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3 Neurotransmitter Receptors

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THE RECEPTOR CONCEPT

All multicellular living things depend on chemical communication between cells to coordinate cellular behaviour. This communication is mediated by protein receptors. Cells send out messengers in the form of hormones and neurotransmitters. Receptors provide both **sensitivity** and **selectivity** in this system. The idea that there are specific receptors for hormones and drugs was developed by Erlich and Langley at the end of the nineteenth century, while Hill, Clark, Gaddum and Schild were pioneers in developing a quantitative understanding of the action of drugs. At that time, there was no evidence regarding the structural nature of receptors, although it was widely supposed they were proteins.

The value of receptors to higher animals becomes most obvious in considering the functioning of the central nervous system. The integration of sensory input, past experience and inborn instinct by the central nervous system in the generation of appropriate behavioural activity is only possible because of the specialised properties and diversity of neurotransmitter receptors in the nervous system which mediate signalling between neurons. It has long been recognised that a detailed knowledge of the neurotransmitter receptors in the brain is crucial to developing specific therapeutic approaches to correcting unwanted nervous system activity.

The aim of this chapter is to consider the structure, distribution and functional properties of neurotransmitter receptors in the brain in general and discuss the principles of how the action of drugs at these receptors can be studied. (*See relevant Chapters for detail of individual NT receptors.*)

IDENTIFICATION AND CLASSIFICATION OF NEUROTRANSMITTER RECEPTORS

EARLY PHARMACOLOGICAL STUDIES AND THE IMPACT OF MOLECULAR GENETIC TECHNIQUES

Traditionally receptors have been classified according to their pharmacology. Each neurotransmitter acts on its own family of receptors and these receptors show a high degree of *specificity* for their transmitter. Thus, the receptors on which acetylcholine (ACh) works do not respond to glutamate (or any other neurotransmitter) and vice versa. Diversity of neurotransmitter action is provided by the presence of multiple receptor subtypes for each neurotransmitter, all of which still remain specific to that neurotransmitter. This principle is illustrated by the simple observations outlined in

Chapter 1 which showed that since muscarine mimicked some of the actions of ACh (but not all) while nicotine mimicked the other actions of ACh, then ACh probably acted on two distinct types of receptors. The fact that atropine antagonised the muscarinic effects of ACh but not the nicotinic effects, while tubocurarine blocked the nicotinic effects provided firm evidence for this concept. These simple qualitative observations by Langley and others at the beginning of the twentieth century led to the development of more quantitative pharmacological methods that were subsequently used to identify and classify receptors. These methods were based on the use of both (1) agonist and (2) antagonist drugs:

(1) If a series of related chemicals, say noradrenaline, adrenaline, methyladrenaline and isoprenaline, are studied on a range of test responses (e.g. blood pressure, heart rate, pupil size, intestinal motility, etc.) and retain exactly the same order of potency in each test system, then it is likely that there is only one type of receptor for all four of these catecholamines. On the other hand, if, as Ahlquist first found in the 1940s, these compounds give a distinct order of potency in some of the tests, but the reverse (or just a different) order in others, then there must be more than one type of receptor for these agonists.

(3) If one set of these responses can be blocked (antagonised) by a drug that does not affect the other responses (e.g. propranolol blocks the increase in heart rate produced by adrenaline, but not the dilation of the pupil evoked by adrenaline) then this is good evidence that adrenoceptors in the pupil are not the same as those in the heart.

In fact, careful quantitative analysis of the order of activity of the agonists in each test, and of the precise potency of antagonists (see Chapter 5 for quantitative detail) has often successfully indicated, although rarely proved, the presence of subclasses of a receptor type (e.g. different muscarinic receptors). The affinity of receptors for selective antagonists determined using the Schild method was a mainstay of receptor classification throughout the second half of the twentieth century. Thus, a muscarinic receptor can be defined as a receptor with an affinity for atropine of around 1 nM and the M1 subtype of muscarinic receptor can be identified as having an affinity of around 10 nM for the selective antagonist, pirenzepine while muscarinic receptors in the heart (M2 subtype) are much less sensitive to pirenzepine block ($K_B \sim 10^{-7}$ M).

Classification of receptors according to agonist potency can be problematic because agonist potency depends partly on the density of receptors in the tissue and therefore use of selective antagonists has become a mainstay of receptor identification and classification. The development of radioligand binding techniques (see Chapter 5 for principles) provided for the first time a means to measure the density of receptors in a tissue in addition to providing a measure of the affinity of drugs for a receptor and allowed the relative proportion of different receptors in a tissue to be estimated.

These approaches to receptor identification and classification were, of course, pioneered by studies with peripheral systems and isolated tissues. They are more difficult to apply to the CNS, especially in *in vivo* experiments, where responses depend on a complex set of interacting systems and the actual drug concentration at the receptors of interest is rarely known. However, the development of *in vitro* preparations (acute brain slices, 'organotypic' brain slice cultures, tissue-cultured neurons and acutely dissociated neuronal and glial cell preparations) has allowed more quantitative pharmacological techniques to be applied to the action of drugs at neurotransmitter receptors while the development of new recording methods such as patch-clamp

recording has allowed the study of drug action at central neurons to be made at ever more detailed levels.

Today we know not only that there is more than one type of receptor for each neurotransmitter, but we also know a great deal about the structural basis for the differences between receptor subtypes which are due to differences in the amino-acid sequence of the proteins which make up the receptor. How do we know this?

Finding the amino-acid sequence of a receptor protein has been approached in three main ways. The final aim of all three methods is to obtain a cDNA clone coding for the protein since the base sequence of this DNA allows the amino-acid sequence of the protein to be predicted:

(1) From purified receptor protein, obtain partial amino-acid sequence information which will allow molecular biologists to isolate the gene (or genes) coding for the receptor.
(2) cDNA library screening. From a receptor-rich tissue, isolate mRNA and create from this, a cDNA library. The library is then screened by, for example, functional expression in *Xenopus* oocytes or mammalian cell lines, for the proteins coded by the library. If positive expression is obtained, the library is subdivided until a single cDNA clone for the receptor is isolated.
(3) Homology screening. Using oligonucleotide probes based on known receptor sequences, search cDNA libraries for homologous sequences which may code for related receptors. The clones are then isolated and sequenced and used in expression studies to confirm the identity of the receptor.

The first tentative steps towards determining the structure of individual receptors were taken by protein chemists. A high-affinity ligand that binds specifically to the receptor (generally an antagonist) was identified by traditional pharmacological methods and attached to the matrix of an appropriate chromatography column. A tissue source, rich in receptors, is homogenised and the cell membranes disrupted with detergents to bring the membrane bound proteins into solution. This solution is then passed through the affinity column and the receptor of interest will stick to the column hence separating it from all the other proteins in the tissue. The receptor is then eluted from the column using a solution of ligand specific for the receptor. This strategy allowed isolation of the nicotinic acetylcholine receptor from the electric organ of the Californian ray (*Torpedo*). Almost 40% of the protein content of this tissue is ACh receptor. The isolation method used a snake toxin from the venom of the Taiwan banded krait (α-bungarotoxin) as the ligand of the affinity column and the purified receptor was eluted from the column using a high concentration of the competitive antagonist, tubocurarine. Following isolation of the protein, a partial N-terminus amino-acid sequence was obtained and from this sequence, oligonucleotide probes were made which were then used to screen a cDNA library to isolate a clone for the receptor. Since DNA sequencing is much faster than protein sequencing, the DNA sequence of the clone is then used to provide the amino-acid sequence of the receptor.

RECEPTOR MECHANISMS

It is often valuable to classify receptors according to their mechanism of action, because this is intimately related to structure. The neurotransmitter receptors in the brain are of two main types classified according to their structure and mechanism of action:

(1) Ion channel receptors
(2) G-protein-coupled receptors

The ion channel receptors are relatively simple in functional terms because the primary response to receptor activation is generated by the ion channel which is an integral part of the protein. Therefore, no accessory proteins are needed to observe the response to nicotinic AChR activation and the full functioning of the receptor can be observed by isolating and purifying the protein biochemically and reconstituting the protein in an artificial lipid membrane. In contrast, the G-protein-coupled receptors require both G-proteins and those elements such as phospholipase-C illustrated in Fig. 3.1, in order to observe the response to receptor activation (in this case a rise in intracellular calcium concentration resulting from the action of IP_3 on intracellular calcium stores).

