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11 Amino Acids: Inhibitory

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INTRODUCTION

Amino acids are the most widely used neurotransmitters in the central nervous system (CNS). Just as glutamate is the major excitatory neurotransmitter in the CNS (Chapter 10), two other amino acids, GABA (γ-aminobutyric acid) and glycine, are the principal inhibitory neurotransmitters. GABA and glycine are found throughout the CNS, but while GABA serves as a neurotransmitter in virtually every brain area, glycine fulfils this role predominantly, although not exclusively, in the caudal portions of the brain, the spinal cord, and the retina. This chapter describes the basic neurochemistry and pharmacology of GABAergic and glycinergic synapses.

GABA

The non-protein amino acid GABA is found in nearly all pro- and eukaryotic organisms, including plants. Its presence in mammalian brain was described 50 years ago, and the progress towards its subsequent acceptance as an important central neurotransmitter has been well documented (Roberts 1986). Over the years, numerous neurochemical, immunocytochemical and electrophysiological studies, as well as more recent investigations made possible by the cloning of GABA receptors, GABA transporters and the enzymes responsible for GABA synthesis, have confirmed the presence of GABAergic synapses throughout the CNS. Many of these synapses arise from local circuit interneurons, which are extremely diverse in both form and function (e.g. Freund and Buzsaki 1996; Gupta, Wang and Markram 2000). In addition, GABA is found in a number of neurons that contribute to identified pathways between brain regions. The majority of these are associated with the basal ganglia and include, for example, projections from the striatum to the globus pallidus and substantia nigra as well as projections from the globus pallidus and substantia nigra zona reticulata to several brain areas. Outside the basal ganglia, one of the best-known GABAergic projection neurons is the Purkinje cell of the cerebellar cortex.

GABA is an 'inhibitory' neurotransmitter in as much as its principal action is to cause membrane hyperpolarisation, thus reducing neuronal activity (see below). The large number and widespread distribution of GABAergic synapses has led to the idea that the nervous system is highly restrained. While this is undoubtedly an over-simplification, the importance of such inhibition for normal brain function is illustrated by experimental or pathological situations in which blocking or impairing the action of

GABA results in uncontrolled neuronal firing or seizures. GABA-mediated inhibition, however, does not act solely as a 'brake' but serves a variety of roles that far exceed the simple suppression of excitability. For example, tonic inhibitory input can transform the underlying firing pattern of a target cell and, by changing its electronic properties, alter both its temporal and spatial integration of excitatory inputs and hence the way information is processed. Moreover, individual neurons should not be considered in isolation. In networks of neurons, inhibitory connections may be organised to provide negative feedback (recurrent inhibition) and so lead to oscillatory behaviour. By controlling the precise timing of firing in multiple target cells inhibitory interneurons may also synchronise activity among neuronal populations and even enhance the effect of excitatory inputs. GABAergic neurons may also contact other GABAergic neurons, producing their ultimate effects through a process of disinhibition. Finally, it is important to note that, while GABA is classically viewed as an inhibitory neurotransmitter, under certain circumstances (described below) it can cause overt excitatory actions, and that this would appear to be its principal role in the developing nervous system.

NEUROCHEMISTRY OF GABA

SYNTHESIS AND CATABOLISM OF GABA

The synthesis and metabolism of GABA is closely linked with that of glutamate and the citric acid or tricarboxylic acid (TCA) cycle (Fig. 11.1). GABA is produced by the decarboxylation of glutamate, a reaction catalysed by the enzyme glutamic acid decarboxylase (GAD). GAD is found in several non-neuronal tissues (including ovary and pancreas) but within the CNS it is a specific marker of GABAergic neurons, where it is present in the cytoplasm as both soluble and membrane-bound forms, principally in the axon terminals. Labelling with antibodies against GAD has thus proved a particularly valuable technique for the identification of these neurons and their synaptic boutons. The breakdown of GABA occurs as a transamination reaction catalysed by the mitochondrial enzyme 4-aminobutyrate aminotransferase (GABA transaminase; GABA-T). In this process the amino group from GABA is transferred onto the TCA cycle intermediate α-ketoglutarate, producing glutamate and succinic semialdehyde. The latter is in turn converted by the enzyme succinic semialdehyde dehydrogenase (SSADH) into succinate, which re-enters the TCA cycle. This synthesis and catabolism of GABA is often referred to as the 'GABA-shunt', as it acts as a shunt of the normal TCA pathway from α-ketoglutarate to succinate. Other potential routes of GABA production have been described — involving deamination and decarboxylation reactions from putrescine, spermine, spermidine and ornithine — but the vast majority of GABA is generated by means of the GABA-shunt.

GABA-T and SSADH are also present in the mitochondria of glial cells and are responsible for the degradation of GABA recovered from the extracellular space (see below). In this case the glutamate formed from the action of GABA-T is converted into glutamine by the cytosolic enzyme glutamine synthetase (GS). Glial glutamine serves as an important precursor for both neuronal glutamate and GABA. It is transported from glia into neurons where the mitochondrial enzyme phosphate-activated glutaminase (PAG) converts it back into glutamate. This neuronal glutamate can then be converted to GABA, either directly or following metabolism via the TCA cycle. The inter-conversion of glutamate and α-ketoglutarate is achieved by two further groups of

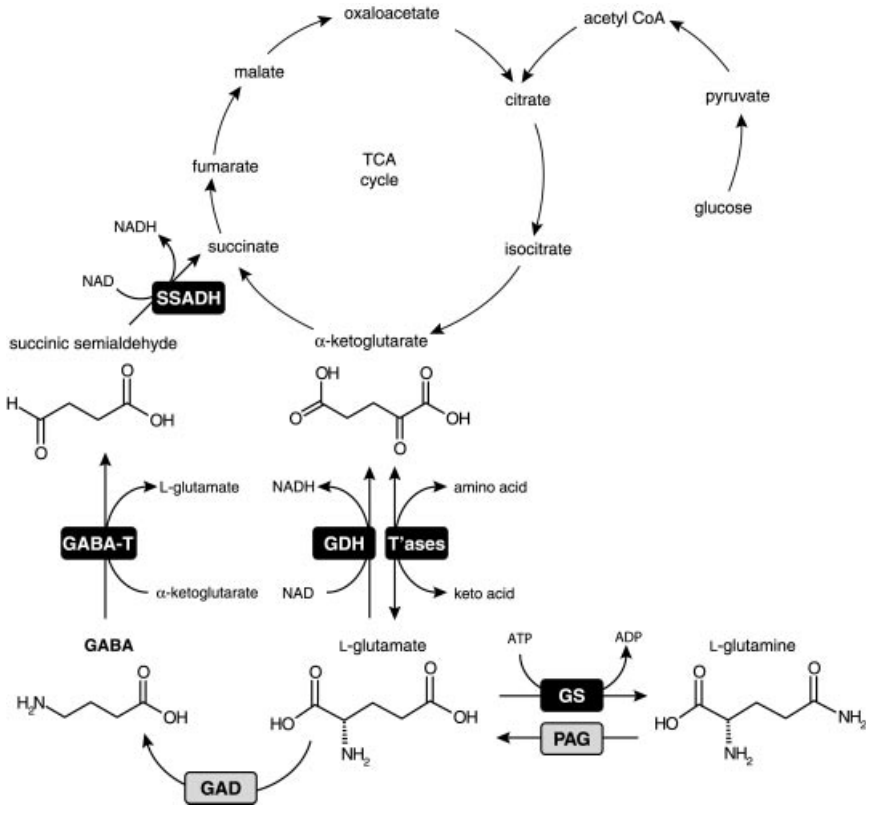

Figure 11.1 Enzymes responsible for the synthesis and metabolism of GABA. Enzymes responsible for the syntheisis (GAD) and metabolism (GABA-T and SSADH) of GABA, and their relationship to the TCA cycle and the amino acids glutamate and glutamine. Precursor glutamate is derived from glutamine by phosphate-activated glutaminase (PAG) and from α-ketoglutarate by aminotransferases, including aspartate and alanine aminotransferases (T'ases) and GABA-T. In glia, glutamate can be converted to glutamine by glutamine synthetase (GS). Other abbreviations are given in the text. Dark-grey boxes denote enzymes present in both neurons and glia, light-grey boxes denote enzymes present only in neurons

enzymes found in the mitochondria of both neurons and glia: the multi-enzyme complex glutamate dehydrogenase (GDH), and several aminotransferases (including aspartate and alanine aminotransferases) whose action is analogous to that of GABA-T. Fig. 11.2 shows the pathways of GABA metabolism in the context of a GABAergic synapse.

Regulation of GAD

Of key importance in the synthesis of GABA is the short-term regulation of GAD activity. Increasing the availability of glutamate does not lead to an increase in the production of GABA, suggesting that GAD may normally be saturated with its substrate. Instead, the control of GAD activity is intimately linked to the enzyme's requirement for the co-factor pyridoxal-5′-phosphate (PLP; a form of vitamin B_6) (Martin and Rimvall 1993). GAD exists in two states; an inactive apoenzyme (apoGAD) lacking the co-factor and active

