

# Receptors, agonists and antagonists

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Receptors are defined pharmacologically as proteins that regulate a particular physiological role in response to recognition of a particular molecular shape. A typical example is acetylcholine activating the nicotinic receptor and causing the contraction of skeletal muscle. Receptors are the targets for most drugs

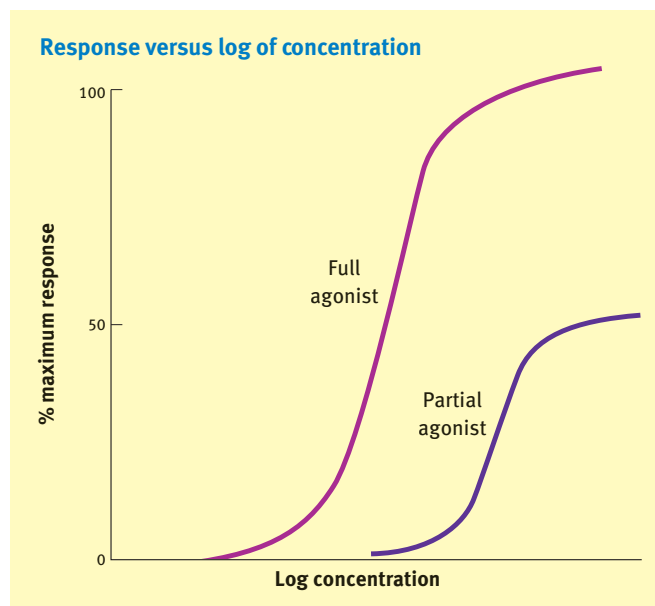
## Agonists and antagonists

The terms agonist (a molecule that binds to a receptor causing activation and resultant cellular changes) and antagonist (a molecule that attenuates the action of an agonist) truly apply only to receptors.

A **full agonist** can produce the largest response that the tissue is capable of giving. The term efficacy has been used to describe the way that agonists can vary in the response they produce even when occupying the same number of receptors. A high-efficacy agonist produces a maximum response even when occupying a small proportion of the available receptors. The magnitude of response to an agonist is usually proportional to the fraction of receptors that are occupied. As the concentration of agonist at its site of action increases so the fraction of occupied receptors rises and, in turn, the magnitude of response rises. The shape of the concentration–response relationship commonly follows a hyperbola as a consequence of this relationship. If this relationship is re-expressed as response versus log of concentration then a sigmoidal curve is usually seen (Figure 1).

A **partial agonist** cannot fully activate the receptors, irrespective of the concentration available. In contrast to a full agonist, a partial agonist cannot exert a maximal response. Partial agonists have lower efficacy and cannot produce a maximal response even when occupying all the receptors (Figure 1).

**Inverse agonists** – the simplest definition is that the compound binds to a receptor but produces the opposite effect from an accepted agonist. The best-described inverse agonists are the  $\beta$ -carboline derivatives at the benzodiazepine receptor. Agonists at this receptor enhance  $\gamma$ -aminobutyric acid (GABA) transmission, inverse agonists reduce GABA transmission and antagonists have no effect on GABA transmission. Flumazenil, a benzodiazepine antagonist, reverses the effects of both agonists and inverse ago-



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nists. However, this classification of individual drugs into agonists, antagonists and inverse agonists may vary with the physiological state of the recipient. Flumazenil can provoke panic attacks in patients with panic disorders, but not in control subjects. This suggests that flumazenil has partial inverse agonist activity in this subgroup of patients. Inverse agonists have been described in many other receptor systems. In addition, the term inverse agonist has been used to describe a ligand that preferentially stabilizes inactive conformations of G-protein-coupled receptors.

A **competitive or surmountable antagonist** binds reversibly to the same receptor as an agonist, but occupies the site without activating the effector mechanism. A competitive antagonist has zero efficacy. Its action may be reversed by increasing the concentration of the agonist. The pharmacological effects seen after the administration of a competitive antagonist depend on the continuing activity of the receptor system affected. For example, the autonomic nervous system is continuously active so adrenoceptor or cholinergic antagonists cause significant changes in function. In contrast, in a fit unstressed person, opioid systems, for example, are seldom active and so an antagonist such as naloxone, when given alone, has no discernible effect. This is not necessarily true in unhealthy individuals.