Most receptors function as mediators of synaptic transmission between neurons. Figure 3.1 illustrates this for the case of a generic glutamatergic synapse. At this synapse glutamate is released from the presynaptic nerve terminal and acts on two different types of fast ionotropic glutamate receptors embedded in the postsynaptic membrane: AMPA receptors mediate an extremely rapid (within 1 ms) response to glutamate release resulting in a rapid depolarisation of the postsynaptic membrane (EPSP). On a slower time scale, the NMDA receptors mediate a slower EPSP which lasts over 100 ms, is carried partly by calcium ions and is voltage-dependent due to blocking of the NMDA receptor channel by Mg ions at negative membrane potentials. The AMPA receptor provides the depolarisation necessary to relieve the Mg block of the NMDA channel and so the calcium influx through the NMDA channel in effect provides a means to integrate synaptic activity mediated by the fast AMPA receptors. The synapse shown in Fig. 3.1 also illustrates that G-protein-coupled glutamate receptors may be located at the synapse, or perisynaptically and therefore can mediate slow synaptic transmission (on a time scale of 100 ms to seconds) whose characteristics will depend on the particular G-protein which is coupled to the metabotropic receptor. In this case, the receptor is coupled by G_q to phospholipase-C and results in IP_3 and diacylglycerol (DAG) production which in turn regulate intracellular calcium concentration and protein kinase-C activity. Thus, at any glutamatergic synapse in the brain there is the potential for a single neurotransmitter to generate fast and slow signals with particular characteristics which depend on the properties of the neurotransmitter receptors expressed in the target cell membrane.

RECEPTOR CLASSIFICATION IN THE POST-GENOMIC ERA

The definitive classification of receptors is by amino-acid sequence analysis. Since all properties of the receptor are determined by the amino-acid sequence of the protein this method has the final say. The explosion in use of molecular genetic techniques in the final decade of the twentieth century has led to the cloning and sequencing of the genes of all the known neurotransmitter receptors in the brain. From the gene sequence, the amino-acid sequence of the receptor protein can be inferred and hence a final classification of all receptors can be made. Ultimately, the human genome sequencing programme will mean that the amino-acid sequence of all human receptors will be known. Does this mean pharmacologists can now retire happy in the knowledge that all is now known that there is to know? Far from it! Gene cloning and sequencing has unveiled an increasingly vast diversity among receptor types which could barely have

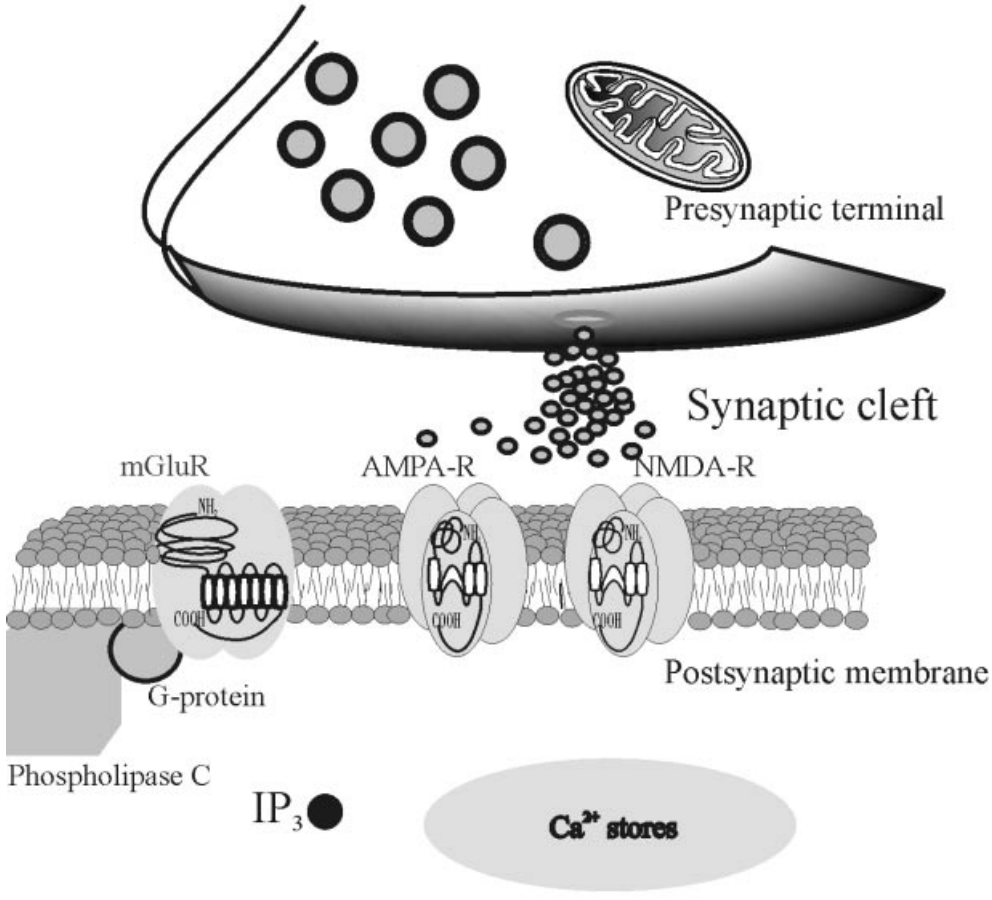

Figure 3.1 Schematic representation of a generic excitatory synapse in the brain. The presynaptic terminal releases the transmitter glutamate by fusion of transmitter vesicles with the nerve terminal membrane. Glutamate diffuses rapidly across the synaptic cleft to bind to and activate AMPA and NMDA receptors. In addition, glutamate may bind to metabotropic G-protein-coupled glutamate receptors located perisynaptically to cause initiation of intracellular signalling via the G-protein, G_q, to activate the enzyme phospholipase and hence produce inositol triphosphate (IP_3) which can release Ca^{2+} from intracellular calcium stores

been imagined by pharmacologists only 20 years ago. The properties and subtle functional differences between receptor subtypes can be studied in increasing detail utilising receptor expression systems such as *Xenopus* oocytes and clonal mammalian cell lines where single receptor populations at high density can be studied without the complications arising from the diversity of receptors present in brain tissue, or the difficulty of recording responses from receptors in the brain. Pharmacology has now entered the era of 'post-genomic' research in which the challenge is to utilise the diversity of receptor types revealed to us by gene cloning techniques in the development of subtype selective drugs. The hope is that if this diversity of receptor subtypes is matched by diversity of function in the brain, then subtype-selective drugs may provide the means to selective therapeutic agents with a minimum of side-effects for use in treating diseases of the brain.

The following sections of this chapter will consider some general and comparative aspects of receptor structure and function. More detailed material on these topics may be found in the relevant chapters on individual neurotransmitters.

ION CHANNEL RECEPTORS

GENERAL

The ligand-gated ion channels are a relatively small group of receptors responsible mainly for fast synaptic transmission at the neuromuscular junction, peripheral autonomic neuroeffector junctions, at autonomic ganglia, and at central synapses. Six different neurotransmitters are known to activate ligand-gated ion channel receptors (Table 3.1). A general principle in the nervous system is that only a few transmitters are used and diversity of effect is achieved by utilising a diversity of receptors. Except for glycine, all fast neurotransmitters have also been found to act at a diversity of G-protein-coupled receptors (Table 3.1) but these are only a small proportion of the huge array of G-protein-coupled receptors which exist in the nervous system and respond to monoamines, peptides and other neurotransmitters and hormones.