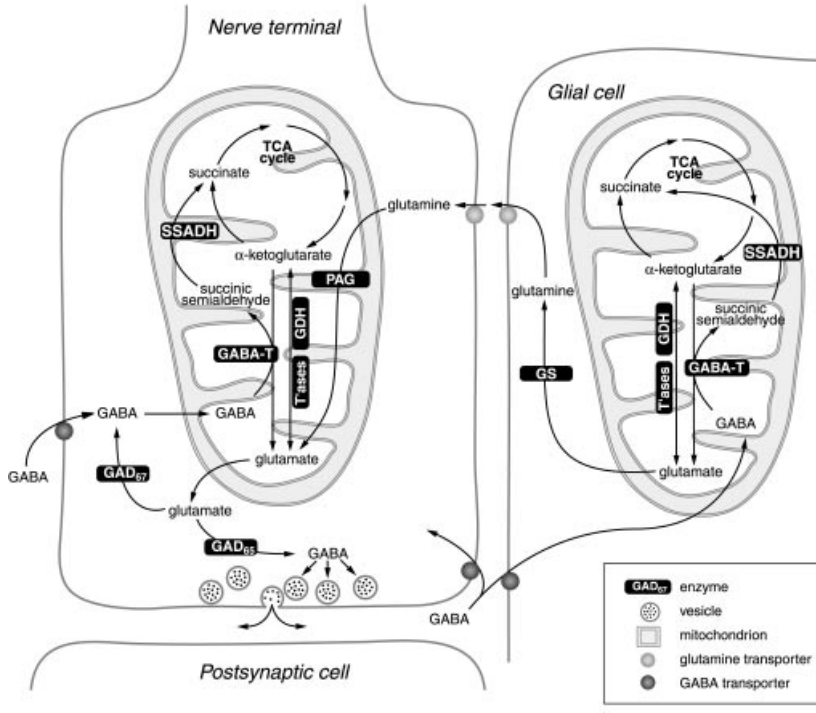

Figure 11.2 Pathways for GABA metabolism. GABA is synthesised in nerve terminals by GAD. GABA produced by both GAD_{67} and GAD_{65} can be used as a neurotransmitter but GAD_{65} is preferentially associated with synaptic vesicles. Synaptically released GABA is recovered into neurons and glia by GABA transporters (not shown is the possible release of GABA by reversal of these transporters). In both neurons and glia, GABA is degraded in mitochondria by GABA-T. Glutamine produced in glial cells is exported to neurons and converted to glutamate (after Soghomonian and Martin 1998)

holoenzyme (holoGAD) complexed with PLP. During the synthetic process GAD can undergo cycles of interconversion between these states. As illustrated in Fig. 11.3, in the primary reaction sequence active holoGAD combines with glutamate to form a complex, which, after decarboxylation, yields an intermediate product that is rapidly converted to GABA and free holoGAD. The intermediate product can also undergo an alternative reaction to produce succinic semialdehyde (SSA) and pyridoxamine-5′-phosphate (PMP) which, unlike PLP, dissociates from GAD to leave inactive apoenzyme, requiring additional PLP to be reactivated.

Traditionally, two processes have been considered important with respect to the regulation of GAD. First, GABA may promote conversion of GAD from its active to its inactive state, and so cause feedback inhibition of GABA synthesis. Second, ATP appears to inhibit, while inorganic phosphate promotes, the reactivation of GAD by PLP. During periods of increased neuronal activity, when the consumption of ATP increases, a rise in the level of phosphate should stimulate the conversion of inactive to active GAD, thereby increasing GABA synthesis. More recently, it has been suggested that soluble and membrane-bound forms of GAD may be differentially regulated. The soluble form of GAD is activated by a phosphatase that causes dephosphorylation while the membrane-bound form is activated following phosphorylation by a vesicular protein kinase (Hsu *et al*. 1999).

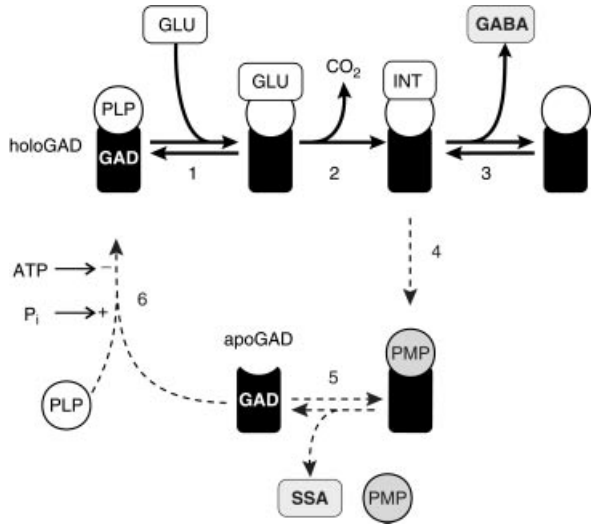

Figure 11.3 Regulation of GAD during the synthesis of GABA. Active GAD (GAD-PLP) combines with glutamate (1) to form a complex (GAD-PLP–GLU). After decarboxylation (2) this yields GABA and GAD-PLP (3). The intermediate product (GAD-INT) can undergo an alternative reaction (4) to produce succinic semialdehyde (SSA) and pyridoxamine-5′-phosphate (PMP). PMP dissociates from GAD (5) leaving inactive enzyme, which requires additional PLP to be reactivated (6), a process that is affected by ATP and inorganic phosphate

Two isoforms of GAD

In addition to the inactive and active states of GAD, there are two distinct forms of the enzyme. The two isoforms, GAD_{67} and GAD_{65}, named for their respective molecular masses ($\sim$67 and $\sim$65 kDa), are encoded by separate, independently regulated genes, *GAD1* and *GAD2* (Erlander *et al.* 1991). GAD_{67} and GAD_{65} differ substantially in their amino-acid sequence, their interaction with PLP, their kinetic properties, and their regulation (Soghomonian and Martin 1998). Individual cells contain both forms of GAD but the ratio of the two differs among different neuronal populations. GAD_{65} is located preferentially in nerve terminals, both in the cytosol and as a membrane-bound form closely associated with synaptic vesicles into which the newly synthesised GABA is accumulated (see below). GAD_{65}, unlike GAD_{67}, is not saturated with PLP and forms the majority of the apoenzyme present in brain (about half of the total GAD). This has led to the view that a proportion of GAD_{65} exists as a pool of inactive enzyme, ready to combine with PLP in response to cellular signals for increased GABA synthesis.

Further insights into the role of GAD isoforms in the synaptic release of GABA have been provided by the techniques of gene manipulation. Mice lacking the GAD_{67} gene have a greatly reduced level of brain GABA (Asada *et al.* 1997). The neurological significance of this reduction is difficult to ascertain: GABA appears essential for the normal development of the palate and one consequence of the reduced production in GABA in these mice is a cleft palate that is responsible for their death soon after birth. In contrast, mice lacking the GAD_{65} gene show only a modest reduction in total brain GABA but exhibit spontaneous seizures and a greater susceptibility to chemical convulsants (Asada *et al.* 1996; Kash *et al.* 1997). In these mice basal GABAergic transmission is normal but GABA release during sustained synaptic activation is reduced (Obata *et al.* 1999; Tian *et al.* 1999). Together these results suggest that GAD_{67} is responsible for the synthesis of

most brain GABA, but GAD_{65} is intimately involved in synthesis of GABA required for the refilling of the releasable pool of synaptic vesicles.

Inhibitors of GAD

Several drugs are known to inhibit GAD, either directly or through interaction with the co-factor PLP. The largest group of inhibitors are the hydrazides, such as isoniazid, semicarbazide and thiosemicarbazide. These are carbonyl-trapping agents that react with the aldehyde group of PLP; as many other enzymes use PLP as a co-factor, these agents are not specific for GAD. Two other agents, allylglycine and 3-mercaptopropionic acid, are competitive inhibitors of GAD. In general, GAD inhibitors reduce the level of GABA in the brain and cause seizures in experimental animals that, in the case of the hydrazides, can be overcome by application of vitamin B_6, the precursor of PLP. Similarly, in humans an inherited defect in pyridoxine metabolism is characterised by a low concentration of GABA in the cerebrospinal fluid, and intrauterine or neonatal seizures that also respond to treatment with vitamin B_6. These findings support the notion that maintained synthesis of GABA is an important factor in the control of overall brain excitability.

STORAGE OF GABA

Within nerve terminals, GABA, like other classical non-peptide neurotransmitters, is stored in synaptic vesicles into which it is accumulated by active transport. The uptake of GABA from the cytosol (where it is present at a concentration of a few millimolar) into the vesicle lumen (where it may reach several 100 millimolar) is dependent on a vesicular protein that transports cytosolic GABA in exchange for lumenal protons. The proton electrochemical gradient that drives this uptake is generated by a H^+-ATPase located in the vesicle membrane. Like vesicular transporters for monoamines and acetylcholine, the 'GABA transporter' recognises more than one substrate, and can also transport glycine (see below).

A gene (*unc-47*) encoding a vesicular GABA transporter was first identified from experiments on the simple nervous system of the nematode worm *C. elegans*. Mammalian homologues were subsequently cloned from rat and mouse; these were named VGAT (for vesicular GABA transporter; McIntire *et al.* 1997) or VIAAT (for vesicular inhibitory amino acid transporter; Sagne *et al.* 1997), respectively. These essentially identical clones have sequences predicting proteins of approximately 520 amino acids with ten transmembrane domains and, when expressed in mammalian cell lines, confer vesicular GABA and glycine transport. Immunohistochemical studies showed that VGAT/VIAAT is concentrated not only in the terminals of GABAergic neurons but also in those of neurons known to use glycine as a neurotransmitter (Gasnier 2000). As yet, no specific blockers or modulators of VGAT/VIAAT activity have been identified.

UPTAKE OF GABA

Once released from a vesicle, GABA molecules are able to activate receptors located on the pre- or postsynaptic membrane before rapidly diffusing out of the synaptic cleft. The ultimate removal of GABA from the extracellular space, and the maintenance of a low extracellular GABA concentration (low micromolar), is achieved by the high-affinity Na^+- and Cl^--dependent uptake of GABA into both GABAergic neurons and glial cells. Like the accumulation of GABA into vesicles, this is a secondary active

transport mechanism, but in this case GABA uptake is coupled to the movement of Na^+ down its electrochemical gradient into the cell.

Drugs which block the uptake of GABA may be beneficial in conditions of reduced GABA function, as they are likely to prolong the action of synaptically released GABA (Thompson and Gahwiler 1992). The uptake of GABA is inhibited by a variety of simple GABA analogues, including nipecotic acid, β-alanine, 2,4-diaminobutyric acid (DABA), *cis*-3-aminocyclohexane-carboxylic acid (ACHC), 4,5,6,7-tetrahydroisoxazolo [4,5-c]pyridin-3-ol (THPO) and guvacine (Fig. 11.4), but as most of these do not cross the blood–brain barrier they have been of experimental interest only. In early studies, a number of compounds were suggested to preferentially inhibit GABA uptake into neurons (DABA and ACHC) or glia (β-alanine and THPO), while others were clearly non-selective (nipecotic acid and guvacine).