**Insurmountable antagonists** – once established, no amount of agonist completely reverses the inhibition induced. If the antagonist binds covalently to the receptor, it may be possible to reverse it with competing agonists provided they are administered before the covalent bond has formed. Phenoxybenzamine is an insurmountable antagonist that covalently binds to  $\alpha$ -adrenoceptors. Antagonists that bind to different sites on the receptor causing a change in the conformation of the agonist-binding site (allosteric antagonism) are also insurmountable.

## Receptor classification

Until relatively recently, receptors were classified on the basis of drug agonist effects and compounds that antagonized those effects. Classical experiments by Dale in 1913 showed that adrenaline

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caused vasoconstriction in some vascular beds and vasodilatation in others. The former, but not the latter, effect was blocked by an ergot derivative. This observation was built on by others and allowed for a classification of  $\alpha$ - and  $\beta$ -adrenoceptors. Further agonist and antagonist discoveries allowed greater subdivisions into  $\alpha_1$ ,  $\alpha_2$  and  $\beta_1$ ,  $\beta_2$  and  $\beta_3$ . The developing science of molecular biology demonstrated that receptors could also be classified according to their amino acid sequences and this has led to a profusion of receptors (e.g.  $\alpha_{2A}$ ) though for many of them the functional correlate has not been determined.

### Families of receptors

Four main families of receptors have been revealed by cloning and structural studies (Figure 2).

#### Ligand-gated ion channels

Several neurotransmitters convey their signals by directly opening ion channels and changing the cell membrane potential or ionic composition. They are multi-subunit receptors in which all subunits traverse the membrane. Those most studied appear to be made up of five subunits (which may or may not be of the same type) surrounding an ion channel. Modulation of channel gating by the binding of ligands to a variety of sites on the receptor complex can significantly affect function; the benzodiazepine enhancement of chloride ion transport via the GABA<sub>A</sub> receptor being an important example. Indeed the GABA<sub>A</sub> receptor may have 11 or more modulatory sites, making its pharmacology one of the most complex so far described (see *Anaesthesia and Intensive Care Medicine* 5:8: 252). There may be several variants of each subunit, which, if incorporated into the receptor, can alter affinity for the neurotransmitter and its modulators.

#### G-protein-coupled receptors

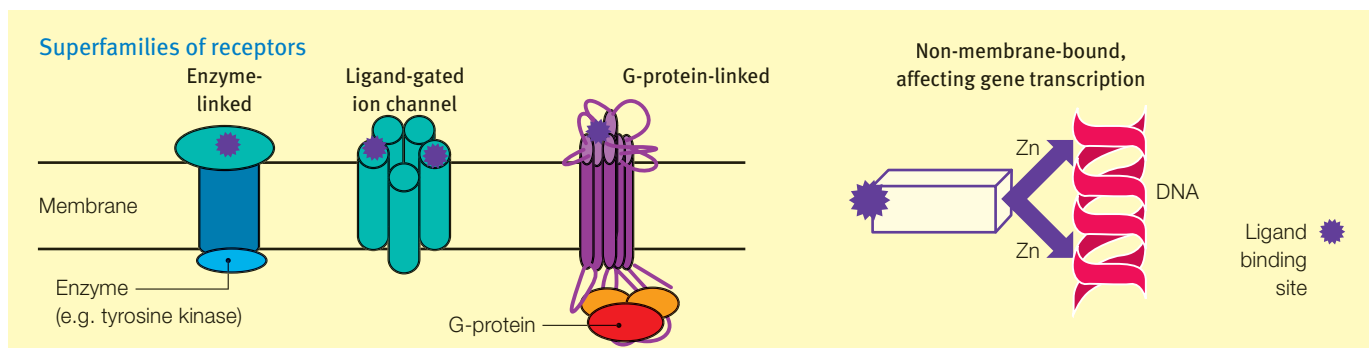
Receptors linked to guanine nucleotide-binding regulatory proteins or G-proteins, make up the largest proportion of the membrane-bound receptors. The receptor consists of a single polypeptide chain with 300–500 amino acids, arranged as seven connected  $\alpha$ -helices that traverse the membrane forming a bundle. The connections between these helices form three extracellular and three intracellular loops. The extracellular amino terminal and the intracellular carboxy terminal vary greatly in sequence and length as does the long third cytoplasmic loop that provides the coupling with the G-proteins. The binding sites for agonists are often buried in pockets within the bundle of  $\alpha$ -helices.

