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$GABA_A$ Receptor Pharmacology

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ABSTRACT. **γ-Aminobutyric acid $(GABA)_A$ receptors for the inhibitory neurotransmitter GABA are likely to be found on most, if not all, neurons in the brain and spinal cord. They appear to be the most complicated of the superfamily of ligand-gated ion channels in terms of the large number of receptor subtypes and also the variety of ligands that interact with specific sites on the receptors. There appear to be at least 11 distinct sites on $GABA_A$ receptors for these ligands.** PHARMACOL THER 69(3): 173–198, 1996.

KEY WORDS. GABA receptors, bicuculline, barbiturates, benzodiazepines, neuroactive steroids.

CONTENTS

ABBREVIATIONS. CHEB, 5-(-2-cyclohexylidine-ethyl)-5-ethyl barbituric acid; DBI, diazepam binding inhibitor; DHP, dihydropicrotoxinin; DMCM, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-caboxylate; DPGL, α,α-di-isopropyl-γ-butyrolactone; α-EMGBL, α-ethyl-α-ethyl-γ-butyrolactone; β-EMGBL, β-ethyl-β-ethyl-γ-butyrolactone; GABA, γ-aminobutyric acid; GABARINS, GABA Receptor INhibitorS; GABOB, γ-amino-β-hydroxybutyric acid; 5-HT, 5-hydroxytryptamine; ipsp, inhibitory postsynaptic potential; NMDA, *N*-methyl-D-aspartate; 3α-OH-DHP, 3α-hydroxy-5α-pregnan-20-one; ORG 20599, (2β,3α,5α)-21-chloro-3-hydroxy-2-(4-morpholinyl)pregnan-20-one methanesulphonate; 4-PIOL, 5-(4-piperidyl)isoxazol-3-ol; RU5135, 3α-hydroxy-16-imino-5β-17-aza-androstan-11-one; (+)-TACP, (+)-*trans*-(1S,3S)-3-aminocyclopentane-1-carboxylic acid; TBPS, [^{35}S]*t*-butylbicyclophosphorothionate; 5α-THDOC, allotetrahydrodeoxycorticosterone, 3α,21-dihydroxy-5α-pregnan-20-one, allotetrahydroDOC; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol; ZAPA, Z-3-[(aminoiminomethyl)thio]prop-2-enoic acid; ZK93423, 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate ethyl ester.

TABLE 1. Agents Acting on $GABA_A$ Receptors

Antagonists

Competitive

Bicuculline, (+)-Hydrastine, SR95531, Pitrazepin, Securinine, RU5135, Benzyl penicillin, (+)-Tubocurarine

Noncompetitive

Picrotoxinin, δ-Guanidinovaleric acid, *m*-Benzenesulfonic acid diazonium chloride, Cunaniol, Dopamine sulfate, Dimefline, Enoxacin, Norfloxacin, Pentylenetetrazole, Furosemide

Agonists

Endogenous

GABA, GABOB, Imidazole-4-acetic acid, β-Alanine, Taurine

Exogenous

Muscimol, THIP, Isoguvacine, ZAPA, (+)-TACP, Pentobarbitone

Partial Agonists

4-PIOL, Thio-THIP

Positive Allosteric Modulators

Endogenous

3α-OH-DHP, 5α-THDOC, Arachidonic acid, Interleukin-1, H^+, NH_4^+, Mg^{2+}

Exogenous

Pentobarbitone, Etomidate, Diazepam, α-EMGBL, Halothane, Diethylether, Enflurane, Isoflurane, Alphaxolone, Ketamine, Propofol, Ethanol, Trichloroethanol, ORG 20599, Cd^{2+}, Mn^{2+}, La^{3+}, Br^-, Dinatin, Chrysin, Amentoflavon, Miltirone

Negative Allosteric Modulators

Endogenous

DBI, Butyl β-carboline-3-carboxylate, Cortisone, Ca^{2+}, Zn^{2+}, Phosphatidylethanolamine, Purines

Exogenous

Ro19-4603, β-Carbolines, β-EMGBL, Cortisone, Dieldrin, Lindane, Deltamethrin, Sr^{2+}, Ba^{2+}, Colchicine, Nocodazole, Vinblastine, Taxol

Bidirectional Allosteric Modulators

Endogenous

Pregnenolone, Pregnenolone sulfate, Cortisol

Exogenous

Avermectin B_{1a}, ICS 205-930, Amitriptyline, Forskolin, 8-Bromo-cAMP, Mefenamic acid, Flufenamic acid

Neutralising Allosteric Modulators

Ro15-1788, ZK93426, DPGL, Epipregnanolone

1. INTRODUCTION

Bucuculline-sensitive receptors for the inhibitory neurotransmitter γ-aminobutyric acid (4-aminobutanoic acid, GABA) are likely to be found on most, if not all, neurons in the brain and spinal cord (Johnston, 1978). These $GABA_A$ receptors are part of a superfamily of ligand-gated ion channels that include nicotinic acetylcholine receptors and strychnine-sensitive glycine receptors, together with ionotropic glutamate and 5-hydroxytryptamine (5-HT) receptor subtypes (Schofield *et al.*, 1987; Ortells and Lunt, 1995).

