Receptor Physiology

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Receptors are proteins. According to Dorland's Medical Dictionary, a receptor may be defined as "a molecular structure within a cell or on the surface characterized by (1) selective binding of a specific substance and (2) a specific physiologic effect that accompanies the binding".

Receptors may be located at various sites:

- Incorporated into or associated with the cell membrane (generally for ligands that do not penetrate the cell); and
- Intracellular sites (generally for lipid-soluble ligands that can diffuse through the cell wall, or for intermediary messengers generated within the cell)
  - membranes of intracellular organelles
  - cytosol
  - nucleus

Ligands may be natural or synthetic, and can be grouped into different efficacy classes:

- *full agonists* are capable of maximal receptor stimulation
- *partial agonists* are unable to elicit full activity even at saturating concentrations
- *antagonists* have no effect on signaling activity but can prevent other ligands from binding to the receptor
- *inverse agonists* reduce the level of basal or constitutive activity below that of the unbound receptor (i.e. are able to exert an effect opposite to that of the ligand)

The interaction of ligands with receptors is an essential element of cellular communication and key in bringing about changes in cell function. As anaesthetists, we use drugs on a daily basis that interact with receptors, and an understanding of receptor physiology is essential knowledge.
It is beyond the scope of this talk to mention all the currently known receptors, but I have tried to keep it relevant to anaesthesia by giving some clinical examples in each broad group.

Classification of receptors:

1. Ion transport proteins
2. Metabotropic or G-protein coupled receptors
3. Catalytic receptors
4. Intracellular receptors

1. **Ion transport proteins** can be broadly divided into 2 classes, the ion channels and carrier-type transporters, most (but not all) of which possess several transmembrane-spanning domains that create a pore through which ions pass.

   a. Ion channels

   Generally, ion channels facilitate rapid trans-membrane translocation of ions down their concentration and electrical gradients with little or no energy expenditure, i.e. ion movement is passive and usually fast over a short time period. Whether the channel is open ("gated") or closed depends on extrinsic factors such as changes in membrane potential (voltage-gated) or the binding of small regulatory molecules (ligand-gated).

   Clinical importance of ligand-gated ion channels:
   Many anaesthetic drugs act on ligand-gated ion channels!

   - Nicotinic acetylcholine receptors (nAchR)
     - Pentameric ligand-gated ion channels opened by Ach and nicotine

- *Ach* is the endogenous neurotransmitter at these receptors
- *Nicotine* is a potentially toxic alkaloid derivative of tobacco. It mimics certain actions of Ach and was used to investigate the physiology of the ANS; at low doses being stimulatory at nAchRs.

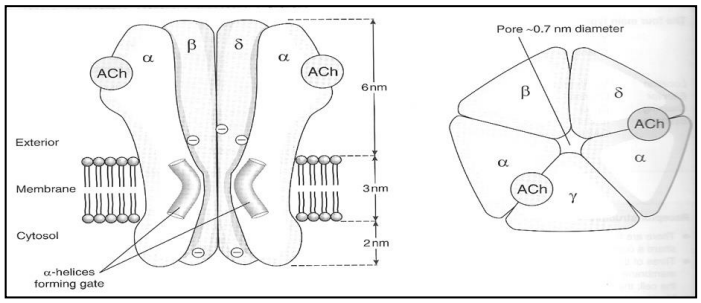


Figure 1: Schematic diagram of the nicotinic acetylcholine receptor

- Location of nAchR:
  - At the neuromuscular junction of skeletal muscle
  - On postganglionic neurons at the ganglion of the autonomic nervous system
- Composed of 5 subunits (2 $\boldsymbol{\alpha}$, and one β, δ and $\boldsymbol{\varepsilon}$ *or* $\boldsymbol{\gamma}$) which span the entire membrane and create a central pore
- Ach binding sites are located on the 2 $\boldsymbol{\alpha}$ subunits; both of which need to be bound to Ach to trigger conformational change which 'opens' the pore and allows for the influx of calcium and sodium ions, and the efflux of potassium
- The $\boldsymbol{\alpha}$ subunit is also the binding site of acetylcholine receptor agonists and antagonists