Subunit transmembrane topology

The ligand-gated ion channel receptors form three distinct super-families based on the number of times the receptor subunits are predicted to cross the cell membrane (Fig. 3.2). For the nicotinic acetylcholine receptors, $GABA_A$, $GABA_C$, $5\text{-}HT_3$ and glycine receptors each subunit is predicted to cross the cell membrane four times (Fig. 3.2(a)). The exact transmembrane topology is only known with certainty for the nAChR (Unwin 1995). For the other 4-TM domain receptors (and for those in the 3-TM domain and 2-TM domain families) the transmembrane topology of each subunit has been inferred by analogy with the nAChR, from hydropathicity analysis of the subunit amino-acid sequence (about 20 hydrophobic amino acids are needed to form an alpha-helix long enough to span the cell membrane) and from experiments manipulating recombinant receptor subunits. All the 4-TM domain receptor subunits have both amino and carboxy terminals located on the outside of the membrane (Fig. 3.2(a)). The

Table 3.1 Fast neurotransmitters in the central nervous system

Transmitter	Ion channel receptors	G-protein-coupled receptors
Glutamate	Kainate AMPA NMDA	mGluR
GABA	$GABA_A$ $GABA_C$	$GABA_B$
Acetylcholine	Nicotinic	Muscarinic
5-HT	$5\text{-}HT_3$	5-HT
ATP	P2X	P2Y
Glycine	Gly-R	

Note:
Except for glycine, all fast transmitters act on both ion channel receptors and G-protein-coupled receptors. Within each receptor class, there may be several subtypes.

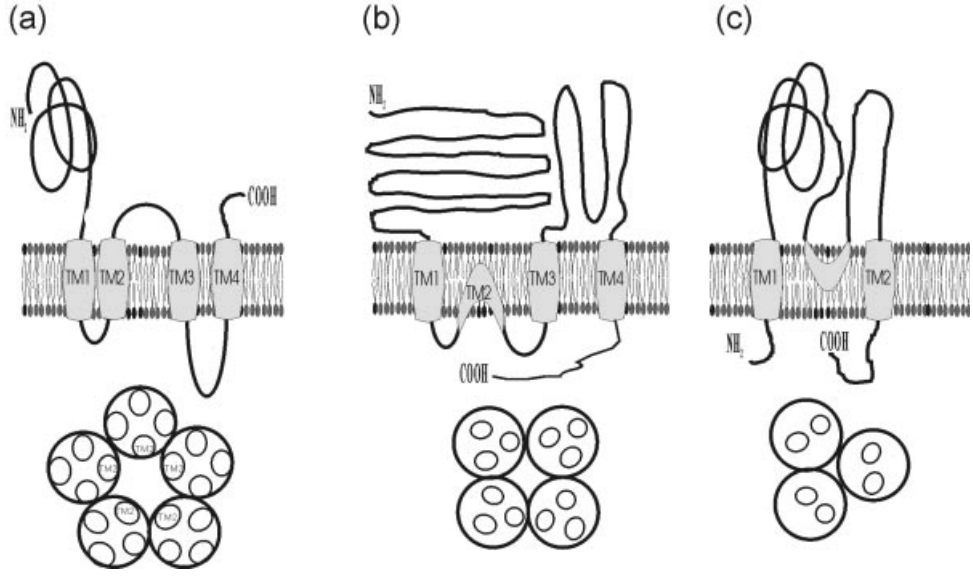

Figure 3.2 Transmembrane (TM) topology of the subunits of three different super-families of ion channel receptors denoted as 4-TM, 3-TM and 2-TM receptors. In (a), the topology of the 4-TM domain subunits is illustrated embedded in the cell membrane. Receptors in this class are the nicotinic acetylcholine receptors, $GABA_A$ and $GABA_C$ receptors, glycine receptors and $5\text{-}HT_3$ receptors. Shown below is the likely pentameric stoichiometry of the 4-TM domain receptors with TM2 of each subunit lining the central ion channel. In (b), the transmembrane topology of the ionotropic glutamate receptors is shown. These have three true transmembrane domains. TM2 creates a pore-forming loop which penetrates into the cell membrane from the intracellular side. As illustrated below, the likely stoichiometry of the glutamate receptors is a tetramer. The exact contribution of TM1, TM3 or TM4 to forming the ion channel is uncertain. In (c), a subunit of the P2X ATP receptors is shown. These subunits cross the cell membrane only twice and the ion channel is probably formed by a short polypeptide loop entering the membrane from the outside. The exact stoichiometry of the P2X receptors is uncertain but current evidence supports the suggestion that they are trimers

agonist binding site is located in the amino terminal domain before the start of TM1 and the ion channel is formed by the TM2 domains of each subunit which come together to make up the complete receptor. Thus the amino acids in TM2 determine the ion conductance properties of the channel. For GABA and glycine receptors a Cl^- channel is formed while for the other ion channel receptors, the channel is largely cation non-selective for monovalent ions such as Na^+ and K^+ and is often also permeable to calcium. One of the key differences between different ion channel receptors for glutamate, ACh, 5-HT and ATP is in their relative permeability to calcium and this is controlled by the amino acids which line the ion channel.

The ionotropic glutamate receptors (kainate, AMPA and NMDA) are formed by subunits which are predicted to cross the cell membrane three times (Fig. 3.2(b)) with the TM2 region forming a loop into the membrane from the intracellular side. The ionotropic glutamate receptor subunits have a large extracellular amino terminal domain and a long intracellular carboxy terminal domain (Fig. 3.2(b)). In the glutamate receptor subunits the agonist binding site is formed by a 'clam-shell' structure where the

amino terminal domain and the extracellular domain between TM3 and TM4 come together to create a pocket for agonist binding.

The P2X receptor subunits are unusual in having only two transmembrane domains with both the amino terminal and carboxy terminal located intracellularly. The ion channel is proposed by analogy with the structure of some potassium channels to be formed by a short loop which enters the membrane from the extracellular side (North and Surprenant 2000).

Subunit stoichiometry

The ion channel receptors are multi-subunit proteins which may be either homomeric (made up of multiple copies of a single type of subunit) or heteromeric (composed of more than one subunit type). These subunits come together after synthesis in the endoplasmic reticulum to form the mature receptor. Notice that stoichiometry is denoted by a subscript number. A receptor composed of two α and three β subunits is therefore denoted as having a stoichiometry of $\alpha_2\beta_3$. This can cause confusion when related subunits are given sequential numbers: $\beta1$, $\beta2$, $\beta3$, etc. The convention is therefore that subunits are numbered normally while stoichiometry is indicated by subscripts so that a pentamer of $\alpha4$ and $\beta3$ subunits might have a stoichiometry of $\alpha4_2\beta3_3$.

NICOTINIC RECEPTORS

All receptors in the 4-TM domain family are thought to form pentameric receptors in which five subunits (Fig. 3.2(a)) surround a central ion channel. Their structure has been most extensively studied in the case of the nicotinic acetylcholine receptor (analogous to the muscle endplate receptor) from *Torpedo* electroplaque (Unwin 2000) where there is now a detailed knowledge of the receptor in both resting and active conformations. The muscle receptor has a subunit stoichiometry of two α subunits, providing the agonist binding sites, and three other subunits (β, γ and δ). In adult muscle an ε subunit is present instead of the γ subunit which is found in the foetal-type receptor. The five subunits are arranged like the staves of a barrel around the central channel. Binding of ACh to the α subunits initiates a conformational change in the protein which, by causing rotation of all five TM2 domains lining the pore, opens the ion channel.

