membrane phase however this is slow 'Hydrophobic pathway'. Charged LA can enter when the channel is open, *through the intracellular side of the pore* ('hydrophilic pathway'). Hence the more that the channel is open, i.e., used, the greater the amount of LA that can enter and hence the greater the block achieved.

Quinidine is a slow in slow out LA, and shows use dependence at low levels of stimulation



From MODA lecture handout (J. Edwardson 2009)

pH dependence

For charged LA, when pH extracellularly is acidic, more LA is charged due to shift in equilibrium, therefore, block is less strong, as smaller amount of LA can cross membrane and hence cause block. As LA blocks in charged form, acidic environment intracellularly would be ideal.

For best block, alkaline extracellular environment and acidic intracellular environment. N.B. Infection leads to acidic extracellular environment and hence poorer action of LA's

Voltage dependence

1) A more hyperpolarising prepulse leads to a greater LA block. A more hyperpolarising pulse will lead to more channels being moved from the inactivated state to the closed but openable state, and thus more channels open upon subsequent depolarisation.

2) Depolarisation alters potential across the membrane such that cations are driven to leave the cell. Positively charged LA can then be driven to enter the channels. So the stronger the depolarisation, the greater the block, due to the greater drive for LA to enter the channels in an attempt to leave the cell.

(Tetrodotoxin blocks Na channel from outside and doesn't exhibit use dependence)

Calcium channel blockers

- Dyhydropyridines (nifedipine) [note Bay K 8644 is a dihydropyridine Ca channel agonist]. They affect the mode of the L-type Ca channel - antagonists favouring mode 0 (closed), agonists favouring mode 2 (long openings) [mode 1 = bursts of opening separated by closed intervals]

- Verapamil (binding of DHP reduced)

- Diltiazem (binding of DHP enhanced)

Potassium channels

- Glibenclamide/tolbutamide – a K_{ATP} channel closer – acts on sulphonylurea subunits leading to closure of the K channel, resulting in cellular depolarisation, activating VGCC, leading to Ca influx triggering insulin secretion. Drugs used to treat diabetes

MCQ – True/False – Negatively marked

1. Receptor – ligand interactions

a) Antagonists demonstrate affinity and a low efficacy
b) EC_{50} and K_{diss} are the same value in most cases
c) Partial agonists have low efficacy but are able to achieve maximum response
d) A competitive antagonist reduces the maximum attainable response
e) Therapeutic index = toxic dose / effective dose
f) Specific binding is saturable

2. Receptor types

a) Ligand gated ion channels are hexameric
b) Charge of the receptor entrance is important for ion selectivity
c) GPCR always function via second messengers
d) GPCR demonstrate negative cooperativity
e) Cholera toxin leads to sustained activity of $G\alpha_i$
f) Insulin receptor only dimerises upon ligand binding
g) Receptor tyrosine kinase receptors have autophosphorylation domains
h) Steroid receptors have DNA binding domains
i) Steroid receptors are usually found in cytoplasm in unbound state
j) VGIC have 6 domains each with 4 TMSD

3. Local anaesthetics

a) Sensory neurones are most affected by LA
b) pH has an effect on ALL local anaesthetics
c) Lignocaine demonstrates use dependence and voltage dependence
d) LA act by blocking the activity of Ca^{2+} channels

MCQ – True/False – Negatively marked

1. Receptor – ligand interactions

g) Antagonists demonstrate affinity and a low efficacy **F no efficacy**
h) EC_{50} and K_{diss} are the same value in most cases **F no – see above**
i) Partial agonists have low efficacy but are able to achieve maximum response **F unable to achieve maximum response**
j) A competitive antagonist reduces the maximum attainable response **F no reduction just needs > concentration to achieve**
k) Therapeutic index = toxic dose / effective dose **T**
l) Specific binding is saturable **T**

2. Receptor types

k) Ligand gated ion channels are hexameric **F pentameric**
l) Charge of the receptor entrance is important for ion selectivity **T**
m) GPCR always function via second messengers **F e.g. K channel**
n) GPCR demonstrate negative Cooperativity **T**
o) Cholera toxin leads to sustained activity of $G\alpha_i$ **F G alpha S**
p) Insulin receptor only dimerises upon ligand binding **F constitutively**
q) Receptor tyrosine kinase receptors have autophosphorylation domains **T**
r) Steroid receptors have DNA binding domains **T**
s) Steroid receptors are usually found in cytoplasm in unbound state **T**
t) VGIC have 6 domains each with 4 TMSD **F 4 domains 6 TMSD**

3. Local anaesthetics

e) Sensory neurones are most affected by LA **T**
f) pH has an effect on ALL local anaesthetics **F some are uncharged**
g) Lignocaine demonstrates use dependence and voltage dependence **T**
h) LA act by blocking the activity of Ca^{2+} channels **F - Na**