$GABA_A$ receptors appear to be the most complicated of the superfamily of ligand-gated ion channels in terms of the large number of receptor subtypes and also the variety of ligands that interact with specific sites on the receptors (Kerr and Ong, 1992). Table 1 lists more than 100 agents known to act on $GABA_A$ receptors. The action of these agents on $GABA_A$ receptors is the subject of this review. It is not known how many different sites there are on $GABA_A$ receptors for such a structurally diverse range of agents. It is anticipated than many of these agents will act at overlapping sites on the various $GABA_A$ receptor protein subunits. An interesting hypothesis is that modulator sites could exist at the interfaces between different pairs of subunits making up the hetero-oligomeric receptors (Galzi and Changeux, 1994). Homo-oligomeric receptors do not seem to exhibit anywhere near the same diversity of modulator responses exhibited by the hetero-oligomeric receptors.

2. DIFFERENT TYPES OF GABA RECEPTORS

The $GABA_{A/B}$ receptor classification, introduced in 1981, defines $GABA_A$ receptors as being sensitive to antagonism by bicuculline and insensitive to baclofen, while $GABA_B$ receptors are insensitive to antagonism by bicuculline and are activated by baclofen (Hill and Bowery, 1981). $GABA_A$ and $GABA_B$ receptors differ not only in their pharmacology, but also in their functionality. $GABA_A$ receptors gate chloride ion channels. $GABA_B$ receptors are linked to second messenger systems and, thus, resemble muscarinic acetylcholine receptors, most 5-HT receptors, and metabotropic glutamate receptors. $GABA_A$ and $GABA_B$ receptors have both pre- and postsynaptic locations on neurons. Both subtypes are heterogeneous.

There is now considerable evidence that GABA can activate receptors that do not fit the 1981 $GABA_{A/B}$ classification, since these receptors are relatively insensitive to the effects of baclofen and bicuculline. Indeed, such "novel" GABA receptors may represent a major subtype of GABA receptors in the animal kingdom. They have been described in vertebrate retina, cerebellum, hippocampus, optic tectum, and spinal cord, as well as in insects and perhaps even in bacteria. These "novel" receptors have been given a variety of descriptions—$GABA_C$, $GABA_{NANB}$ ("non-A, non-B"), and GABA ϱ-receptors (cloned from retina), and have been collectively termed $GABA_C$ receptors as an extension of the $GABA_{A/B}$ nomenclature (Johnston, 1994). These "novel" receptors may represent a relatively simple form of ligand-gated ion channels, which are made up of homo-oligomeric subunits, in contrast to the hetero-oligomeric $GABA_A$ receptors. Their overall pharmacology appears simpler than that of the classic $GABA_A$ receptors, especially with respect to lack of modulation by neurosteroids and benzodiazepines, and there are substantial differences in agonist and antagonist specificity. The more complex $GABA_A$ receptors may have evolved from the simpler $GABA_C$ receptors.

The heterogeneity of $GABA_A$ receptors results from the association of five subunits in a range of combinations to form a single ligand-gated ion channel complex (Macdonald and Olsen, 1994; Nayeem *et al.*, 1994). More than 15 different, but structurally related, gene products coding for $GABA_A$ receptor protein subunits have been described. On the basis of predicted amino acid sequences, five distinct classes of glycoproteins have emerged, most containing isoforms as follows: α_1–α_6, β_1–β_3, γ_1–γ_3, δ, and ϱ_1–ϱ_2,though the ϱ-subunits may constitute the homomeric $GABA_C$ rather than heteromeric $GABA_A$ receptors. To these must be added splice variants and differing phosphorylation states of β- and γ-subunits. If we limit all possible combinations of these native isoforms to those with 2α-, 2β-, and one other subunit, then there could be still more than 2000 different subtypes of $GABA_A$ receptors—an unlikely number indeed!