❖ $\boldsymbol{\gamma}$-Aminobutyric acid (GABA) type A receptors
- These receptors are a pentameric collection of 3 different subunits: $\boldsymbol{\alpha}$, β, $\boldsymbol{\gamma}$
- $GABA_A$ receptors mediate fast postsynaptic inhibition
- These ion channels are selectively permeable to chloride. The resultant influx of chloride ions results in hyperpolarization which inhibits action potential firing, and hence decreased excitability
- Apart from binding its endogenous ligand GABA, which is the main inhibitory neurotransmitter in the CNS, the receptor has multiple binding sites which facilitate the action of GABA. Drugs acting here include benzodiazepines (which augment the opening induced by GABA), barbiturates, propofol, etomidate, and possibly inhaled anaesthetic agents. The different anaesthetic agents are thought to interact with different subunits of the receptor.

❖ N-Methyl-D-aspartate (NMDA) receptors
- Named after its potent exogenous agonist
- Ligands: excitatory neurotransmitter glutamate which requires glycine as a co-agonist. Glutamine and glycine bind different subunits of the receptor
- Activation leads to opening of a cation-selective ion channel, resulting in the influx of Na+ and Ca++ and efflux of K+ ions. Also, activation results in an increased response to glutamate by a positive feedback mechanism, thought to lead to a hyper-excitable state ('wind-up') whereby repeated stimuli cause increasing degrees of pain sensation and expansion of sensory neurons involved in pain pathways
  - NMDA receptors can be phosphorylated by the serine/threonine kinases namely protein kinase C (PKC),

PKA and calcium/calmodulin-dependent protein kinase II, as well as tyrosine kinases
- In general, phosphorylation *enhances* NMDA receptor function

- Exhibits a voltage-dependent block by magnesium ions
- Antagonist: Ketamine

❖ 5-HT_3 receptors
- 5-hydroxytryptamine or *serotonin* is widespread throughout the body
- The 5-HT_3 receptor is the only of the 7 families of serotonin receptors which is an ion-gated channel (the rest are all G-protein coupled receptors)
- Activation results in excitatory effects mediated via ion channels which control the influx of Na^+ and K^+ ions
- Receptors are located in the CNS and PNS. Those located in the area postrema/vomiting centre in the medulla play an important role in nausea and vomiting
  - MOA of antagonists such as ondansetron and granisetron

Clinical significance of voltage-gated ion channels:

❖ Voltage-gated channels include Na+ channels and Cl^- channels
- Local anaesthetics work by blocking voltage-gated Na+ channels

b. Transporters

Transporters include exchangers, co-transporters, and ATP-driven ion pumps. Functionally, transporters are excitable membrane proteins that, after relatively selective binding of transporter ions, undergo conformational changes to allow physical movement of ions across the membrane. Transporters facilitate active transport of certain ions against their electrochemical gradients, thus establishing and maintaining transmembrane electrochemical gradients.

Primary active transporters are dependent on the hydrolysis of ATP to ADP by ATP-ase. ATPase-coupled ion pumps are typified by the Na^+-K^+-ATPase which actively transports three Na^+ extracellularly against its concentration gradient in exchange for two K^+.

Na^+-K^+-ATPase is a heterodimer made up of an α subunit and a β subunit. There are 3 α subunits ($\alpha_{1,2,3}$) and 3 β subunits described. α_1 is found in most cellular membranes; α_2 in muscle, heart, adipose tissue and brain; and α_3 in heart and brain. β_1 is found in most tissues, but *absent* astrocytes, vestibular cells and glycolytic fast-twitch muscles which only contain β_2 subunits.
The β subunit is a glycoprotein with a single membrane-spanning domain and 3 extracellular glycosylation sites. The α subunit is where the ion transport occurs; it is thought to span the cell membrane 10 times and when Na^+ binds to the α subunit, ATP also binds and is converted to ADP. The phosphate generated binds to the phosphorylation site on the α subunit, and this causes a conformational change in the protein with Na^+ extrusion into the ECF. K^+ then binds extracellularly which dephosphorylates the α subunit, and as it returns to its previous conformation, K^+ is released into the cytoplasm.