Diversity among neuronal nicotinic receptors is generated by having nine more different α subunits ($\alpha2$–$\alpha10$) and three further β subunits ($\beta2$–$\beta4$). These receptors are activated by nicotine and blocked by the antagonists hexamethonium, mecamylamine and trimetaphan, and the erythrina alkaloid dihydro-β-erythroidine. The neuronal nicotinic receptors are found in autonomic ganglia and in the brain may be either heteromeric (e.g. $\alpha4_2\beta2_3$) or homomeric ($\alpha7_5$). The $\alpha7$ receptor is likely to be the source of the α-bungarotoxin binding sites in the brain observed in autoradiograms of ^{123}I-α-bungarotoxin binding to brain sections (Clarke 1992) and α-bungarotoxin sensitive nicotinic receptors have been shown in a number of studies to stimulate transmitter release from nerve terminals such as dopaminergic terminals in the striatum and glutamatergic terminals in the cortex. Its main functional role may therefore be as a presynaptic receptor regulating transmitter release. It has a high affinity for nicotine and so may mediate some of the central effects of nicotine. Notice that a homomeric receptor has implications for the interpretation of functional studies since the number

of agonist (and antagonist) binding sites on the receptor must equal the number of subunits. In the case of $\alpha 7$ receptors, Hill coefficients (see Appendix) of around 1.8–2.0 have been observed suggesting that only any two of the five binding sites on the receptor need be occupied for efficient activation of the receptor.

GLYCINE RECEPTORS

Inhibitory glycine receptors with high affinity for the antagonist strychnine are predominantly found in the spinal cord and brainstem. Three different α subunits have been cloned ($\alpha 1$–$\alpha 3$) and a single β subunit ($\beta 1$). Interestingly, the foetal-type of glycine receptor which is a homomer (the adult stoichiometry is likely to be $\alpha_3\beta_2$) has Hill coefficients nearer 3.0, suggesting three agonist sites need to be occupied for receptor activation.

GABA RECEPTORS

The GABA receptor subunits are one of the most diverse groups of ion channel receptor subunits in the brain. Six different α subunits ($\alpha 1$–$\alpha 6$), four β subunits ($\beta 1$–$\beta 4$), four γ subunits ($\gamma 1$–$\gamma 4$), an ε subunit, a π subunit, and three ρ subunits ($\rho 1$–$\rho 3$) have been found. This diversity of subunits is reflected in the complicated pharmacology of the GABA receptors. Functional GABA receptors can be formed by co-expression of any α subunit with any β subunit probably in the stoichiometry $\alpha_2\beta_3$. The agonist binding site is on the β subunit. However, these receptors, although activated by GABA and muscimol, potentiated by barbiturates and neurosteroids, and blocked by bicuculline and picrotoxin, lack classical benzodiazepine sensitivity which can be restored to the molecule by co-expressing a γ subunit with α and β.

The ρ subunits form $GABA_C$ receptors. These are activated by the weak agonist CACA, relatively insensitive to bicuculline, not sensitive to barbiturates or neurosteroids, and blocked by TPMPA. They have been described in the retina (where $\rho 1$ is expressed) but relatively little is known of their function in the brain although they are widely expressed (Bormann 2000). For example, all three ρ subunits are found in cerebellar Purkinje cells. Native $GABA_C$ receptors may be homomeric pentamers of a single ρ subunit or heteromeric pentamers of more than one of the ρ subunits. $GABA_C$ receptor dose–response curves have Hill slopes around 3 or greater, unlike $GABA_A$ receptor dose–response curves where the Hill slope is usually around 2.

5-HT_3 RECEPTORS

5-HT_3 receptors are an exception to the general nomenclature of numbering subunits and denoting stoichiometry by subscripts. The 5-HT_3 receptor subtype is denoted by the subscript '3' and the two known subunits are referred to as 'A' and 'B'. When expressed alone, 5-HT_3-A subunits form functional homomeric receptors but these have a much smaller single channel conductance (less than 1.0 pS) and altered pharmacology compared to native 5-HT_3 receptors. Native 5-HT_3 receptors are likely to be pentameric heteromers of 5-HT_3-A and 5-HT_3-B subunits. 5-HT_3-B subunits, unlike 5-HT_3-A subunits, do not form functional receptors when expressed alone but when co-expressed with 5-HT_3-A subunits, the receptors formed have functional properties similar to native 5-HT_3 receptors. Synaptic transmission mediated by 5-HT_3 receptors has been

described in only a few places in the brain, such as in the locus coeruleus and it has been suggested that most 5-HT_3 receptors are located presynaptically on nerve terminals where their calcium permeability may be important in potentiating transmitter release.

GLUTAMATE RECEPTORS

Glutamate receptors mediate fast synaptic transmission throughout the nervous system. They have been classified pharmacologically as three main types according to their sensitivity to three specific agonists: kainate, AMPA and NMDA. The NMDA receptors are unique among the ion channel receptors in requiring the binding of two separate agonists, glutamate and glycine in order to activate the receptor. Selective antagonists for both the glutamate binding site (AP5) and the glycine binding site (7-chloro-kynurenate) have been developed. AMPA and kainate receptors are often referred to as non-NMDA receptors and are blocked by the non-NMDA receptor antagonists CNQX and DNQX. Gene cloning has identified five kainate receptor subunits, four AMPA receptor subunits and six NMDA receptor subunits (Table 3.2).

Kainate receptors

Kainate receptors may be formed by homomeric combination of GluR5, GluR6 or GluR7 or by heteromeric combination of any of GluR5–7 with the kainate binding proteins, KA1 or KA2. KA1 and KA2 do not form functional homomeric receptors.

AMPA receptors

AMPA receptors can be formed by homomeric or heteromeric combinations of GluR1–4. However, most native AMPA receptors are more likely to be heteromeric assemblies

Table 3.2 Ion channel neurotransmitter receptor subunits

Acetylcholine	GABA	Glutamate
Muscle nicotinic AChR	*$GABA_A$*	*AMPA*
$\alpha1$ $\beta1$ γ ε δ	$\alpha1$ $\beta1$ $\gamma1$ δ ε π	GluR1
Neuronal nicotinic AChR	$\alpha2$ $\beta2$ $\gamma2$	GluR2
$\alpha2$ $\beta2$	$\alpha3$ $\beta3$ $\gamma3$	GluR3
$\alpha3$ $\beta3$	$\alpha4$ $\beta4$ $\gamma4$	GluR4
$\alpha4$ $\beta4$	$\alpha5$	*Kainate*
$\alpha5$	$\alpha6$	GluR5 KA1
$\alpha6$	*$GABA_C$*	GluR6 KA2
$\alpha7$	$\rho1$	GluR7
$\alpha8$	$\rho2$	*NMDA*
$\alpha9$	$\rho3$	NR1 (ξ) NR2A ($\varepsilon1$) NR3A
$\alpha10$		NR2B ($\varepsilon2$)
		NR2C ($\varepsilon3$)
		NR2D ($\varepsilon4$)

Notes:
Receptor subunits are numbered $\alpha1$, $\beta1$, etc. where there are more than one subunit of that type. Between different α subunits in any family there is around 60–70% amino-acid sequence homology whereas between α and β subtypes, for example, there is normally around only 40% sequence homology. Mouse NMDA receptor subunits are denoted by the Greek letters ξ and ε while rat and human are indicated as shown.

of GluR2 with one or more of the other AMPA receptor subunits. The main evidence for this came from the discovery that the mRNA for GluR2 is edited at the position coding for a glutamine residue in the TM2 region. mRNA editing produces an arginine residue and changes the functional properties of the receptor such that the receptor is no longer calcium permeable and the resulting synaptic current has a linear current–voltage relationship. This change in the GluR2 subunit dominates the functional properties of the receptor when heteromeric combinations of GluR subunits are expressed in oocytes or cell lines. Thus, wherever GluR2 subunits are expressed, the AMPA receptors so formed have a linear current–voltage relation and are Na^+ permeable but not Ca^{2+} permeable. This is the case at most synapses which have been investigated in the brain but interestingly, does not appear to be so at excitatory synapses onto interneurons where the AMPA receptors are probably heteromeric assemblies of GluR1, GluR3 and GluR4. mRNA for GluR2 is found to be almost 100% edited in the brain. The mRNAs for GluR5 and GluR6 are also edited at the equivalent position to that of GluR2 but the extent of editing varies between 50% and 90% in different brain regions. Thus kainate receptors are likely to be more heterogeneous than AMPA receptors in terms of their calcium permeability.