Cloned GABA transporters

This simple distinction between glial and neuronal uptake has required revision following the molecular cloning of a family of four Na^+- and Cl^--dependent GABA transporters, each encoded by a different gene: GAT-1, GAT-2, GAT-3 and BGT-1 (reviewed by Palacin *et al*. 1998). The nucleotide sequence of GAT-1 predicts a protein of 599 amino acids with a presumed structure containing twelve membrane-spanning regions. The transport of each GABA molecule into the cell is coupled to the movement of 2 Na^+ and 1 Cl^-. All the GABA transporters share a similar structure, with approximately 50% amino acid identity. GAT-1 appears to be mainly neuronal in origin as its mRNA is found in neurons and it is inhibited more effectively by neuronal than by glial uptake inhibitors. Nevertheless, immunohistochemical studies suggest some expression in glial cells. GAT-2 is found in cells of the ependyma and arachnoid membrane surrounding the brain and may play a role in the regulation of GABA in cerebrospinal fluid (it is also found in other tissues such as liver). GAT-3 is present in brain, principally in glia, but also in some neurons. BGT-1 was isolated from kidney and transports the osmolyte betaine as well as GABA (hence betaine/GABA transporter). It is present in the brain but its precise location and role are unclear.

In parallel with the identification of distinct transporters for GABA there has been continued interest in the development of selective blockers of these transporters and the therapeutic potential that could result from prolonging the action of synaptically released GABA. It has been known for a long time that certain pro-drugs of nipecotic acid (e.g. nipecotic acid ethyl ester) are able to cross the blood–brain barrier and are effective anticonvulsants in experimental models of epilepsy. More recently, several different systemically active lipophillic compounds have been described that act selectively on GAT-1, GAT-2 or GAT-3 (Fig. 11.4). Of these, tiagabine (gabitril), a derivative of nipecotic acid that acts preferentially on GAT-1, has proved clinically useful in cases of refractory epilepsy.

METABOLISM OF GABA

Once recovered into GABAergic nerve terminals or glia, GABA is metabolised to succinic semialdehyde and then to succinate. As detailed above, these reactions are catalysed by GABA-T and SSADH, respectively. The actions of these two enzymes are closely linked. Aminotransferase reactions are reversible but GABA-T breaks down GABA, rather than producing it, because the irreversible action of SSADH rapidly oxidises the product SSA to succinate (Fig. 11.1). SSA may also be reduced by the enzyme succinic semialdehyde reductase (SSAR) to form γ-hydroxybutyric acid (GHB).

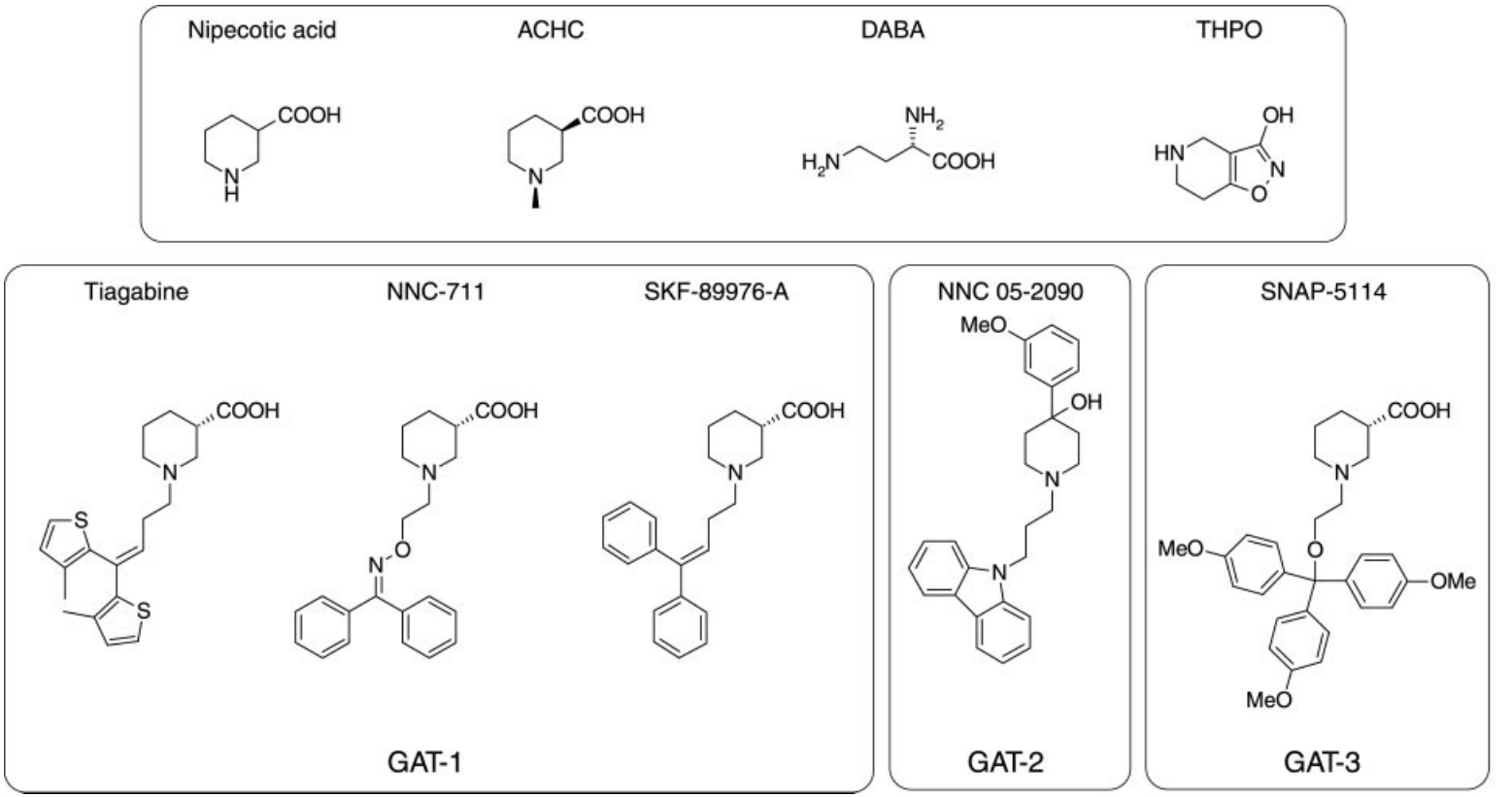

Figure 11.4 Blockers of GABA transport.The upper panel shows the structure of several GABA analogues that inhibit GABA transport into neurons or glia (see text). The lower panels show more recently developed compounds that exhibit selectivity for various cloned GABA transporters

Inhibitors of GABA-T

Inhibition of GABA-T leads to an elevation of brain GABA and, presumably because of an enhanced presynaptic availability of the transmitter, this has an anticonvulsant effect. Inhibitors of GABA-T include aminooxyacetic acid, 5-amino-1,3-cyclohexadienenecarboxylic acid (gabaculine), γ-vinyl GABA (vigabatrin) and 2-propylpenatanoic acid (valproate). The first two are PLP antagonists and are of experimental interest only. Vigabatrin is an irreversible inhibitor of GABA-T and has been used clinically as an anticonvulsant. Valproate is a widely used anticonvulsant but it is not clear to what extent inhibition of GABA-T contributes to its therapeutic properties, as it also inhibits SSADH and SSAR, and inhibits Na^+ currents, thus limiting neuronal firing.

GABA RECEPTORS

The actions of GABA are mediated by receptors belonging to three distinct classes, termed $GABA_A$, $GABA_B$ and $GABA_C$. $GABA_A$ and $GABA_C$ receptors form membrane channels (ionotropic receptors) and their activation leads to an increased permeability to chloride (Cl^-) and bicarbonate (HCO_3^-) ions. $GABA_B$ receptors belong to the family of G-protein-coupled receptors (metabotropic receptors) and can modify the activity of the enzyme adenylate cyclase, suppress transmitter release by directly inhibiting calcium channels or hyperpolarise postsynaptic cells by directly activating potassium channels.

$GABA_A$ RECEPTORS

$GABA_A$ receptors are the most widely expressed of the GABA receptors in the CNS and are found at the vast majority of GABAergic synapses. Binding of two molecules of GABA to the receptor causes the opening of an integral transmembrane anion channel (Bormann, Hamill and Sakmann 1987). As the Cl^- permeability of the channel is approximately five times that of HCO_3^-, under most circumstances the net flux is dominated by Cl^-. Because of this the amplitude and direction of GABA-gated currents, and the resultant transmembrane potential changes, are determined largely by the sign and magnitude of the difference between the membrane potential (V_m) and the chloride equilibrium potential (E_{Cl}).

If Cl^- were passively distributed across the neuronal membrane E_{Cl} would equal V_m. However, neurons possess a variety of transport mechanisms for extrusion or uptake of Cl^- (Kaila 1994). The value of E_{Cl} is dictated by the net result of these chloride-extruding or accumulating mechanisms. Mature central neurons tend to maintain a low intracellular Cl^- through the activity of a Cl^--extruding K^+/Cl^- co-transporter (KCC2). Thus, in many neurons, E_{Cl} is more negative than V_m (although variable, typical values would be -70 and -65 mV, respectively). Under these circumstances, an increase in chloride conductance (g_{Cl}) leads to an influx of Cl^- that results in membrane hyperpolarisation (a movement towards E_{Cl}). This is the classic GABA-mediated inhibitory postsynaptic potential (IPSP). The IPSP transiently (tens of milliseconds) moves the membrane potential to a more hyperpolarised value, away from the threshold for action potential initiation (Fig. 11.5). In cells in which the net Cl^- extrusion is less (because KCC2 or other Cl^--extruding mechanisms are less active), E_{Cl} may be very close to, or slightly positive to, V_m. The effect of GABA is still inhibitory,

as the increase in g_{Cl} tends to hold the membrane potential close to E_{Cl}, thus making it more difficult to trigger an action potential. This is often referred to as 'silent' or 'shunting' inhibition, and may play a role at both pre- and postsynaptic sites.