Clinical importance of Na^+-K^+-ATPase

❖ Drugs acting on these ion pumps alter the resting membrane potential
- Digitalis
  - Inhibits Na^+-K^+-ATPase which has particular importance in the heart where the Na^+-K^+ exchange is replaced by Na^+-Ca^{++}

exchange. This results in increased intracellular calcium which increases myocardial contractility

Secondary active transporters couple movement of one ion against its electrochemical gradient to the movement of another down its electrochemical gradient.
Examples of secondary active transporters are the Na^+-K^+-$2Cl^-$ co-transporter and Na^+/H^+ exchanger.

2. Metabotropic or G-protein coupled receptors (GPCRs)

GPCRs are the largest family of membrane receptors and mediate most cellular responses to hormones, ions, photons, neurotransmitters and other stimuli. Approximately 40-50% of drugs on the market target GPCRs!

Simplistically speaking, these membrane-bound proteins have a serpentine structure and traverse the cell membrane 7 times with alternating intracellular and extracellular loop regions. The binding of a ligand to the extra-cellular side results in activation of a G-protein on the cytosolic side, which in turn activates intermediate messengers, to bring about an often amplified intra-cellular change. Signal amplification occurs as a result of the intra-cellular messenger potentially being reused after the initial stimulus. The extracellular surfaces and ligand-binding sites have significant structural divergence, and a wide variety of ligand-binding "modes" will have quite different effects, from full or partial agonism through to antagonism.

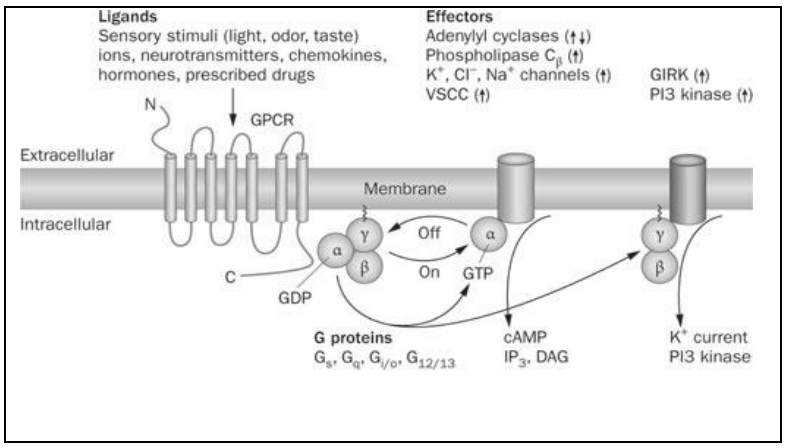


Figure 2: schematic illustration of the effects of GPCRs

The larger metabotropic G-protein coupled receptors are heterotrimeric having three subunits: $\boldsymbol{\alpha}$, β & $\boldsymbol{\gamma}$. In the inactive form, the $\boldsymbol{\alpha}$-subunit is bound to GDP. When a ligand binds and activates the receptor, GDP is exchanged for GTP. The $\boldsymbol{\alpha}$-GTP subunit dissociates from the β$\boldsymbol{\gamma}$ dimer and either activates or inhibits an effector protein (most commonly adenyl cyclase or phospholipase C). Thereafter the $\boldsymbol{\alpha}$-subunit acts as GTPase and regenerates the inactive $\boldsymbol{\alpha}$-GDP subunit. $\boldsymbol{\alpha}$-GDP and the β$\boldsymbol{\gamma}$ dimer recombine to form the heterotrimeric receptor complex and the process may start over. The β$\boldsymbol{\gamma}$ dimer also has the potential to exert an effect in certain scenarios such as activating G-protein-regulated inwardly rectifying potassium (GIRK) channels, and plays a role in anchoring regulatory kinases to the cell membrane. Apart from activating potassium channels, intracellular G-protein subunits may also mediate calcium, sodium and chloride channel activity in certain tissues.
Now, just as you're thinking this all makes perfect sense, please remain aware of the fact that these G-proteins relay signals from over a thousand ligands, and the effects within the cell are widely varied. They are not a simple "two-state switch", and many ligands may even activate more than one class of GPCRs with differing effects.
Also to be considered is the concept of desensitization, which can be defined as "waning of physiologic responsiveness to a drug over time". Here stimulation of receptor pathways leads to phosphorylation of specific regions of the receptor (due to the activation of kinases such as protein kinase A, G-protein coupled receptor kinase or protein kinase C) which prevent further receptor and/or second messenger activity. Phosphorylation by a G-protein coupled receptor kinase may also result in coupling to arrestin. Arrestin is a signaling and regulatory protein that

promotes the activation of extracellular signal-regulated kinases (ERK), prevents the activation of G-proteins, and promotes the internalization of receptors through clathrin-coated pits. Acute desensitization is termed tachyphylaxis.