NMDA receptors

The NMDA receptors come in four main flavours depending on which NR2 subunit (or subunits) is involved in forming the receptor (Table 3.2). Functional NMDA receptors result from the co-assembly of one or more members of three different subfamilies of subunits: NR1, NR2 and NR3.

The NR1 family is composed of one subunit with nine different alternatively spliced variants. Block by NMDA channel blockers such as ketamine, MK-801 and phencyclidine is affected by which splice variant of the NR1 subunit is involved, probably because the NR1 splice variant affects the kinetics of channel activation (the effectiveness of any channel blocker being dependent on how much the channel is open). The glycine binding site is on the NR1 subunit and the glutamate binding site is on the NR2 and NR3 subunits.

The NR2 family is composed of four related subunits termed NR2A, NR2B, NR2C and NR2D and the NR3 family composed of two subunits termed NR3A and NR3B (Table 3.2). It is likely that the receptor is a tetramer composed of two NR1 subunits and two NR2 subunits or an NR2 plus an NR3 subunit. Expression of NR1 alone does not result in functional receptors, except in oocytes where it is now thought likely that the oocyte expresses a low level of native frog NR2 subunits. Expression of NR1 with an NR3 subunit in oocytes or cell lines does not result in functional receptors in contrast to expressing NR1 with an NR2 subunit which results in robust functional responses. An NR2 subunit is essential for function. None of the NR2 subunits form functional homomeric NMDA receptors.

Like the NR1 subunit, the NR2 subunits carry an asparagine residue in the pore-forming TM2 region (in an analogous position to the edited glutamine–arginine position of GluR2) that is important in regulating voltage-dependent Mg^{2+} block and calcium permeability of the channel and affects the affinity of non-competitive antagonists like ketamine. At present, subunit-selective antagonists have only been developed for NR2B-containing receptors. These antagonists such as ifenprodil and eliprodil act to inhibit NMDA receptors by increasing the proton sensitivity of the

NMDA receptors and have been widely investigated as possible neuroprotective agents for stroke and head injury patients (during stroke or mechanical injury pH falls which may result in enhanced effectiveness of this class of blocking drugs in the damaged region). However, the results of clinical trials with these drugs have so far been equivocal despite the known role of NMDA receptor activation in excitotoxic cell death. Compared to other glutamate receptor subunits, the NR2 subunits have the longest cytoplasmic carboxy terminal (>500 amino acids) which contains a conserved SXV sequence (where S is serine, X is any amino acid, and V is valine) that interacts with PSD-95, a postsynaptic anchoring protein and is thought to serve to anchor NMDA receptors at the synapse.

NR2 subunit expression is highly regulated during development and subunit segregation is observed between brain regions. In general, NR2B and NR2D are expressed early in development and NR2A and NR2C expression occurs in rats in the weeks after birth and continues into the adult in selected areas. In the adult cortex, hippocampus and thalamus NR2A and NR2B predominate while in the cerebellum and spinal cord, NR2C and NR2D are also expressed in the adult and their expression is specific to particular cell types.

Transgenic mice technology is increasingly used to investigate the functional significance of different receptor subunits to the development and behaviour of the whole animal. Mice lacking the $\varepsilon 1$ (rat NR2A) subunit show apparently normal growth and mating behaviour, but an enhanced startle response and reduced hippocampal long-term potentiation (LTP) and spatial learning. Mice lacking the $\varepsilon 2$ (rat NR2B) subunit die soon after birth showing impairment of suckling response, impairment of trigeminal neuronal pattern formation and impairment of hippocampal long-term depression (LTD). Mice lacking the $\varepsilon 3$ (NR2C) subunit show apparently normal development and behaviour. Mice lacking the $\varepsilon 4$ (NR2D) subunit show apparently normal growth and mating behaviour, but reduced spontaneous behavioural activity.

The details of NMDA receptor subunit expression during development are still only partly known at the single-cell level. Good examples of this can be found in the cerebellum where NMDA receptors have been investigated extensively by Cull-Candy and coworkers. In young animals, cerebellar granule cells express NR1 and NR2B. By three weeks old the rat granule cells express NR1, NR2A and NR2C. In contrast, cerebellar Golgi cells express NR1, NR2B and NR2D in both young and adult animals but it appears NR2D is not involved in synaptic transmission. In cerebellar Purkinje cells, young animals express NR1 and NR2D but in the adult, only NR1 is found and the cerebellar Purkinje cells are no longer responsive to NMDA, one of the few neurons in the brain to lack functional NMDA receptors.

The NR2 subunits confer diversity to the functional and pharmacological properties of NMDA receptors. They modulate properties such as the strength of Mg^{2+} block (NR2A and NR2B subunits are most sensitive and NR2C and NR2D are less sensitive to Mg^{2+}), glycine sensitivity (NR2D-containing receptors have the highest affinity for glycine) and kinetics of deactivation. NR2A-containing receptors have relatively rapid deactivation kinetics with a time constant of around 100 ms and mediate relatively fast synaptic currents, while NR2D-containing receptors have extremely slow deactivation kinetics with a time constant of around 4 s and have not yet been shown to be involved in synaptic transmission. The NMDA receptor single-channel properties have been shown to be dependent on the NR2 subunit. NR2A- and NR2B-containing receptors have a main single-channel conductance of 50 pS while NR2C- and NR2D-containing

Table 3.3 G-protein-coupled receptors

Receptor class	Neurotransmitter or hormone receptor
Amino acid receptors	Metabotropic glutamate and $GABA_B$ receptors
Monoamine receptors	Adrenoceptors, dopamine and 5-HT receptors, muscarinic and histamine receptors
Lipid receptors	Prostaglandin, thromboxane and PAF receptors
Purine receptors	Adenosine and ATP (P2Y) receptors
Neuropeptide receptors	Neuropeptide Y, opiate, cholecystokinin VIP, etc.
Peptide hormone receptors	Angiotensin, bradykinin, glucagon, calcitonin, parathyroid, etc.
Chemokine receptors	Interleukin-8
Glycoprotein receptors	TSH, LH/FSH, chorionic gonadotropin, etc.
Protease receptors	Thrombin

receptors have a main conductance level of 36 pS and a subconductance level of 18 pS.

G-PROTEIN-COUPLED RECEPTORS

The G-protein-coupled receptors generate a response by linking drug binding at the extracellular part of the receptor protein to activation of particular intracellular GTP binding proteins (G-proteins). The G-proteins act as transducers between the receptors and the effector systems. G-protein-coupled receptors represent the largest and most diverse class of membrane receptors: a super-family of receptors which ranges from rhodopsin to the odorant receptors (Table 3.3). These receptors are by far the biggest targets for drug development and particularly in the central nervous system they provide the opportunity to alter neuronal firing properties or release of transmitter from nerve terminals by inhibition of voltage-gated Ca^{2+} and K^+ channels, or by changing cAMP levels or by regulation of intracellular Ca^{2+} levels.

TRANSMEMBRANE TOPOLOGY AND TERTIARY STRUCTURE

X-ray crystallography has so far been unable to elucidate the structure of any membrane receptor. However, cryoelectron microscopy of two-dimensional arrays of receptors embedded in membrane lipid has produced electron density maps of both bacteriorhodopsin and bovine rhodopsin (Schertler, Villa and Henderson 1993). Bacteriorhodopsin is not a G-protein-coupled receptor but a proton pump while bovine rhodopsin is a true G-protein-coupled receptor. Both proteins are folded into seven helical bundles as illustrated in Fig. 3.3(a). Each of these seven alpha helices crosses the cell membrane giving rise to the fingerprint of the seven transmembrane (7-TM) domain receptors. The electron density map of rhodopsin can be compared with a helical-wheel diagram showing the amino acids of the 7-TM domains spiralling through the cell membrane (Fig. 3.3(b)). Molecular modelling suggests a particular packing arrangement for the alpha helices which is stabilised by a disulphide bridge between the second and third extracellular loops. The structure of other G-protein-coupled receptors is inferred to be similar to rhodopsin due to the presence of seven hydrophobic amino acid domains in all members of this receptor family so far sequenced.