There is evidence for preferred stoichiometries of recombinant $GABA_A$ receptors, which would limit the likely number of distinct $GABA_A$ receptors found *in vivo* (e.g., Backus *et al.*, 1993). Different subunits of the same subclass, e.g., α-subunits, are known to be

coexpressed in the one functional $GABA_A$ receptor complex (Ebert *et al.*, 1994). Different $GABA_A$ receptor isoforms are known to be expressed in an apparently homogeneous cell type (Stephanson, 1995). The assembly of different receptor isoforms codes for sorting and localisation in polarised cells (Perezvelazquez and Angelides, 1993). It is clear that differing combinations of these protein subunits give rise to $GABA_A$ receptor complexes with differing pharmacology and physiology, e.g., differing sensitivity to modulation by ethanol and different channel properties. There is good evidence for regional heterogeneity of protein subunits of $GABA_A$ receptors in the brain (e.g., Endo and Olsen, 1993). There is considerable pharmacological and physiological evidence in the literature indicating the heterogeneity of $GABA_A$ receptors, e.g., differences in agonist profiles between "synaptic" and "nonsynaptic" $GABA_A$ receptors in the spinal cord (Allan *et al.*, 1980), novel properties of $GABA_A$ autoreceptors (Minchin *et al.*, 1992) and differences in time courses of $GABA_A$ receptor-mediated synaptic currents (e.g., Pearce, 1993; Puia *et al.*, 1994). Noise analysis of $GABA_A$-mediated miniature inhibitory postsynaptic currents in the hippocampus reveals three different time constants of 0.3, 1.6, and 22 msec (Dekoninck and Mody, 1994). The functional heterogeneity of $GABA_A$ receptors is clearly demonstrated in the hippocampus, where low affinity GABA responses are more strongly affected by benzodiazepines as compared with cells exhibiting high affinity responses (Schönrock and Bormann, 1993a).

$GABA_A$ receptors are sometimes referred to as GABA-benzodiazepine-ionophore receptor complexes, or the like, but it is important to note that not all $GABA_A$ receptors are influenced by benzodiazepines. The enhancement of $GABA_A$ responses by diazepam requires the presence of the γ_2-subunit in the receptor complex (Pritchett *et al.*, 1989). $GABA_A$ receptors susceptible to modulation by barbiturates and neuroactive steroids are much more widespread in the brain than $GABA_A$ receptors susceptible to benzodiazepine modulation.

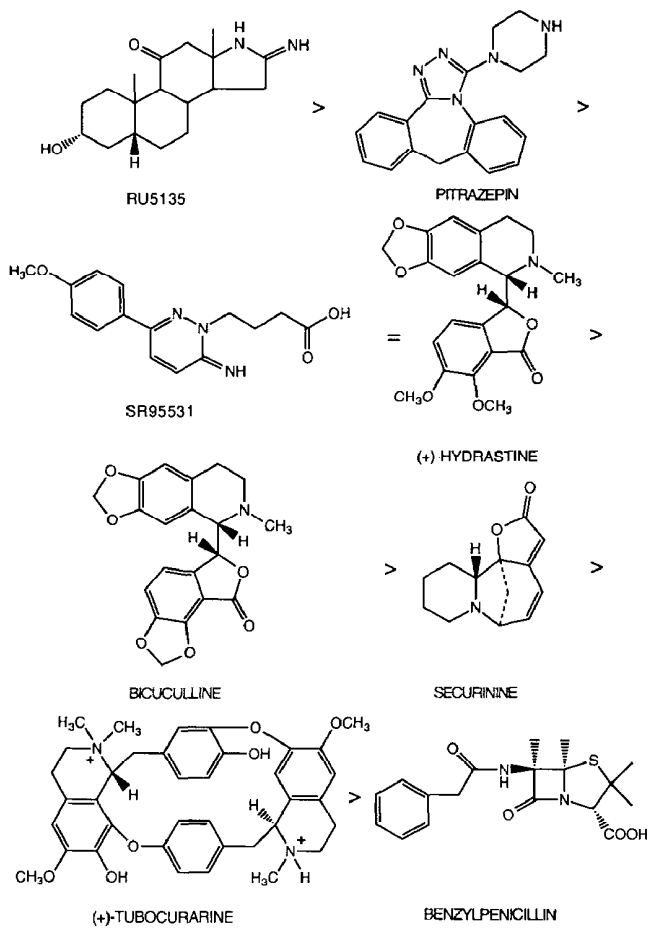

FIGURE 1. Some competitive antagonists of $GABA_A$ receptors in approximate order of potency.