A host of different sub-classes of G-proteins exist, 4 of which (determined by the α-subunit) will be discussed here, namely G_s, G_i, G_K and G_q.

i. **<u>G_s: stimulate adenyl cyclase (AC)</u>** resulting in increases in cyclic adenosine 3',5'-monophosphate (cAMP). cAMP exerts the effects of protein synthesis, gene activation or alterations in permeability through the stimulation of the R unit of protein kinase A and the phosphorylation of proteins involved in muscle contraction.

cAMP is broken down by phosphodiesterases (this should turn on a little 'pharmacology light bulb' in your head regarding actions and effects of the phosphodiesterase inhibitors!).

<u>Clinical importance of G_s-proteins:</u>

- β-adrenergic receptors (β_1, β_2, β_3)
  - All act via G_s proteins to increase the cellular level of cAMP which is indirectly responsible for inotropic and electrophysiologic effects
  - β_1 receptors:
    - heart: increase rate and force of cardiac contraction – cAMP activates a specific PKA that phosphorylates several important cardiac ion channels including L-type Ca^{2+} channels, Na^+ channels, voltage-dependent K^+ channels, and Cl^- channels. Ultimately, the increased intracellular calcium enhances inotropy
    - adipose tissue: lipolysis
    - kidney (juxtaglomerular apparatus): renin release
  - β_2 receptors:
    - vascular smooth muscle (sm): relaxation occurs as cAMP-dependent protein kinase phosphorylates myosin light chain kinase
    - bronchial sm; intestinal sm; bladder sphincter: relaxation
    - salivary glands: watery secretion
    - liver: glycogenolysis
    - pancreas: increased insulin & glucagon secretion
    - *<u>To highlight the complexity of the GPCR system</u>: β_2 adrenergic receptors can activate G_i as well as G_s proteins, which differentially regulate AC*
  - β_3 receptors:
    - adipose tissue: lipolysis

- cAMP second messengers are downregulated by specific phosphodiesterase proteins. Phosphodiesterase III inhibitors indirectly increase cAMP levels by inhibiting its breakdown
  - Phosphodiesterase is also inhibited by methylxanthines such as caffeine and theophylline

- The cholera toxin alters the alpha subunit of the G_s protein which inhibits its GTPase activity, resulting in prolonged stimulation of adenylyl cyclase

- 5-HT_4 receptors:
  - located in the CNS, GIT, bladder heart
  - stimulation mainly results in increased GIT motility
  - Pharmacologic agonists include metoclopramide
- Other receptors include DA_1, H_1, H_2, HT_2, glucagon, ACTH, LH, FSH, VIP, GHRH, TRH and prostacyclin

ii. **<u>G_i: inhibits AC</u>** thus reducing intracellular cAMP. G_i also activates phospholipase A_2 which activates the arachidonic acid cascade

Clinical importance of G_i-proteins:

- α_2-adrenergic receptors
  - located in presynaptic adrenergic nerve terminals where they inhibit the release of NA and Ach (negative feedback loop). Also found on platelets, lipocytes and smooth muscle
  - receptors are further subdivided into:
    - α_{2A} : responsible for central regulation of BP, sympathetic activity, pain processing and alertness
    - α_{2B} : cause vasoconstriction
    - α_{2C} : thought to be involved in behavioural responses
  - Pharmacologic agonists include clonidine and dexmedetomidine