Neurons also possess mechanisms for Cl^- accumulation. One such is a $Na^+/K^+/2Cl^-$ co-transporter (NKCC1). Activity of NKCC1 can result in E_{Cl} being positive to V_m, such that an increase in g_{Cl} leads to chloride exit from the cell and membrane depolarisation. GABA may still be inhibitory, as long as E_{Cl} is below the threshold for spike initiation. This is thought to be the mechanism underlying one form of $GABA_A$ receptor-mediated presynaptic inhibition, originally identified in primary afferents to spinal neurons and best characterised in nerve terminals of the pituitary (Zhang and Jackson 1995). Here, modest depolarisation of the nerve terminal promotes inactivation of Na^+ channels, limiting the invasion of the action potential and reducing the effectiveness with which it triggers Ca^{2+} influx and transmitter release. NKCC1 is also present in developing neurons, prior to the expression of KCC2. As a result, the hyperpolarising or shunting inhibition, typical of the postsynaptic action of GABA in the adult, is absent. Instead, E_{Cl} is substantially more positive than V_m and GABA may produce sufficient depolarisation to activate voltage-dependent Ca^{2+} channels or trigger action potentials. Thus, in some situations in the developing nervous system GABA may function effectively as an excitatory neurotransmitter. The shift during development to an inhibitory action reflects the increased expression of the Cl^--extruding KCC2 (Rivera *et al.* 1999). One interesting example of the flexibility of this system is seen in cells of the suprachiasmatic nucleus of the hypothalamus (a brain region involved in the generation of circadian rhythms). Here, GABA is inhibitory during the night but excitatory during the day. This seems to result from diurnal variations in E_{Cl} such that during the night the GABA reversal potential is negative to V_m while during the day it is more positive than V_m so that GABA depolarises the cell above spike threshold (Wagner *et al.* 1997).

$GABA_A$ RECEPTOR PHARMACOLOGY

$GABA_A$ receptors are classically defined by their sensitivity to the antagonist bicuculline. Other widely used antagonists include SR-95531 (gabazine), picrotoxin, and cage convulsants such as TBPS (*t*-butylbicyclophosphorothionate). Like bicuculline, SR-95531 is a competitive antagonist acting at the GABA binding site while picrotoxin and TBPS are non-competitive, acting at a site which may be more closely associated with the chloride ion channel. Of these antagonists, only bicuculline is selective, as the others also act at $GABA_C$ receptors (see below). Similarly, no truly selective $GABA_A$ agonist is known. Muscimol is commonly used to activate $GABA_A$ receptors but it is also a partial agonist at $GABA_C$ receptors, while THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) is a partial agonist at $GABA_A$ receptors and an antagonist at $GABA_C$ receptors (Chebib and Johnston 2000). In addition to these agonists and antagonists, an enormous range of structurally diverse compounds can affect $GABA_A$ receptors. The most important of these are the benzodiazepines, barbiturates, neuroactive steroids and various general anaesthetics. The various sites of action of these drugs are depicted schematically in Fig. 11.6.

Benzodiazepines

Benzodiazepines such as chlordiazepoxide (Librium) and diazepam (Valium) were discovered in the early 1960s and found to have clinically important anxiolytic,

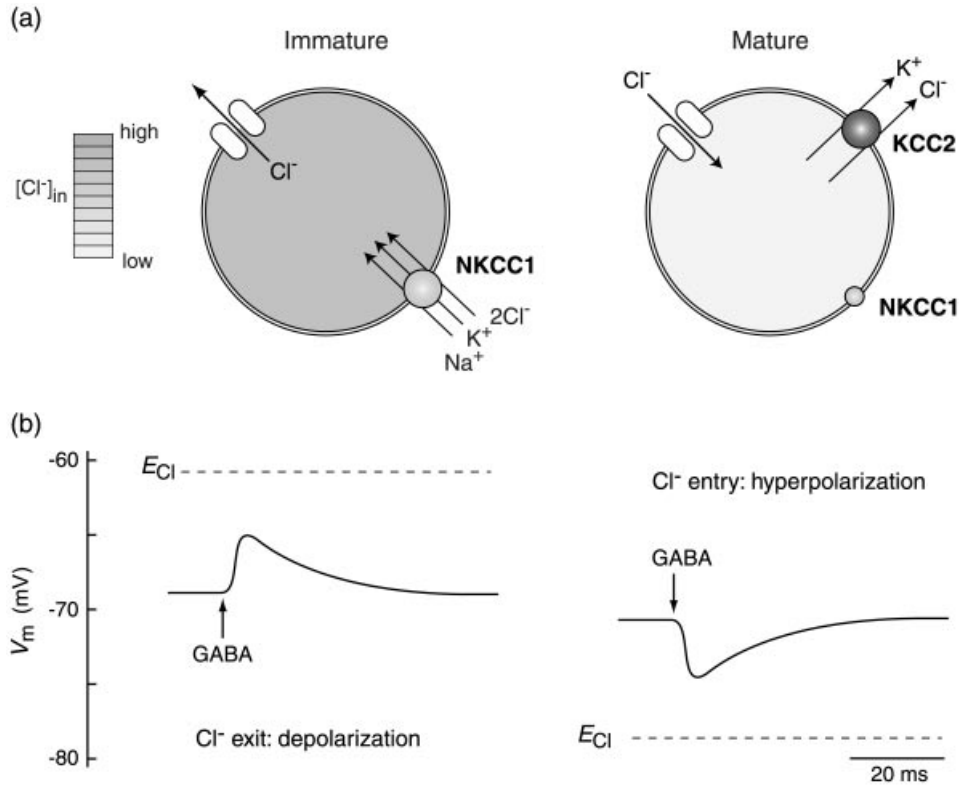

Figure 11.5 Chloride distribution and the $GABA_A$ response. The change in membrane voltage (V_m) that results from an increase in chloride conductance following activation of $GABA_A$ receptors is determined by the resting membrane potential and the chloride equilibrium potential (E_{Cl}). (a) Immature neurons accumulate Cl^- via NKCC, while mature neurons possess a Cl^--extruding transporter (KCC2). (b) In immature neurons $GABA_A$ receptor activation leads to Cl^- exit and membrane depolarisation while in mature neurons the principal response is Cl^- entry and hyperpolarisation. This is the classic inhibitory postsynaptic potential (IPSP)

anticonvulsant and muscle relaxant properties. It was not until the late 1970s, however, that their mode of action was established. Then, in studies of cultured neurons, benzodiazepines were found to increase the conductance change produced by GABA, shifting the GABA concentration–response curve to the left. They had no effect on conductance in the absence of GABA. At approximately the same time, specific high-affinity binding sites for benzodiazepines were identified in the CNS using radiolabelled compounds. A significant correlation between the binding affinity of various benzodiazepines and their potency in behavioural tests suggested that these sites mediated the central effects of benzodiazepines. A close association between benzodiazepine binding sites and the GABA receptor was indicated by the discovery of increased binding of benzodiazepines in the presence of GABA and increased binding of GABA in the presence of benzodiazepines.

The term 'benzodiazepine' refers to a specific chemical structure. Numerous benzodiazepine-receptor ligands exist which have different structures. These include the β-carbolines (e.g. methyl-6,7-dimethoxy-4-ethyl-β-carboline 3-carboxylate; DMCM), triazolopyridazines (e.g. CL 218872), imidazopyridines (e.g. zolpidem), and pyrazoloquinolinones (e.g. CGS 8216). In experimental animals these compounds produce

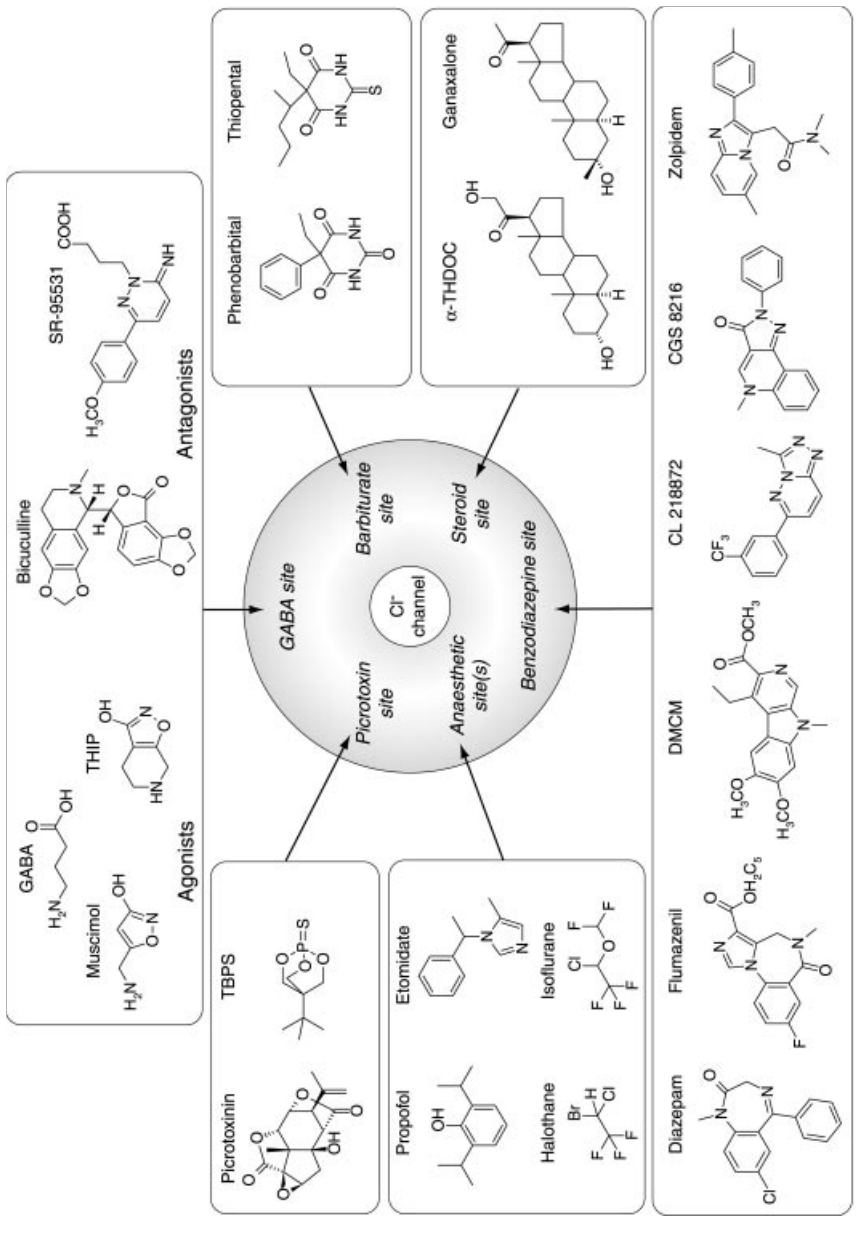

Figure 11.6 Schematic representation of the $GABA_A$ receptor complex. Examples of the many structurally diverse compounds that act at different sites on the receptor (see text for details). Picrotoxinin, the active component of picrotoxin, and TBPS act as non-competitive antagonists. The barbiturates, steroids and anaesthetics are positive allosteric modulators, as are the benzodiazepine site ligands shown, with the exception of DMCM (negative allosteric modulator) and flumazenil (benzodiazepine site antagonist)