3. $GABA_A$ RECEPTOR ANTAGONISTS

The discovery in 1970 that the convulsant alkaloid bicuculline could antagonise certain inhibitory actions of GABA in the CNS provided a vital pharmacological agent with which to probe GABA-mediated inhibition (Curtis *et al.*, 1970). By 1974, GABA was well established as an inhibitory neurotransmitter of widespread significance in the mammalian CNS (Curtis and Johnston, 1974a). Further progress in the development of GABA antagonists was relatively slow with the introduction in 1981 of the $GABA_{A/B}$ receptor classification, highlighting that not all GABA receptors could be antagonised by bicuculline (Hill and Bowery, 1991). The next major development in $GABA_A$ antagonists was the introduction in 1986 of SR95531 ("gabazine"), which offered some advantages over bicuculline in terms of ease of use.

With the increasing realisation of the molecular diversity of $GABA_A$ receptors coming from molecular biological studies, there is an urgent need for antagonists that distinguish between different subtypes of $GABA_A$ receptors.

3.1. *Competitive $GABA_A$ Receptor Antagonists*

It is considered likely that competitive antagonists of $GABA_A$ receptors act at GABA recognition sites. Thus, structural similarities between the competitive $GABA_A$ antagonist bicuculline and the $GABA_A$ agonist muscimol have been described (Andrews and Johnston, 1979). The structures of representative competitive $GABA_A$ receptor antagonists in approximate order of potency are shown in Fig. 1. There have been a number of studies aimed at a further understanding of the interactions between $GABA_A$ antagonists and $GABA_A$ receptors (Pooler and Steward, 1988; Aprison and Lipkowitz, 1989) with varying degrees of success (Kerr and Ong, 1992). Molecular modelling studies on bicuculline, SR95531, securinine, tubocurarine, pitrazepin, 3α-hydroxy-16-imino-5β-17-aza-androstan-11-one (RU5135) and iso-THAZ have shown common structural features of these $GABA_A$ antagonists, which suggests that they share some common binding sites on $GABA_A$ receptors (Rognan *et al.*, 1992).

There is evidence for agonist and competitive antagonist conformations of $GABA_A$ binding sites (Möhler and Okada, 1978), with differences in the thermodynamics of agonist and antagonist binding, the binding of antagonists being driven by changes in both enthalpy and entropy, whereas agonist binding is driven entirely by entropy changes (Maksay, 1994).

Point mutations of α_1-subunits of rat brain $GABA_A$ receptors alter both agonist and competitive antagonist properties, suggesting a close structural association of α_1 Phe64 with agonist/antagonist binding sites (Sigel *et al.*, 1992). Substitution of this Phe by Leu results in a large decrease in the apparent affinity for GABA, bicuculline, and SR95531.

3.1.1. Bicuculline and related phthalide isoquinoline alkaloids. Bicuculline is a phthalide isoquinoline alkaloid first isolated from the plant *Dicentra cucullaria* (known as "Dutchman's breeches") and subsequently from a variety of *Corydalis*, *Dicentra*, and *Adlumia* species. Its convulsant action was reported in 1934, and

several investigators are now known to have examined the action of bicuculline on various synaptic processes to explain its convulsant action. Early studies include one carried out in China in 1965 showing that bicuculline could block synaptic inhibition, which was not published until 1976 due to the "Cultural Revolution" (see Johnston, 1985). The discovery in 1970 of the GABA antagonist action of bicuculline (Curtis *et al.*, 1970) came from a systematic study of convulsant alkaloids following the discovery of the glycine antagonist action of strychnine (Curtis *et al.*, 1967). The 3 years of investigation of convulsant alkaloids showed that while many isoquinoline alkaloids are convulsants, most are glycine antagonists, with GABA antagonism being restricted to the phthalide isoquinoline alkaloids that have the 1*S*,9*R* configuration, i.e., bicuculline, corlumine and (+)-hydrastine (Curtis and Johnston, 1974b).

The potency of the 1*S*,9*R*-phthalide isoquinoline alkaloids as $GABA_A$ antagonists and as convulsants is (+)-hydrastine > bicuculline > corlumine (Huang and Johnston, 1990). Structure–activity studies show the importance of the γ-lactone moiety of the phthalide isoquinolines (Johnston, 1991), opening of the lactone ring of bicuculline to give bicucine resulting in a loss of $GABA_A$ antagonist activity. Some activity is restored on esterification of bicucine to afford bicucine methyl ester. As the lactone ring of bicuculline readily opens under physiological conditions (Olsen *et al.*, 1975), this led to considerable confusion over early attempts to use bicuculline as a GABA antagonist and possibly led to a delay in the recognition of truly bicuculline-insensitive GABA receptors. The problem persists, e.g., with commercial samples of [^{3}H]-bicuculline methochloride that are not active as GABA antagonists due to storage at neutral pH (Johnston, 1991). Lactone ring formation takes place at acid pH and thus, activity can be restored to an inactivated bicuculline sample over 24 hr at pH 2 or lower. The lactone ring in bicuculline is stable at neutral pH for many hours at 0°C, 45 min at 24°C, and only a few minutes at 37°C.