- Opioid receptors:
  - Opioid receptor nomenclature is based on their founding anatomical location and pharmacological profile, namely, *m*orphine (*m*u or MOP), *k*etocyclazocine (*k*appa or KOP), and vas *d*eferens (*d*elta or DOP). Each of these receptors is the product of a single gene and are regarded as the 'classical' opioid receptors because they are sensitive to the antagonist naloxone. There is a fourth receptor, the nociception (NOP) receptor whose endogenous ligand is nociception/orphanin FQ (N/OFQ), which is currently classified as a non-opioid member of the family because it is *not* sensitive to naloxone.
  - Most opioid drugs used clinically work through the MOP receptor
  - All four opioid receptor subtypes bind to G_i proteins to reduce the activity of adenylyl cyclase, reducing levels of intracellular cAMP. This also results in closure of voltage-sensitive calcium channels (VSCCs) and stimulation of potassium efflux which effectively hyperpolarizes the cell. The overall effect is reduced neurotransmission and reduced neuronal excitability, as well as inhibition of neurotransmitter release.
  - I would highly recommend a really great article on this topic in BJA Education from 2014 titled *Opioid Receptors* (see references)

- Muscarinic acetylcholine receptors (mAchR) M_2 & M_4
  - Agonists for all subtypes include Ach and muscarine
  - Antagonists vary between subtypes but include atropine, scopolamine, diphenhydramine, ipratropium, chlorpromazine, haloperidol and mamba toxin
  - M_2 receptors:
    - In the CNS, M_2 receptors play an important role in muscarinic agonist-mediated tremor and temperature control
    - In the PNS, receptors in the heart control cardiac myocyte contractility resulting in slowing of the heart rate, reduced contractile force of the atrium, and reduced conduction velocity of the AV node

      - **A subtype of G_i receptors, the G_k class or atrial muscarinic M_2 receptors, is linked to K^+ channels**
      - These receptors were initially thought to occur only in the atria, however it is now known that they also exist in the ventricles but in lower concentrations
      - The more important signaling mechanism than changes in cAMP at the cardiac atrial location is opening of an inwardly rectifying K^+ channel in the plasma membrane. Here the $\beta\gamma$ subunit activates the K^+ channel
      - Cardiac adenosine receptors are also coupled to this channel

- M_4 receptors:
  - located in the CNS where overall effects are inhibitory

❖ $GABA_B$ receptors
- Reduced formation of cAMP after receptor activation ultimately results in inhibition of voltage-gated calcium channels. There is thus reduced neurotransmitter release
  - Pharmacological agonists include baclofen

❖ 5-HT_1 receptors
- act mainly as inhibitory pre-synaptic receptors and result in neural inhibition and vasoconstriction
- subtypes 5-HT_{1A} & 5-HT_{1D}
  - The 5-HT_{1D} receptor agonist, sumatriptan, is used in the treatment of migraine

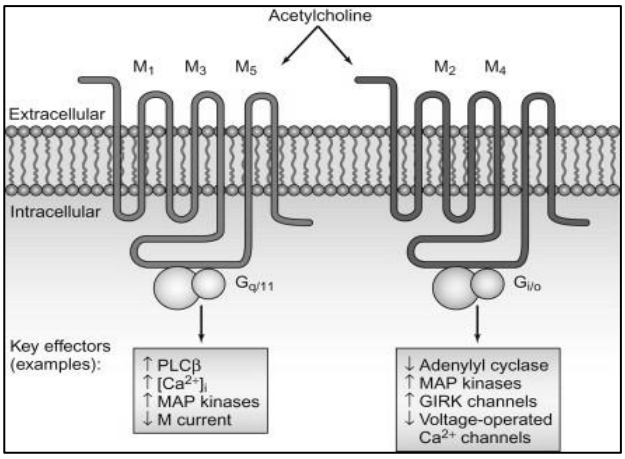
❖ The pertussis toxin interferes with the alpha subunit of G_i

iii. **<u>G_q: activate phospholipase C (PLC)</u>** which controls the breakdown of phosphoinositides to form inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 causes calcium release from the endoplasmic reticulum which then causes membrane hyperpolarization or enzyme release. Calcium then binds calmodulin which further activates kinases. DAG causes activation of protein kinase C which has various cellular effects based on interactions with ion channels, transporters, glycolytic enzymes and transcription factors.