effects ranging from anticonvulsant and anxiolytic (benzodiazepine-like) to proconvulsant/convulsant and anxiogenic. Between these two extremes are compounds, such as the imidazodiazepine flumazenil, that display only a limited degree of intrinsic activity but which are capable of antagonising the effects of the clinically useful benzodiazepines as well as those of the convulsant ligands or so-called 'inverse agonists' (negative allosteric modulators). All the compounds appear to act at the same or overlapping sites on the receptor complex. In studies of $GABA_A$ receptor single-channel currents, anxiolytic benzodiazepines, such as diazepam, increase the response to GABA but do not generally change the conductance of individual Cl^- channels. Instead they increase the affinity of the receptor for GABA and, in steady-state experiments, increase the frequency of channel opening, in a manner equivalent to increasing the concentration of GABA. At GABAergic synapses such compounds prolong the decay of the postsynaptic current and may also increase its peak amplitude. Inverse agonists such as DMCM reduce the response to GABA by decreasing the frequency of channel opening.

Barbiturates

Like benzodiazepine agonists, barbiturates possess sedative, anxiolytic and anticonvulsant properties. Although certainly not their only site of action, sedative barbiturates, such as phenobarbitone or the clinically used intravenous anaesthetic thiopentone, cause a marked potentiation of GABA responses. Unlike benzodiazepines, barbiturates increase the time for which GABA-activated channels are open and increase the length of bursts of openings. At higher concentrations barbiturates can activate Cl^- channels even in the absence of GABA. Neither effect is due to an action at the benzodiazepine binding site, as they are not blocked by the benzodiazepine antagonist flumazenil.

Steroids

It has been known since the 1940s that steroids of the pregnane series have anaesthetic properties. These early studies led to the development of the intravenous anaesthetic althesin, the active component of which is alphaxolone (3α-hydroxy-5α-pregnan-11,20 dione). This compound has barbiturate-like actions and is also able to directly activate Cl^- channels in the absence of GABA. These properties are shared by several endogenous steroids (synthesised in the brain or adrenal glands), the most potent being the reduced metabolite of progesterone, 3α-hydroxy-5α-pregnan-20-one (allopregnanolone or 3α,5α-THP) and the reduced metabolite of dexoycorticosterone, 3α,21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone or α-THDOC). Particular interest in these compounds stems from the fact that they may act as endogenous modulators of $GABA_A$ receptors and their levels are altered by stress as well as during the menstrual cycle and pregnancy. For example, during menstruation decreasing levels of progesterone result in a decline in the production of allopregnanolone. Recently it has been demonstrated that such an abrupt decline (akin to drug withdrawal) can cause changes in the properties of $GABA_A$ receptors that may underlie the symptoms associated with premenstrual syndrome, including increased susceptibility to seizures and insensitivity to benzodiazepine agonists. Steroids appear to act at a distinct site on the GABA-receptor complex, as flumazenil does not block their action, and the Cl^- currents they evoke directly can be potentiated by barbiturates (and vice versa). The 3β-methyl-substituted synthetic analogue of allopregnanolone, ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one) is less easily metabolised than its endogenous parent

compound, allowing activity following oral administration, and is currently under investigation as an anticonvulsant.

Anaesthetics

Steroids, such as alphaxolone, and barbiturates, such as thiopentone, represent only two classes of the many structurally diverse molecules found to induce general anaesthesia. Although a number of these clearly have actions on a range of targets, including glycine, 5-HT_3, nicotinic and glutamate receptors, all, with the exception of the dissociative anaesthetic ketamine, have an effect on $GABA_A$ receptors at relevant concentrations. For example, the intravenous anaesthetic agents propofol, propanidid and etomidate markedly enhance responses to GABA (apparently by prolonging bursts of Cl^- channel openings) and are capable of directly evoking Cl^- currents. The currents produced by these agents at high doses, as well as those caused by steroids and barbiturates, are blocked by bicuculline, indicating that they are due to activation of the Cl^- channel associated with the $GABA_A$ receptor. It is also now clear that volatile anaesthetics such as halothane and isoflurane as well as alcohols (including ethanol), rather than having non-specific membrane-disrupting actions, owe at least some of their properties to a potentiation of GABA responses, through a direct interaction with sites on $GABA_A$ receptors.

STRUCTURE OF $GABA_A$ RECEPTORS

Over the past decade or so significant advances have been made in our understanding of the structure of the $GABA_A$ receptor, which is now known to be formed by the assembly of multiple subunit proteins. In 1987 two subunits of the receptor, designated α and β, were cloned (Schofield *et al.* 1987). Following on from this work, 16 mammalian subunits encoded by distinct genes have now been identified. These genes encode proteins of approximately 450–550 amino acids (depending on the species) which, according to their sequence similarities, have been grouped into seven families — α, β, γ, δ, ε, π and θ (Barnard *et al.* 1998; Bonnert *et al.* 1999). The α, β and γ families contain multiple isoforms ($\alpha1$–$\alpha6$, $\beta1$–$\beta3$ and $\gamma1$–$\gamma3$) and in a number of cases additional complexity is generated by alternative mRNA splicing.

The subunits share varying degrees of sequence identity but have a similar predicted tertiary structure. This consists of four membrane-spanning α-helices (M1–M4), a large extracellular N-terminal region, a large intracellular domain between M3 and M4 and a short extracellular C-terminal portion (Fig. 11.7). The highest degree of conservation is in the transmembrane regions and the greatest variation in the intracellular loop between M3 and M4. The extracellular domain contains potential N-linked glycosylation sites and a β-loop formed by a disulphide bridge between two cysteine residues. The intracellular loops of β and γ subunits contain sites for phosphorylation by a variety of protein kinases, including cAMP-dependent protein kinase, cGMP-dependent protein kinase, protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase and tyrosine kinase, which may be important in the regulation of receptor function. These general features are very similar to those of two other ligand-gated ion channels, the nicotinic acetylcholine receptor and the glycine receptor (see below) and there is a considerable degree of sequence homology among these proteins. By analogy with the nicotinic acetylcholine receptor, it is thought that the $GABA_A$ receptor is formed by the assembly of five subunits around a central ion channel, with the M2 region of each subunit forming the lining of the channel (Fig. 11.7).

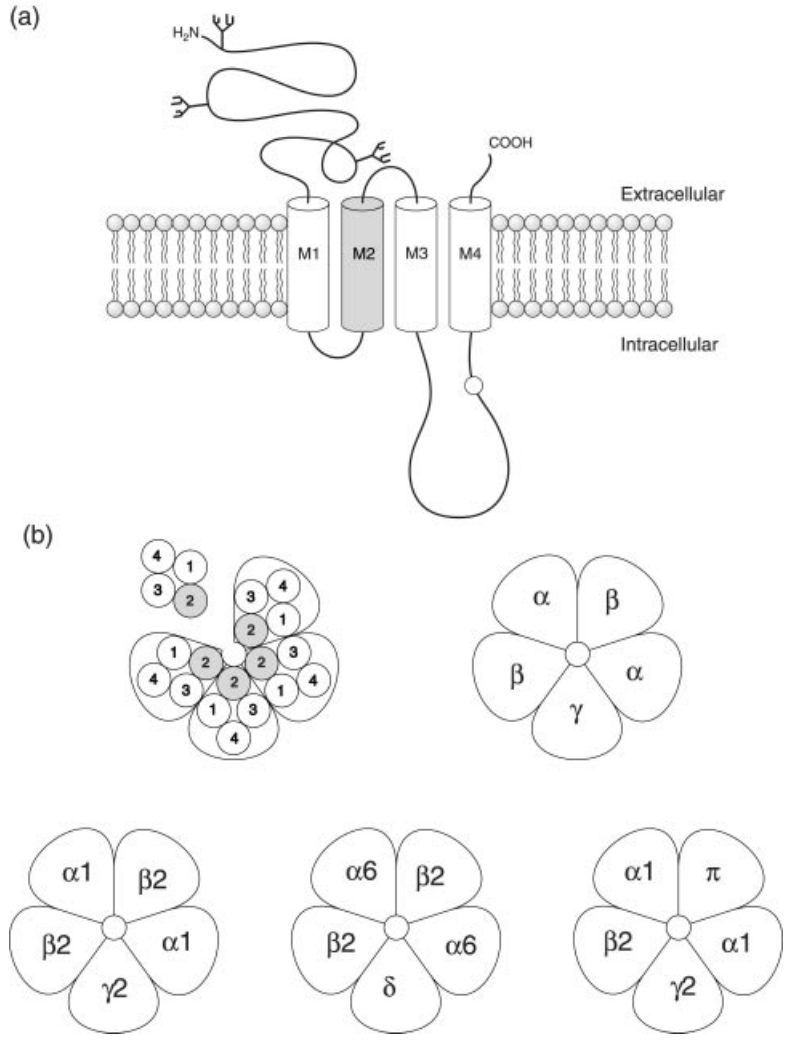

Figure 11.7 Presumed arrangement of $GABA_A$ receptor subunits to form a receptor–channel complex. (a) Diagrammatic representation of an individual subunit with four transmembrane regions, extracellular sites for glycosylation and a site for phosphorylation on the intracellular loop between M3 and M4. (b) Association of five subunits to form a central ionophore bounded by the M2 region of each subunit. The suggested stoichiometry of the most widely expressed form of receptor is 2α, 2β and 1γ. Shown below are the possible subunit combinations of one such benzodiazepine-sensitive receptor together with a benzodiazepine-insensitive receptor in which the γ subunit is replaced by a δ, and a π-containing receptor with four different subunit types

Subunit combinations and receptor function

Expression studies in *Xenopus* oocytes or transfected cell lines originally suggested that functional GABA-activated chloride channels could be formed by receptor subunits of each class in isolation. However, much better expression occurs with two or more subunit types in combination and it is likely that most native receptors contain at least three different subunits. Co-expression of α and β subunits results in the assembly of

functional receptors that can be activated by GABA and are sensitive to the antagonists bicuculline and picrotoxin and show modulation by barbiturates. But only when a γ subunit is expressed in conjunction with an α and a β subunit is benzodiazepine binding and potentiation of GABA seen. As benzodiazepines do not bind to γ subunits alone, it is likely that the conformation of the receptor is appropriate for benzodiazepine binding only when all three subunit types are present.