The existence of G-proteins came to light when it was observed that stimulation of second messenger systems such as adenylyl cyclase required not only the receptor agonist but also the presence of guanosine triphosphate (GTP). The G-protein was eventually isolated and found to be a heterotrimer with subunits  $\alpha$ ,  $\beta$  and  $\gamma$ , in order of decreasing molecular weight. In its resting state the G-protein complex is not associated with any particular receptor and is freely diffusible in the plane of the membrane and can interact with several different receptors and effectors. Specificity for a particular agonist–receptor complex, always producing the same end biochemical change in the cell, is guaranteed by the variability of the structure of the G-protein  $\alpha$  subunit. Many variants of the subunits have been described and the number is still growing. Two bacterial toxins (pertussis and cholera toxins) have been particularly useful in helping to distinguish which type of G-protein is involved in a particular situation. Cholera toxin causes persistent activation of the G-protein that stimulates adenylyl cyclase (Gs), thus causing the excessive secretion of fluid from the gastrointestinal epithelium that is characteristic of cholera. Pertussis toxin has no effect on Gs but prevents the actions of other G-proteins such as Gi that inhibits adenylyl cyclase activity. Several G-proteins are inhibited by pertussis toxin and thus its functional effects are less obviously explicable in terms of G-protein inhibition. The agonist–receptor complex causes a conformation change in the intracellular domain to a form that has high affinity for the G-protein. The process of binding the receptor to the G-protein catalyses the disassociation of guanosine diphosphate from the  $\alpha$  subunit of the G-protein and it is exchanged for intracellular GTP. This, in turn, causes the dissociation of the  $\alpha$  subunit from the  $\beta$  and  $\gamma$  subunits. The latter two proteins appear to be involved in anchoring the protein to the membrane. They also have a signal transduction role since some isoenzymes of adenylyl cyclase are modulated by the combined  $\beta$  and  $\gamma$  subunits of the G-protein and not by the  $\alpha$  subunit.

The  $\alpha$  subunit that modulates the action of a given effector is not always adenylyl cyclase (Figure 3). The  $\alpha$  subunit has GTPase activity and converts GTP to GDP followed by reassociation with the other two subunits and the cessation of the signal.

#### Direct enzyme-linked receptors

These receptors possess large intracellular and extracellular domains joined by a single membrane-spanning helix. The extracellular domain provides the binding site for peptide agonists, usually hormones, that regulate differentiation, development and growth. The intracellular domain is often a protein kinase that



### G-protein effectors

#### Adenylyl cyclase

- Stimulated by G<sub>s</sub>
- Inhibited by G<sub>i</sub>
- Produces second messenger cAMP

#### Guanylyl cyclase

- Produces second messenger cGMP

#### Phospholipase C

- Activated by G<sub>q</sub>
- Produces second messengers IP<sub>3</sub> and DAG

#### Phospholipase A<sub>2</sub>

- Produces second messenger arachidonic acid

#### Ion channels

- Particularly Ca<sup>2+</sup>, Na<sup>+</sup> and K<sup>+</sup>

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phosphorylates amino acid residues both of its own cytoplasmic domain and of target proteins. Many phosphorylate tyrosine, and the group has been called the tyrosine kinase-linked receptors, of which receptors for insulin are a typical example. However, some receptors are serine/threonine kinase linked and others are linked to guanylyl cyclase (e.g. the atrial natriuretic peptide receptor). The phosphorylated residues provide binding sites for other intracellular proteins and eventually lead to altered gene transcription.