<u>Clinical importance of G_q-proteins:</u>

❖ α_1- adrenergic receptors:
- vascular & bladder sm sphincter: contraction
- iris: contraction
- intestinal sm: relaxation (but sphincter contraction)
- uterus: variable
- salivary glands: viscous secretion
- liver: glycogenolysis
- pancreas: decreases secretion of enzymes, insulin & glucagon

❖ mAchR M_1, M_3 & M_5
- Agonists for all subtypes include Ach and muscarine
- Antagonists vary between subtypes but include atropine, scopolamine, diphenhydramine, ipratropium, chlorpromazine, haloperidol and mamba toxin
- M_1
  - Receptors are located in the autonomic ganglia, salivary glands and stomach
  - They predominate in the CNS, being found in the hippocampus, cerebral cortex, and striatum. The functioning of these receptors are critical for memory processes.
- M_3
  - widespread location and play an important role in the contraction of smooth muscle (airway, ileum, iris, bladder)
  - saliva and insulin secretion
  - Induce emesis
  - Paradoxical vasodilation is due to increased nitric oxide production by vascular endothelial cells
- M_5
  - Located in vascular endothelium (especially cerebral vessels), and CNS



- 5-HT_2 receptors
  - 5-HT_{2A} mediates excitatory effects on platelets and sm

3. <u>Catalytic receptors</u>

<u>Tyrosine kinases</u> are located within the cell membrane. Insulin, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) act through this receptor, as well as many drugs. These receptors have a single membrane-spanning domain with an intracellular tyrosine kinase (TK) domain. Binding of a ligand results in autophosphorylation or cross-phosphorylation of the TK domain. The result is the production of transcription factors in the nucleus which alter gene expression. <u>Serine/threonine kinase receptors</u> have a similar mechanism of action.

<u>Clinical importance of tyrosine kinases:</u>

- Insulin receptor:
  - Found in primary target tissues: liver, muscle, adipose tissue
  - Receptors bind insulin with high specificity and affinity
  - The insulin receptor consists of 2$\boldsymbol{\alpha}$ and 2β subunits which are covalently linked. The $\boldsymbol{\alpha}$ subunit is extracellular and is essentially the 'binding' site. The β subunit spans the membrane and contains a tyrosine kinase. Binding of insulin to the $\boldsymbol{\alpha}$ subunits activates the receptor through a conformational change which bring the β subunits closer together, facilitating mutual phosphorylation of tyrosine residues and activation of tyrosine kinase. Activated tyrosine kinases phosphorylate docking proteins, insulin receptor substrate 1 to 6 (IRS-1 to IRS-6), which results in a complex cascade of further phosphorylation within the cell which represent insulin's second message and results in:
    - translocation of glucose transporters (esp. GLUT 4) to the cell membrane which results in increased glucose uptake
    - increased glycogen synthase activity and increased glycogen formation
    - effects of protein synthesis, lipolysis, lipogenesis
    - activation of transcription factors that enhance cell growth and division
  - Glucocorticoids lower the affinity of insulin receptors for insulin
  - Growth hormone increases the affinity

<u>Guanylyl cyclases:</u> Just as adenylyl cyclase catalyses the formation of cAMP, guanylyl cyclases are a family of enzymes that catalyze the formation of cGMP which then activates cGMP-dependent kinases with a number of physiologic effects. Guanylyl cyclases exist in 2 forms as illustrated by these two examples:

- ❖ Receptors for atrial natriuretic peptide (ANP) have an extracellular amino terminal domain (the receptor portion) and a single transmembrane domain. The cytoplasmic portion has guanylyl cyclase catalytic activity which increases intracellular cGMP.
- ❖ The other form of guanylyl cyclase is intracellular and is activated by nitric oxide (NO) and NO-containing compounds.

4. Intracellular receptors

When certain ligands such as steroid hormones, thyroid hormones or retinoic acid bind receptors within the cell (either within the cytoplasm or the cell nucleus), a receptor-hormone complex is formed. This complex interacts with DNA via zinc fingers and binds to certain genes. This increases the transcription of encoded mRNAs which are in turn translated in the ribosomes. The result is increased production of proteins that alter cell function.

The receptors are mostly similar in structure, having cysteine-rich DNA-binding domain; a ligand-binding domain near the carboxyl terminal; and a nondescript amino terminal region.

Conclusion:

I hope this talk highlights the relevance of this highly complex system with specific reference to anaesthesia and the drugs we use on a daily basis.
Good luck!

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