The large number of cloned subunit proteins makes it clear that $GABA_A$ receptors themselves must be diverse. An illustration of this diversity is provided by the pharmacology of benzodiazepine ligands. Even before the existence of $GABA_A$ receptor subunits was recognised, variations in the binding of radiolabelled drugs to native benzodiazepine receptors had led to the suggestion that not all GABA receptors were the same. Two types of benzodiazepine receptor were postulated — BZI and BZII. These had similar affinity for agonists such as diazepam and antagonists such as flumazenil, but BZI receptors showed a higher affinity for triazolopyridazines (e.g. CL 218872) and β-carbolines (e.g. β-CCM). It is now clear that the molecular basis for these differences resides in the variety of α subunits. Thus, while γ subunits are required for benzodiazepine binding, the precise nature of this interaction depends on the type of α subunit present. Heteromeric recombinant receptors ($\alpha\beta\gamma$) containing an α1 subunit exhibit BZI-type pharmacology, receptors containing α2, α3 or α5 subunits exhibit BZII pharmacology, while receptors containing α4 or α6 subunits have a low affinity for both benzodiazepines and β-carbolines. Studies involving site-directed mutagenesis of the various subunits have narrowed down even further the precise amino acid residues responsible for these differences in benzodiazepine pharmacology, as well as those involved in the binding of GABA. Altogether, such data suggest that GABA molecules bind at the interface of α and β subunits while benzodiazepines bind at the interface of α and γ subunits.

The complexity afforded by different α, β and γ subunits is increased further by the existence of the δ, ε, θ and π subunits. The δ subunit preferentially associates with the α4 and α6 subunits. Receptors containing this subunit are unusual in having a particularly high affinity for GABA and muscimol and a reduced sensitivity to benzodiazepines and neurosteroids. The most recently cloned subunits, ε, θ and π, are the least well understood. The sequence of the ε subunit is most closely related to that of the γ subunits but studies in recombinant expression systems show that it assembles with α and β subunits to form receptors that are insensitive to benzodiazepines and show altered sensitivity to anaesthetics (pregnanolone, pentobarbital and propofol). The θ subunit is most closely related to the β subunits; it coassembles with α, β and γ subunits to form receptors with a low affinity for GABA, although other subunit combinations (notably $\alpha\beta\theta\varepsilon$ or $\alpha\theta\varepsilon$) have been suggested. The ε and θ subunits have a fairly restricted pattern of expression that includes the hypothalamus and brainstem nuclei such as the locus coeruleus. The sequence of the π subunit is most closely related to that of the β subunits. Unlike the other $GABA_A$ subunits it is principally found in peripheral tissues, including lung, thymus, prostate and particularly the uterus.

Heterogeneity of native $GABA_A$ receptors

Given that the pharmacological and biophysical properties of recombinant $GABA_A$ receptors have been shown to depend critically on their subunit composition, much effort has been directed towards understanding the assembly of native receptors. This could provide a rational basis for the design of compounds able to interact with specific

subpopulations of $GABA_A$ receptors in different brain regions that may be involved in different aspects of brain function. Clearly, many hundreds of different receptor types could arise from the assembly of 16 different subunits into a pentameric structure. However, numerous studies, involving the use of subunit-specific antibodies to localise or to purify receptor populations, have suggested that the restricted distribution and preferential assembly of these subunits results in the generation of no more than a dozen favoured receptor types. Of these, the most common receptor type is composed of $\alpha 1$, $\beta 2$ and $\gamma 2$ subunits. Several lines of evidence suggest that the most likely stoichiometry of these receptors is 2α, 2β and 1γ (although assemblies containing 2α, 1β and 2γ have also been described). As indicated above, in less widely expressed assemblies, the δ or ε subunits can substitute for the γ subunit, while the π and θ subunits may co-assemble with α, β *and* γ subunits.

Advances in our understanding of the functional significance of $GABA_A$ receptor heterogeneity have also come from studies of mice lacking specific subunit genes or expressing altered receptor subunits. To date, mutant mice have been generated that lack the $\alpha 1$, $\alpha 6$, $\beta 2$, $\beta 3$, $\gamma 2$ or δ subunits (Rudolph *et al.* 2001; Sur *et al.* 2001). In the case of the $\gamma 2$ subunit deletion, neurons cultured from newborn mice show a complete lack of sensitivity to benzodiazepines (Gunther *et al.* 1995). By introducing a histidine residue (instead of the normal arginine) at position 101 in the $\alpha 1$ subunit of mice — making receptors containing this subunit insensitive to benzodiazepines — it has also been possible to determine which of the various effects of benzodiazepines are mediated by $\alpha 1$-containing receptors and which by receptors containing $\alpha 2$, $\alpha 3$ or $\alpha 5$ subunits. This approach showed that $\alpha 1$-containing $GABA_A$ receptors are involved in the sedative and amnesic actions of benzodiazepines (McKernan *et al.* 2000; Rudolph *et al.* 1999). Complementary experiments have shown that the anxiolytic actions of benzodiazepines are mediated by $\alpha 2$-containing receptors and the muscle-relaxant actions by $\alpha 2$- and $\alpha 3$-containing receptors (Rudolph *et al.* 2001; Crestani *et al.* 2001).

$GABA_B$ RECEPTORS

$GABA_B$ receptors are found in both the peripheral nervous system and CNS. They were first identified in the late 1970s, during studies of noradrenaline release from axon terminals of sympathetic post-ganglionic fibres in rat atria. GABA was found to reduce the evoked release of transmitter but this action was not blocked by the conventional antagonists bicuculline and picrotoxin. The effect of GABA was mimicked not by muscimol but by the compound (*R*)-4-amino-3-(4-chlorophenyl)butanoic acid (baclofen), a GABA analogue that has no effect on GABA receptors linked to Cl^- channels. To distinguish between the established receptors and the newly identified bicuculline-insensitive receptors the terms $GABA_A$ and $GABA_B$ were introduced (reviewed in Bowery 1993).

$GABA_B$ RECEPTOR PHARMACOLOGY

Baclofen and the phosphinic analogue of GABA, 3-aminopropyl phosphinic acid (APPA), selectively activate $GABA_B$ receptors. The first antagonists identified were 2-OH-saclofen and phaclofen, the sulphonic and phosphonic acid analogues of baclofen, respectively (Kerr and Ong 1995). In recent years, a number of more potent and systemically active antagonists have been developed. These include CGP 54626 and GCP 62349 (Fig. 11.8), the high affinity of the latter proving instrumental in the

eventual cloning of $GABA_B$ receptors (see below). As yet, no modulatory compounds of the type described for $GABA_A$ receptors have been identified.

$GABA_B$ RECEPTOR MECHANISMS

Depending on cell type and the location of the receptor on neurons, $GABA_B$ receptors act via G-proteins to affect the activity of either Ca^{2+} channels, K^+ channels or adenylate cyclase (Bowery and Enna 2000). For example, in dorsal root ganglion (DRG) neurons baclofen was found to inhibit the Ca^{2+}-dependent phase of the DRG action potential, an effect attributed to block of voltage-activated Ca^{2+} currents. A similar action on presynaptic Ca^{2+} channels was presumed to underlie the block of neurotransmitter release by baclofen. This has now been demonstrated by recording Ca^{2+} currents from presynaptic terminals directly (Takahashi, Kajikawa and Tsujimoto 1998). In the CNS such presynaptic $GABA_B$ receptors are found on terminals releasing a variety of transmitters, as well as on GABAergic terminals themselves, where they may act as autoreceptors.

In several neuronal types baclofen was also shown to cause a postsynaptic hyperpolarisation due to the opening of K^+ channels (E_K is more negative than V_m). These postsynaptic $GABA_B$ receptors can be activated by synaptically released GABA and give rise to a delayed and long-lasting (hundreds of milliseconds) hyperpolarisation which, following as it does the $GABA_A$ receptor-mediated fast IPSP, is often referred to as the 'late IPSP' (Dutar and Nicoll 1988). In general, both types of $GABA_B$ response can be blocked by the same antagonists or by treatment with *Pertussis* toxin (which blocks the activation of G-proteins of the $G_{i/o}$ class). However, at some sites presynaptic effects of baclofen are not blocked by all agents, indicating that multiple types of $GABA_B$ receptor may exist (see below).