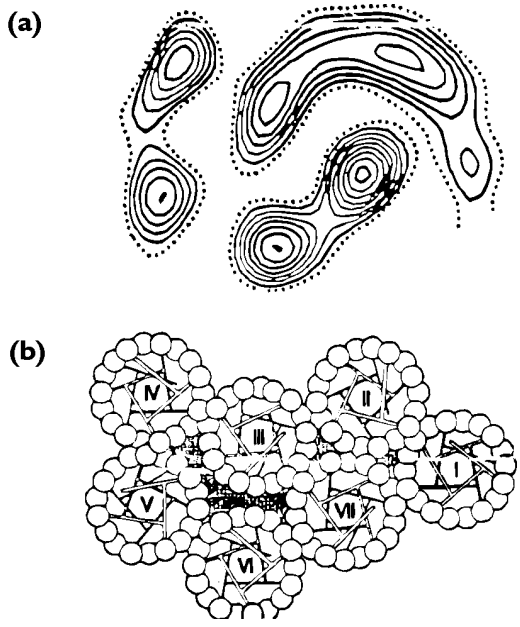

Figure 3.3 Molecular structure of G-protein-coupled receptors. In (a) the electron density map of bovine rhodopsin is shown as obtained by cryoelectron microscopy of two-dimensional arrays of receptors embedded in lipid membrane. The electron densities show seven peaks reflecting the seven α-helices which are predicted to cross the cell membrane. In (b) is shown a helical-wheel diagram of the receptor orientated according to the electron density map shown in (a). The diagram is seen as the receptor would be viewed from outside the cell membrane. The agonist binding pocket is illustrated by the hatched region between TM3, TM5 and TM6. (From Schertler *et al.* 1993 and Baldwin 1993, reproduced from Schwartz 1996). Reprinted with permission from *Textbook of Receptor Pharmacology*. Eds Foreman, JC and Johansen, T. Copyright CRC Press, Boca Raton, Florida

RECEPTOR ACTIVATION

Most structure–function information for the G-protein-coupled receptors has been inferred from molecular genetic experiments where single amino acids or groups of amino acids in the protein have been changed to investigate their role. The β-adrenoceptor was the first G-protein-coupled receptor to be cloned and a detailed picture of the receptor structure has emerged using the techniques of molecular biology combined with radioligand binding and classical pharmacology to study receptor function (see, for example, Lefkowitz *et al.* 1993). An outline structure of the β-adrenoceptor is shown in Fig. 3.4. Two main structural domains are recognised in all G-protein-coupled receptors:

(1) Ligand-binding domain
(2) G-protein-binding domain on the third intracellular loop

Ligand-binding domain

In the monoamine receptors the ligand-binding domain is located within the transmembrane helices. A pocket is formed between TM3, TM5 and TM6 where the agonist binds. A conserved aspartate residue in TM3 (Asp-113 in the β-adrenoceptor) and a

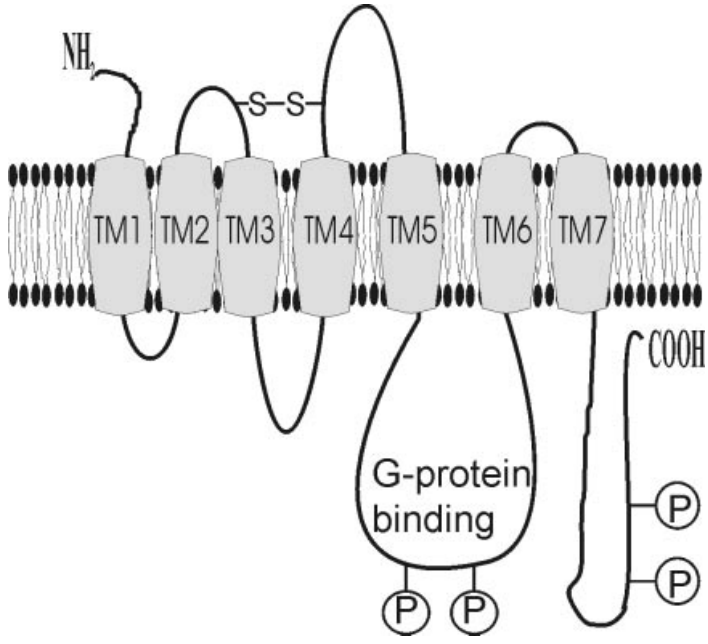

Figure 3.4 Transmembrane topology of a 7-TM domain G-protein receptor such as the β-adrenoceptor. Agonist binding is predicted to be within the transmembrane domains. The extracellular structure is stabilised by the disulphide bond joining the first and second extracellular loop. The third intracellular loop is the main site of G-protein interaction while the third intracellular loop and carboxy tail are targets for phosphorylation by kinases responsible for initiating receptor desensitisation

conserved phenylalanine in TM6 (Phe-290) and two serine residues in TM5 (Ser-204 and 207) are known to be crucial for agonist binding. Antagonists have been shown to have extra interaction points on TM4 and TM7 but are thought to largely share the same binding sites as the agonist and so can act by simple competition.

G-protein coupling

All rhodopsin-like G-protein-coupled receptors have a conserved arginine residue at the intracellular end of TM3 and this residue is thought to be crucial for G-protein activation. The third intracellular loop determines the class of G-protein activated by the receptor with the second intracellular loop and C-terminus also influencing G-protein binding in some cases. Four classes of G-protein are known:

(1) G_s — activates adenylyl cyclase (irreversibly activated by cholera toxin)
(2) G_i — inhibits adenylyl cyclase (inactivated by *Pertussis* toxin)
(3) G_q — activates phospholipase-C (not activated by *Pertussis* toxin or cholera toxin)
(4) G_o — inhibits voltage-dependent Ca^{2+} and K^+ channels (inactivated by *Pertussis* toxin)

Using chimaeric receptors it has been shown that swapping the third intracellular loop between receptors also swaps their G-protein selectivity. The G-protein-binding

Table 3.4 G-protein receptor-mediated responses

Receptor	G-protein activation	Effector mechanism
ACh-muscarinic		
M_1, M_3, M_5	G_q	Phospholipase-C to IP_3 to $[Ca^{2+}]_i$ regulation
M_2, M_4	G_o, G_i	Inhibits adenylyl cyclase, Ca^{2+}/K^+ channels
Histamine		
H_1, H_3	G_q	Phospholipase-C to IP_3 to $[Ca^{2+}]_i$ regulation
H_2	G_s	Stimulates adenylyl cyclase raising cAMP
Adrenoceptors		
α_1, α_2	G_o, G_i	Iinhibits adenylyl cyclase, Ca^{2+}/K^+ channels
β_1, β_2, β_3	G_s	Stimulates adenylyl cyclase raising cAMP
Opiate		
μ, δ, κ	G_o, G_i	Inhibits adenylyl cyclase, Ca^{2+}/K^+ channels
Dopamine		
D_1	G_s	Stimulates adenylyl cyclase raising cAMP
D_2, D_4	G_o, G_i	Inhibits adenylyl cyclase, Ca^{2+}/K^+ channels
D_3	G_q	Phospholipase-C to IP_3 to $[Ca^{2+}]_i$ regulation
$GABA_B$	G_o, G_i	Inhibits adenylyl cyclase, Ca^{2+}/K^+ channels
Glutamate-metabotropic		
mGluR1, 3, 5	G_q	Phospholipase-C to IP_3 to $[Ca^{2+}]_i$ regulation
mGluR2, 4, 6, 7	G_o, G_i	Inhibits adenylyl cyclase, Ca^{2+}/K^+ channels

domain and the carboxy terminal domain also contain a variable number of serine and threonine residues which are the target of protein kinases involved in receptor desensitisation. Different subtypes of G-protein-coupled receptor have evolved which couple to different G-proteins (Table 3.4) and it has been shown in heterologous expression systems that when a G-protein-coupled receptor is expressed at very high levels, the selectivity of G-protein coupling can be overruled and, for example, a receptor which normally couples to G_o can be made to activate G_i.