#### Intracellular receptors affecting gene transcription

These non-membrane-bound receptors may be nuclear (e.g. thyroid hormone receptor), predominantly cytosolic, or form high molecular weight complexes with heat shock proteins (e.g. glucocorticoid receptor). In the latter, the presence of a ligand leads to the dissociation of the receptor from the complex and the movement of the receptor–ligand complex into the nucleus. It is obvious from the intracellular location of these receptors that ligands for these receptors must be lipid soluble and pass into the cell. The binding of the ligand to the receptor results in an uncurling of the receptor protein to reveal the DNA binding domain. The DNA binding domain consists of two ‘zinc fingers’, which contain cysteine residues surrounding a zinc atom. These fingers are believed to wrap around the DNA helix at hormone-responsive elements in the strand. RNA polymerase activity can be modulated and protein production is enhanced or reduced. A well-documented example of this is the increase in lipocortin production and the decrease in COX-2 production induced by glucocorticosteroid hormones. ♦

#### FURTHER READING

Drug targets suite of programmes from the British Pharmacological Society, <http://www.bps.ac.uk>. These computer-assisted learning packages deal with molecular mechanisms of action.

Rang H P, Dale M M, Ritter J M, Moore P K. How drugs act: general principles. In: *Pharmacology*. 5th ed. Edinburgh: Churchill Livingstone, 2003: 7–21.

Rang H P, Dale M M, Ritter J M, Moore P K. How drugs act: molecular aspects. In: *Pharmacology*. 5th ed. Edinburgh: Churchill Livingstone, 2003: 22–50.

## Mechanism of action of general anaesthetic drugs

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General anaesthesia is a loss of sensation with a loss of consciousness. The mechanisms by which drugs can produce this state are uncertain, but recent advances hold out the promise that it may be possible to produce agents with greater selectivity for the wanted effects of general anaesthesia and less selectivity for the unwanted effects, such as cardiovascular and ventilatory depression. Anaesthetic agents have a variety of effects on the lipids and proteins in neuronal membranes and most researchers believe that this is their site of action.

The membrane consists of a phospholipid bilayer with proteins embedded in it (Figure 1). The proteins associated with the membrane may be associated with the internal or external surface of the membrane or embedded in the membrane and they have polar regions in contact with the aqueous media. There are five main classes of membrane proteins including enzymes (e.g. acetylcholinesterase) and neurotransmitter receptors.

The correlation of the oil/gas partition coefficient with anaesthetic potency has been repeatedly confirmed for a range of compounds that have general anaesthetic properties, ranging from the inert gas xenon to complex steroids. The membrane lipid bilayer and the integral proteins both contain hydrophobic sites that could be the target of anaesthetic action.

#### Interactions with the membrane lipid bilayer

The lipid bilayer consists of two rows of closely packed phospholipid heads, with extended tails (Figure 1), which exist in a gel-like state that is highly ordered and where there is little movement. Transition from this state to a more fluid, liquid-like state can occur by a small increase in temperature or the insertion of small molecules, such as anaesthetic gases and vapours, into the bilayer.

There is a positive correlation between the fluidization of the phospholipid heads and anaesthetic potency. It is logical to assume that fluidization would be reversed by increases in pressure. Thus, the well-established pressure reversal of anaesthesia would be consistent with fluidization of the lipid bilayer being relevant to anaesthetic action. While changes in fluidity could cause a change in membrane function that equates with anaesthesia, most scientists have discarded this hypothesis. At anaesthetic concentrations, the fluidization produced by anaesthetic agents is equivalent to that produced by a 1°C increase in temperature. Minor elevations of temperature do not cause anaesthesia; indeed cooling, rather than heating, increases anaesthetic potency. In addition, some alcohols, which cause anaesthesia, do not appear to fluidize lipid bilayers and there is some structural dependency of some molecules with anaesthetic action that is not compatible with a simple fluidization hypothesis.

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