STRUCTURE OF $GABA_B$ RECEPTORS

$GABA_B$ receptors long resisted attempts at expression cloning of the type used to identify $GABA_A$ receptor subunits, partly because the requirement for G-protein coupling to ion channels made functional assays in cell lines or oocytes difficult to devise. Recently, however, a $GABA_B$ receptor ($GABA_BR1$) was successfully isolated from a rat cDNA library by screening transfected cells with a high-affinity radiolabelled $GABA_B$ receptor antagonists, binding of which does not require the presence of G-proteins (Kaupmann *et al.* 1997). Two isoforms of the receptor were identified which differed only in the length of their amino-terminals — these were termed $GABA_BR1a$ (960 amino acids) and $GABA_BR1b$ (844 amino acids). Additional isoforms, both termed $GABA_BRIc$, have also been identified in rat (Pfaff *et al.* 1999) and human (Ng *et al.* 2001). Each protein has a predicted structure consisting of a large N-terminal and seven transmembrane domains, similar to metabotropic glutamate receptors.

The cloned receptors, when expressed in cell lines and studied by radioligand binding assays, showed some of the expected pharmacology of $GABA_B$ receptors. However, the affinity of agonists was much lower than seen with native receptors and not all expected coupling to effector systems could be demonstrated (possibly because of inappropriate or inefficient linkage to G-proteins). Subsequently, a second receptor protein, $GABA_BR2$, was identified (Jones *et al.* 1998; Kaupmann *et al.* 1998; White *et al.* 1998), and shown to interact with $GABA_BR1$, through the coiled regions of their intracellular C-termini, to form fully functional heterodimers (Fig. 11.8). $GABA_BR1$

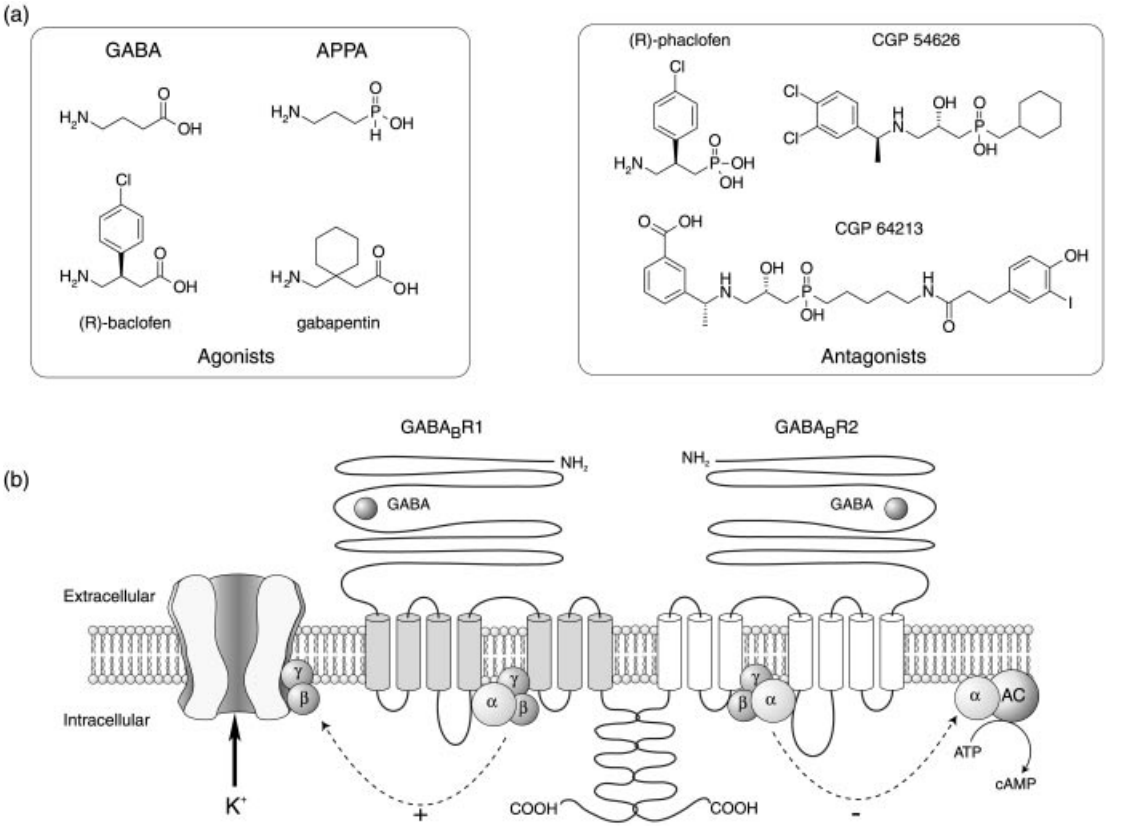

Figure 11.8 Pharmacology and structure of $GABA_B$ receptors. (a) Various $GABA_B$ agonists and antagonists described in the text. (b) A $GABA_B$ receptor shown as a dimer containing one copy of $GABA_BR1$ and one copy of $GABA_BR2$, joined by their coiled intracellular carboxy-terminals. The large extracellular amino-terminals are the proposed sites of GABA binding. A G-protein is shown linked to each of the $GABA_BR$ proteins. The activated β/γ subunits trigger the opening of a K^+ channel while the α subunit is shown inhibiting the activity of adenylate cyclase (AC). At presynaptc sites the β/γ subunits would inhibit a Ca^{2+} channel (after Bowery and Enna 2000)

and $GABA_BR2$ are found in areas of the brain known to contain $GABA_B$ receptors, including hippocampus, cerebellum and cortex, but some differences in their distribution suggest that in certain cases homomeric $GABA_BRs$ may be functional or that dimerisation may occur with other unidentified $GABA_BRs$ (Bowery and Enna 2000). The existence of structurally and pharmacologically distinct pre- and postsynaptic $GABA_B$ receptors is supported by the recent demonstration that gabapentin, an anticonvulsant GABA analog, is a selective agonist for postsynaptic $GABA_BR1a/R2$ heterodimers coupled to K^+ channels (Ng *et al.* 2001).

$GABA_C$ RECEPTORS

Early studies of the action of GABA and its analogues on spinal neurons revealed that the depressant action of one of these, *cis*-4-aminocrotonic acid (CACA), was not blocked by bicuculline. Several analogues of GABA shared the same properties and did not interact with the then newly described $GABA_B$ receptors. In 1984, the term $GABA_C$ was introduced to distinguish this third class of GABA receptor (Johnston 1996). Like $GABA_A$ receptors, $GABA_C$ receptors activate anion channels permeable to Cl^- (and HCO_3^-) and the responses are similarly governed by the distribution of Cl^- across the neuronal membrane.

$GABA_C$ RECEPTOR PHARMACOLOGY

$GABA_C$ receptors are defined by their insensitivity to bicuculline and their activation by conformationally restricted analogues of GABA such as CACA and (+)-CAMP (1*S*,2*R*-2-(aminomethyl)cyclopropanecarboxylic acid). They are blocked by picrotoxin but can be selectively antagonised by TPMPA (1,2,5,6-tetrahydropyridin4-ylphosphinic acid). Unlike $GABA_A$ receptors, they are not affected by benzodiazepines, barbiturates or anaesthetics (Barnard *et al.* 1998; Bormann 2000; Chebib and Johnston 2000).

STRUCTURE OF $GABA_C$ RECEPTORS

The best evidence for the existence of functional $GABA_C$ receptors and the clearest indication as to their molecular identity comes from work on the retina. Expression of retinal mRNA in *Xenopus* oocytes produces GABA-gated chloride channels with conventional $GABA_A$ receptor pharmacology as well as channels with characteristics of $GABA_C$ receptors (i.e. blocked by picrotoxin but not bicuculline). The basis of this distinction was made clear with the cloning from a retinal cDNA library of a new GABA receptor subunit termed ρ (Cutting *et al.* 1991). To date, three ρ subunits have been identified in mammals ($\rho 1$–$\rho 3$). Originally classed as $GABA_A$ subunits, with which they have $\sim$35% sequence identity, they are now accepted as a distinct group of subunits, forming the basis of the relatively simple, and evolutionarily older, $GABA_C$ receptors. Unlike subunits of the $GABA_A$ receptor, ρ subunits form fully functional homomeric receptors and do not co-assemble with α or β subunits. These homomeric receptors are similar to native $GABA_C$ receptors, in that they are activated by GABA and CACA, blocked by picrotoxin and TPMPA but not bicuculline, and unaffected by barbiturates, benzodiazepines or anaesthetics (Fig. 11.9). Receptors formed from ρ subunits have a higher affinity for GABA than many $GABA_A$ receptors formed from $\alpha\beta\gamma$ combinations, have a lower single-channel conductance and produce currents that decay more slowly after removal of GABA.

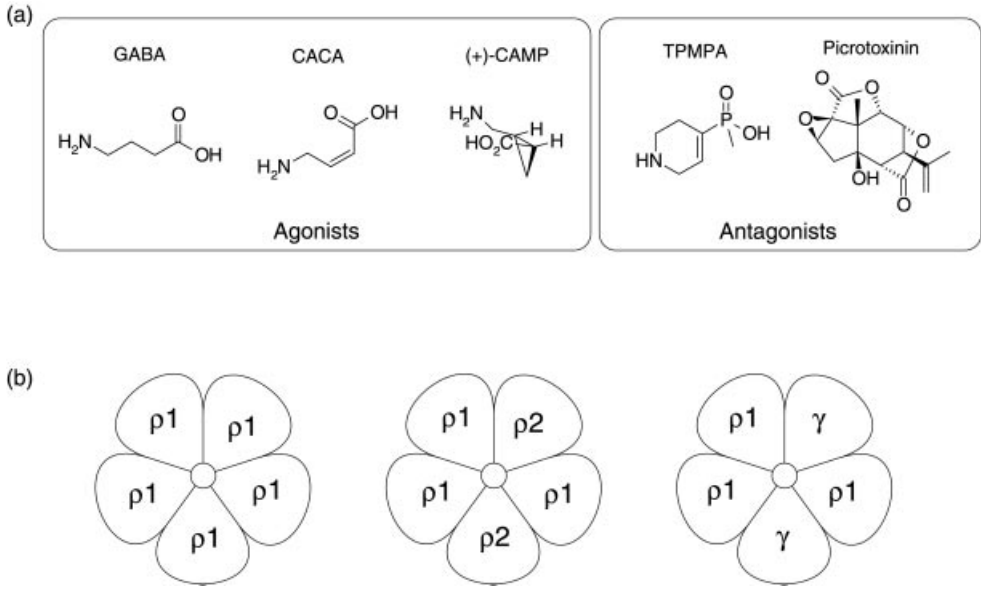

Figure 11.9 $GABA_C$ receptor pharmacology and structure. (a) Various $GABA_C$ agonists and antagonists described in the text. Picrotoxinin is the active component of picrotoxin and also acts at $GABA_A$ receptors. (b) Presumed subunit structures of $GABA_C$ receptors. The receptors can form as homomeric assemblies of ρ subunits but native receptors may be heteromeric assemblies of ρ subunits (e.g. $\rho 1$ and $\rho 2$) or may contain both ρ and γ subunits