Receptor dimerisation

The $GABA_B$ receptors were the first G-protein-coupled receptors to be observed to form functional heterodimers (Bowery and Enna 2000) where two G-protein molecules come together to act as a dimer to enhance their combined response. A similar effect has recently also been described for dopamine and somatostatin receptors (Rocheville *et al*. 2000) and it is likely that this may occur with other G-protein-coupled receptors. The significance of this in terms of the pharmacology of the receptors is unclear, or indeed whether dimerisation affects mechanisms such as desensitisation.

7-TM DOMAIN RECEPTOR FAMILIES

Figure 3.5 shows diagrammatic illustrations of the transmembrane topology of the G-protein-coupled receptor families. Three main families have been identified:

(1) Rhodopsin-like 7-TM receptors
   - Ligand binding within the transmembrane domains
   - Monoamine, nucleotide and lipid receptors

(2) Glucagon, VIP and calcitonin family
   - Ligand binding outside the transmembrane domains on cell surface

(3) Metabotropic glutamate receptors and chemosensor (Ca^{2+}) receptors
   - Ligand binding on large extracellular N-terminus

Rhodopsin-like 7-TM receptors

By far the most studied family of the G-protein-coupled receptors are the rhodopsin-like receptors. These are also the largest group of receptors in number as they include receptors not only for the monoamines, nucleotides, neuropeptides and peptide hormones, but they also include the odorant receptors which number several hundreds of related receptors. These receptors have short N-termini, a conserved disulphide bridge between the TM2–TM3 and TM4–TM5 extracellular domains, and variable-length C-termini. In some cases the C-terminus is myristolyated which by tying the C-terminus to the cell membrane generates a fourth intracellular loop.

While the agonist binding domain is thought to be within the transmembrane domains for the monoamine and nucleotide receptors, neuropeptides are thought to bind close to the membrane surface on the extracellular domains of the receptor. It is still not clear whether non-peptide antagonists bind at the same or a different site on the receptor.

Glucagon, VIP and calcitonin family

These receptors are unlike the well-characterised rhodopsin-like family in that they have a large extracellular N-terminus and hormone binding seems to be dominated by this domain rather than the transmembrane domains. Receptors in this class include

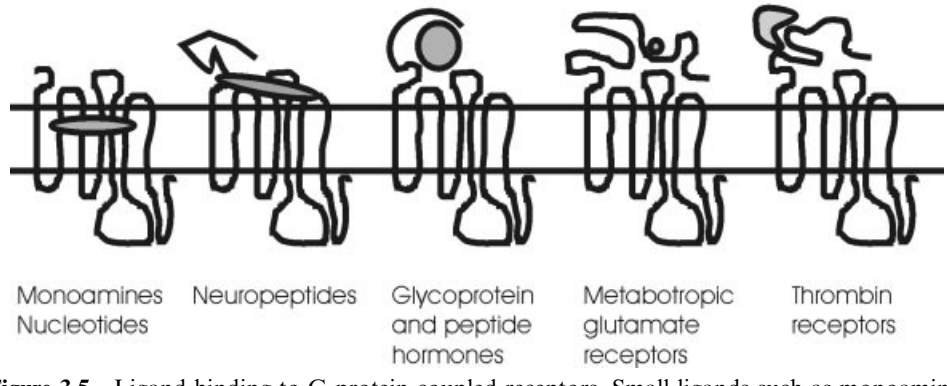

Figure 3.5 Ligand binding to G-protein-coupled receptors. Small ligands such as monoamines, nucleotides and lipids bind within the transmembrane domains while peptide and glycoprotein hormones bind outside the transmembrane region. Metabotropic glutamate receptors have agonist binding on the large N-terminal domain while the thrombin receptor is activated by cleavage of the N-terminal domain by thrombin (reproduced from Schwartz 1996). Reprinted with permission from *Textbook of Receptor Pharmacology*. Eds Foreman, JC and Johansen, T. Copyright CRC Press, Boca Raton, Florida

growth hormone-releasing hormone (GHRH), adrenocorticotrophic hormone-releasing factor and the neuropeptide, vasoactive intestinal polypeptide (VIP). All receptors in this family couple to G_s to stimulate adenylyl cyclase and hence raise cAMP levels.

Metabotropic glutamate receptors and chemosensor (Ca^{2+}) receptors

The metabotropic glutamate receptors are a distinct family of G-protein-coupled receptors which are homologous only to the Ca^{2+} sensors of the parathyroid and kidney. These receptors have an extremely large extracellular N-terminal domain of 500–600 amino acids (cf. 30–40 amino acids for the monoamine receptors). The binding of glutamate is thought to be within a 'clam-shell' region formed by two subdomains of the N-terminal region in an analogous manner to the binding of glutamate to the extracellular domain of the kainate, AMPA and NMDA receptor NR2 subunits.

Thrombin receptors

The thrombin receptor is unusual in that the receptor is activated by the enzymatic action of thrombin which cleaves the N-terminus of the receptor leaving the receptor constitutively active.

G-PROTEIN-COUPLED RECEPTOR DESENSITISATION

Desensitisation is a general phenomenon which serves as a negative feedback on receptor activity. The mechanisms of G-protein-coupled receptor desensitisation have been particularly investigated by Lefkowitz's group working on the β-adrenoceptors. In general, G-protein-coupled receptors desensitise following phosphorylation of specific serine and threonine residues on the third intracellular loop and on the COOH tail. These amino acids are targets for phosphorylation by protein kinases and specific G-protein receptor kinases (GRKs) which result in receptor desensitisation by inhibiting G-protein coupling, stabilising receptor *arrestin* binding, and controlling receptor internalisation. Two forms of desensitisation have been characterised: *homologous* and *heterologous*. Homologous desensitisation refers to desensitisation of the response to an agonist due to prior application of the same agonist. Homologous desensitisation is mediated by specific GRKs which are activated by the generation of free G-protein $\beta\gamma$ subunits. GRKs only phosphorylate agonist occupied receptors making their action relatively specific to the G-protein-coupled receptor involved in G-protein stimulation.

Heterologous desensitisation refers to the desensitisation of the response to one agonist by the application of a different agonist. For example, desensitisation of a response to adrenaline by application of 5-HT is mediated by protein kinase A or protein kinase C because these kinases can phosphorylate receptors which are not occupied by agonist. Phosphorylation disrupts the receptor–G-protein interaction and induces the binding of specific proteins, *arrestins* which enhance receptors internalisation via clathrin-coated pits. Thus desensitisation of G-protein-coupled receptors results in a decrease in the *number* of functional receptors on the cell surface.

CONSTITUTIVELY ACTIVE RECEPTORS

One current model of G-protein receptor activation is the **allosteric ternary complex** model of Lefkowitz and Costa. The agonist, receptor and G-protein must combine to

form a *ternary* complex in order to generate a response. Thermodynamically, in this freely reversible mechanism receptors may occasionally adopt the active conformation in the absence of ligand and may then cause G-protein activation. This constitutive activity can be demonstrated by the effect of 'antagonists' which inhibit the unstimulated activity of the receptor and so are known as 'inverse agonists' (see Appendix). Constitutive activity has been produced by specific point mutations of the β-adrenoceptor where conversion of Ala-293 to glu-293 results in a tenfold increase in constitutive activity. In contrast, some antagonists ('pure antagonists') do not alter the constitutive activity of the receptor.

GENETIC DISEASE

Mutations producing constitutively active receptors have been found in several rare genetic diseases (Coughlin 1994) such as hyperthyroidism (TSH receptor), precocious puberty (LH receptor), and retinitis pigmentosa (rhodopsin). Clearly the ability to manipulate the degree of constitutive receptor activity using drugs could provide a therapeutic strategy in these diseases.

CONCLUSIONS

Neurotransmitter receptors have evolved as one of the key components in the ability of the central nervous system to coordinate the behaviour of the whole animal, to process and respond to sensory input, and to adapt to change in the environment. These same receptors are therefore ideal targets for drug action because of their central role in the activity of the nervous system. A rational approach to the development of new therapeutic strategies involving the action of drugs at receptors in the nervous system is based on knowledge of receptor structure, distribution and function.