In the retina, electrophysiological data indicate that $GABA_C$ receptors are present on horizontal and bipolar cells, and ρ subunits have been localised to subsets of synapses formed by amacrine cells onto the axon terminals of rod bipolar cells. Activation of these presynaptic receptors inhibits glutamate release from the bipolar cells. However, the true molecular composition of native $GABA_C$ receptors is still under investigation. While homomeric receptors formed from ρ subunits share many features of retinal $GABA_C$ receptors, a number of discrepancies have been noted in the details of ion permeability, single-channel conductance and channel open time (Wotring, Chang and Weiss 1999). Thus, it has been suggested that native $GABA_C$ receptors may be composed of heteromeric assemblies of ρ subunits or, in certain cases, that such assemblies may also contain a $\gamma 2$ subunit (Qian and Ripps 1999). All three ρ subunits have been identified in brain, but their precise location and the functional significance of this expression is unclear. In particular, the basis of $GABA_C$ receptor-like responses seen, for example, in the spinal cord, cerebellum, optic tectum and hippocampus is yet to be determined.

GLYCINE

Glycine is the simplest of all amino acids. It is involved in many metabolic pathways, is an essential component of proteins, and is found throughout the brain. A neurotransmitter role for glycine was first identified in the spinal cord, where it was found to be differentially distributed between dorsal and ventral regions and shown to cause hyperpolarisation of motoneurons (Werman *et al*. 1967). This inhibitory action of glycine is distinct from its

subsequently identified role as a co-agonist at NMDA-type glutamate receptors (Chapter 10), and is mediated by receptors that share many features with $GABA_A$ receptors (see below). Glycine-mediated neurotransmission plays a key role in spinal cord reflexes, mediating reciprocal and recurrent inhibition of motoneurons by Renshaw cells, and is important in motor control and sensory pathways. Glycine receptors are also found in higher brain centres including the hippocampus, cortex and cerebellum.

NEUROCHEMISTRY OF GLYCINE

SYNTHESIS AND CATABOLISM OF GLYCINE

The details of glycine metabolism within neural tissue are poorly understood, and it is unclear to what extent neurons depend on *de novo* synthesis or uptake of glycine. Two enzymes are important in glycine metabolism; serine hydroxymethyltransferase (SHMT), which is thought to be present in the mitochondria of both neurons and glia, and the four-enzyme complex known as the glycine cleavage system (GCS), present in glia. SHMT catalyses the interconversion of L-serine and glycine while GCS catalyses the breakdown of glycine. Within neurons the action of SHMT leads to the conversion of L-serine to glycine, while in glia the coupling of SHMT and GCS results in the conversion of glycine to L-serine (Verleysdonk *et al*. 1999). The L-serine derived from glycine may be further metabolised, or released from glial cells to be taken up into neurons, forming a cycle analogous to the glutamine–glutamate cycle shown in Fig. 11.2. Glycine can also be formed by the action of aminotransferases (such as alanine-glyoxylate transaminase or glycine transaminase), in which the amino group from a donor amino acid is transferred onto glyoxlate, producing glycine and a keto acid.

STORAGE OF GLYCINE

Glycine, like GABA, is stored in synaptic vesicles. As described above, it seems likely that a common transport mechanism (VIAAT) is responsible for the accumulation of both amino acids. This lack of absolute specificity in the vesicular transporter means that the 'phenotype' of a neuron (GABAergic or glycinergic) is dictated by the relative concentrations of GABA and glycine in the cytosol. This will be determined by the expression of the respective biosynthetic enzymes and plasma membrane transporters. In certain cases neurons may release both GABA *and* glycine, which have been packaged into the same vesicles (Jonas, Bischofberger and Sandkuhler 1998; O'Brien and Berger 1999). The extent and significance of such co-release is unclear, but its effects will obviously depend on the types of pre- and postsynaptic receptors present at the synapse. Possible benefits of co-release may stem from the different kinetic properties of $GABA_A$ and glycine receptors, the ability to activate $GABA_B$ receptors or the modulatory action of glycine at NMDA receptors.

UPTAKE OF GLYCINE

Glycine is removed from the extracelluar space by high-affinity uptake into neurons and glia. Five glycine transporters have been identified in the CNS of mammals. All are members of the Na^+- and Cl^--dependent family transporters and are encoded by two independently regulated genes, *GLYT1* and *GLYT2*. Three GLYT1 isoforms (1a, b and c) and two GLYT2 isoforms (2a and b) are generated by alternative splicing (reviewed by

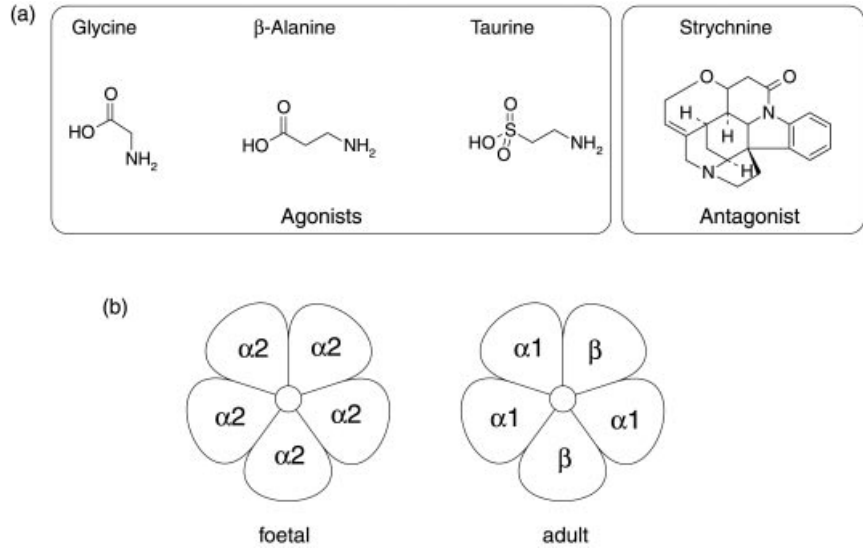

Figure 11.10 Glycine receptor pharmacology and structure. (a) Amino acids that act as agonists at glycine receptors, and strychnine a competitive antagonist. (b) Subunit composition of foetal and adult glycine receptors in the spinal cord. The receptors are shown with a pentameric assembly but the α and β subunits are distinct from those that form $GABA_A$ receptors. Picrotoxin is also an effective glycine antagonist and in recombinant systems is selective for homomeric receptors

Palacin *et al.* 1998). GLYT2 is found in neurons and GLYT1 is found predominantly in glia. The distribution of the transporters with respect to glycine receptors has led to the suggestion that both transporters are associated with glycinergic synapses, while GLYT1 may also regulate extracellular glycine levels at glutamatergic synapses and hence affect the activity of NMDA receptors. Relatively few selective blockers of glycine uptake have been described. GLYT1 isoforms are inhibited by sarcosine (*N*-methyl glycine) and various lipophillic derivatives of sarcosine, including NFPS (*N*[3-(4′-fluorophenyl)-3-(4′-phenyl-phenoxy)propyl]sarcosine) and ORG 24598 (Bergeron *et al.* 1998; Roux and Supplisson 2000). GLYT2a is inhibited by ORG 26176 but not by sarcosine.

GLYCINE RECEPTORS

Glycine receptors can be activated by a range of simple amino acids including glycine, β-alanine and taurine, and are selectively blocked by the high-affinity competitive antagonist strychnine (Fig. 11.10). Glycine receptors were originally isolated from spinal cord membranes on the basis of strychnine binding, and found to be composed of two membrane-spanning polypeptides (termed α and β) and an associated cytoplasmic protein (gephyrin). To date, four α subunit genes (α1–4) and a single β subunit gene have been identified, with several additional variants of the α1 and α2 isoforms produced by alternative splicing (reviewed by Kuhse, Betz and Kirsch 1995; Rajendra, Lynch and Schofield 1997). The α and β subunits are formed from approximately 420 and 470 amino acids, respectively, are similar in structure to $GABA_A$ subunits, and likewise form pentameric receptors with a central ion channel permeable to Cl^- and HCO_3^-. In recombinant expression systems the α subunits give rise to functional homomeric receptors or co-assemble to form heteromeric receptors. The β subunit is only

incorporated into receptors when co-expressed with α subunits. Native receptors in the adult spinal cord contain 3 α1 and 2 β subunits whereas neonatal receptors are homomeric receptors formed from α2 subunits. The cytoplasmic protein gephyrin is not needed for the formation of functional receptors but plays an important role in the clustering of both glycine and $GABA_A$ receptors (Moss and Smart 2001).

Glycine receptors in the postsynaptic membrane, like $GABA_A$ receptors, most commonly generate a hyperpolarizing IPSP. In the brainstem, glycine receptors have also been shown to be present on presynaptic terminals, where they induce a small depolarisation that activates Ca^{2+} channels and *increases* neurotransmitter release (Turecek and Trussell 2001). This differs from the action of presynaptic $GABA_A$ receptors described above, where the depolarisation induced is sufficient to inactivate Na^+ channels and *decrease* neurotransmitter release. Unlike $GABA_A$ receptors, glycine receptors are inhibited by some steroids, unaffected by benzodiazepines and are relatively insensitive to barbiturates. However, native and recombinant glycine receptors are positively modulated by a wide range of general anaesthetics, including diethyl ether, halothane, isoflurane, chloral hydrate, brometone and trichloroethylene.

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