APPENDIX

1 DERIVATION OF THE HILL COEFFICIENT (OR SLOPE) AS A DETERMINANT OF THE NUMBER OF BINDING SITES FOR AN AGONIST (NEUROTRANSMITTER) ON ITS RECEPTOR

The relationship between drug concentration and receptor occupancy

The simplest model for the reversible combination of a drug, A, with its receptor, R, is:

$$A + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AR \tag{A3.1}$$

The law of mass action states that the rate of a reaction is proportional to the product of the concentrations of the reactants. Thus the rate of the forward reaction is proportional to $[A][R] = k_{+1}[A][R]$, where k_{+1} is the *association rate constant* (with units of $M^{-1}\ s^{-1}$). Likewise, the rate of the backward reaction is proportional to $[AR] = k_{-1}[AR]$, where k_{-1} is the *dissociation rate constant* (with units of s^{-1}). At equilibrium, the rates of the forward and backward reactions will be equal so

$$k_{+1}[A][R] = k_{-1}[AR]$$

Since receptors are embedded in cell membranes it is more convenient to consider the amount of receptor either free or bound as a proportion of the total receptor present. The proportion of receptors in state R (free) is

$$p_R = [R]/[R]_T$$

where $[R]_T$ is the total concentration of receptors and

$$p_{AR} = [AR]/[R]_T$$

is the proportion of receptors occupied by the drug. As the system is at equilibrium we can write

$$k_{+1}[A][R]/[R]_T = k_{-1}[AR]/[R]_T$$

and hence

$$k_{+1}[A]p_R = k_{-1}p_{AR}$$

Rearranging gives

$$[A]p_R = k_{-1}/k_{+1}p_{AR}$$

$k_{-1}/k_{+1} = K_A$ is defined as the *dissociation equilibrium constant*. This is often referred to as the *equilibrium constant*. Substitution for k_{-1}/k_{+1} gives

$$[A]p_R = K_A p_{AR}$$

Rearranging gives

$$p_R = \{K_A/[A]\}\, p_{AR}$$

In the simple two-state model shown in equation (A3.1) the receptor can only exist in either free or bound states and so

$$p_R + p_{AR} = 1$$

Substituting for p_R gives

$$\frac{K_A}{[A]}\, p_{AR} + p_{AR} = 1$$

Hence

$$p_{AR} = \frac{[A]}{K_A + [A]} \tag{A3.2}$$

This is known as the **Hill–Langmuir equation**. Its derivation assumes that the concentration of A does not change as drug receptor complexes are formed. In effect, the drug is considered to be present in such excess that the number of drug molecules in solution is many times greater than the number of receptor molecules available for the drug to bind to. It can be rearranged to

$$\frac{p_{AR}}{1 - p_{AR}} = \frac{[A]}{K_A}$$

and taking logs gives

$$\log\left(\frac{p_{AR}}{1-p_{AR}}\right) = \log[A] - \log K_A \tag{A3.3}$$

Hence a plot of log $(p_{AR}/(1-p_{AR}))$ against log[A] gives a straight line of unit slope. This is known as a **Hill plot**.

In practice, it is not often possible to directly measure p_{AR} except in radioligand binding experiments. In many experiments it is the relationship between agonist concentration [A] and percentage maximum response (y) which is measured (a dose–response curve) and the Hill plot is made by plotting

$$\log\left(\frac{y}{100-y}\right) = \log[A] - \log K_A \tag{A3.4}$$

The slope of this log–log plot is known as the **Hill coefficient** (n_H) or **Hill slope**. If the slope is 1 this may imply there is only one agonist binding site on the receptor, while a slope approaching 2 implies two binding sites. In practice, the slope of the line may be greater or less than unity and is rarely an integer. Factors which can affect the Hill slope are particularly the presence of more than one population of receptors with different affinities for the agonist contributing to the response ($n_H < 1$), occurrence of receptor desensitisation ($n_H < 1$), the presence of more than one agonist binding site on the receptor (as occurs with the ligand-gated ion channel receptors) where more than one site needs to be occupied for efficient activation of the receptor ($n_H > 1$), and the presence of spare receptors in the tissue ($n_H > 1$).

Concentration–response curves are often fitted *empirically* by the expression

$$y = y_{max}\frac{[A]^{n_H}}{[A]_{50}^{n_H} + [A]^{n_H}} \tag{A3.5}$$

where n_H is the Hill coefficient and y_{max} is the maximum response. $[A]_{50}$ is the concentration of A at which y is half maximal. Equation (A3.5) is known as the **Hill equation**. $[A]_{50}$ is sometimes denoted by K. However, the constant K obtained by fitting the Hill equation does not correspond to an equilibrium constant as defined above when deriving the Hill–Langmuir equation.

2 KINETICS AND MECHANISMS OF AGONIST ACTION

(a) Ion channel receptors

Agonist responses at ligand-gated ion channels and drug effects at ion channels are often more amenable to mechanistic investigation because the response (ionic current through open ion channels when measured with voltage or patch-clamp techniques) is directly proportional to receptor activation. This is a great advantage and has allowed electrophysiological techniques to be used to study ion channel activation and drug block of ion channels in great detail.

The first physically plausible mechanism for receptor activation was proposed by del Castillo and Katz (1957). They made the important distinction that agonist binding and channel opening of the AChR must occur as two separate steps:

$$A + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AR \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} AR^* \tag{A3.6}$$

In the del Castillo and Katz model it is important to notice that the fraction of receptors occupied is the sum of both active (AR*) and occupied, but inactive (AR) receptors:

$$\begin{aligned} p_{occ} &= p_{AR} + p_{AR^*}, = (1 + K_2)p_{AR^*} \\ &= \frac{(1 + K_2)[A]}{K_1K_2 + (1 + K_2)[A]} \\ &= \frac{[A]}{\dfrac{K_1K_2}{1 + K_2} + [A]} \end{aligned}$$

This can be rewritten as

$$p_{occ} = \frac{[A]}{K_{eff} + [A]}$$

where K_{eff} is the **effective dissociation equilibrium constant**. Thus, most macroscopic estimates of the equilibrium constant for an agonist (radioligand binding, EC_{50} from the occupancy versus agonist concentration–response curve) the estimated equilibrium constant will depend on both affinity for the receptor and subsequent activation steps on the receptor.

(b) G-protein-coupled receptors

The simplest possible model for the action of such receptors is as follows:

$$A + R \overset{K_A}{\rightleftharpoons} AR$$
$$AR + G \overset{K_{ARG}}{\rightleftharpoons} ARG^*$$

where the binding of a G-protein (G) to AR to form a ternary complex (ARG*) is described by the equilibrium constant K_{ARG}. However, recent results suggest that G-protein-coupled receptors (and potentially other receptors) can exist in the active state in the absence of agonist. These **constitutively active receptors** give rise to interesting new predictions for the shape of the dose–response curve and an alternative interpretation for the difference between agonists, partial agonists and antagonists (Lefkowitz *et al.* 1993; Jenkinson 1996). Suppose the receptors can isomerise spontaneously to and from an active form:

$$\underset{\text{(inactive)}}{R} \rightleftharpoons \underset{\text{(active)}}{R^*}$$

In principle, both states of the receptor could be occupied by a ligand, L:

$$\begin{array}{ccc} \text{(inactive) } R & \overset{K_A}{\rightleftharpoons} & R^*\text{(active)} \\ + & & + \\ L & & L \\ K_{LR} \Updownarrow & & \Updownarrow K_{LR^*} \\ \text{(inactive) } LR & & LR^* \text{ (active)} \end{array}$$

If L combines only with R, then the presence of L will *reduce* the proportion of active receptors. L is said to be an **inverse agonist** or **negative antagonist**. If L combines with R* there will be an increase in active receptors and so L will behave as a conventional agonist. Where L has equal affinity for R and R*, it will not affect the fraction of receptors in the active state. However, it will reduce the binding of either a conventional or an inverse agonist, and so will behave as an antagonist.

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