Quaternary salts of bicuculline, such as bicuculline methochloride and bicuculline methiodide, are more easy to use than the hydrochloride in that they are more water-soluble and more stable, although the lactone ring can still open in these derivatives, rendering them inactive as GABA antagonists (Johnston *et al.*, 1972). The quaternary salts are, however, more potent inhibitors of acetylcholinesterase than the hydrochlorides (Breuker and Johnston, 1975). Other actions of bicuculline and its derivatives include effects on certain 5-HT (Mayer and Straughan, 1981), nicotinic (Zhang and Feltz, 1991), and perhaps *N*-methyl-D-aspartate (NMDA) receptors (Krebs *et al.*, 1994).

Both bicuculline and (+)-hydrastine interact preferentially with low affinity $GABA_A$ receptors (Olsen and Snowman, 1983; Huang and Johnston, 1990). Chaotropic agents, such as thiocyanate, enhance the ability of bicuculline to displace GABA from low affinity binding sites, perhaps by promoting the interconversion of these receptors from a relatively hydrophilic agonist state to a relatively hydrophobic antagonist state (Maksay and Ticku, 1984). Since bicuculline appears to prefer binding to the antagonist state of low affinity $GABA_A$ binding sites, there is considerable interest in affinity labels or irreversible binding ligands as tools to investigate such sites. The further development of bicuculline derivatives substituted in the 5-position would be worthwhile (Allan and Apostopoulos, 1990).

3.1.2. SR95531 and related pyridazinyl GABA derivatives. A series of pyridazinyl derivatives of GABA are potent competitive antagonists of $GABA_A$ receptors (Wermuth *et al.*, 1987). The most widely used is SR95531 ("gabazine," 2-(3-carboxypropyl)-3-amino-6-*p*-methoxyphenylpyridazinium bromide), which is a selective $GABA_A$ antagonist in the spinal cord *in vivo* (Gynther and Curtis, 1986) and in the cuneate nucleus *in vitro*, being approximately equipotent with bicuculline methochloride (Michaud *et al.*, 1986). Binding studies using [^{3}H]-GABA and GABA-stimulated [^{3}H]-diazepam binding to rat brain membranes indicate that SR95531 is a competitive inhibitor of high affinity GABA binding sites and a noncompetitive inhibitor of low affinity binding sites (Heaulme *et al.*, 1986). This indicates a difference between SR95531 and bicuculline in their relative potencies for high and low affinity $GABA_A$ binding sites, with SR95531 being more potent at high affinity sites and bicuculline being more potent at low affinity sites (Johnston, 1991).

[^{3}H]-SR95531 binds to two distinct populations of binding sites in rat brain membranes (Maksay, 1994). SR95531 is not selective for $GABA_A$ receptors since it is a substrate inhibitor of monoamine oxidase A (Luque *et al.*, 1994). This has led to the suggestion that the capability of SR95531 to disinhibit noradrenergic neurons by blocking GABA-mediated inhibition, together with the potentiation of noradrenergic neurons via monoamine oxidase A inhibition, could result in the development of more effective therapies for depression.

Extensive structure–activity studies of the pyridazinyl GABA derivatives have been carried out (Wermuth *et al.*, 1987). Isosteric substitution of the pyridazine ring to produce thiadiazole analogues of SR95531 results in a 5-fold decrease in potency as a $GABA_A$ antagonist (Allan *et al.*, 1990).

Recently, a series of pyridazinyl derivatives of GABA have been examined as antagonists of GABA receptors in the nematode *Ascaris* (Martin *et al.*, 1995). These GABA receptors show a similar agonist profile to vertebrate $GABA_A$ receptors, but a very different antagonist profile in that bicuculline, securinine, pitrazepin, and RU5135 are inactive. While SR95531 is weakly active in *Ascaris*, other pyridazinyl derivatives are much more potent as competitive GABA antagonists, the most potent being NCS 281-93 (2-(3-carboxypropyl)-3-amino-4-phenylpropyl-6-phenyl pyridazine).

Pyridazinyl derivatives of GABA might be very useful in probing different subtypes of $GABA_A$ receptors made up of different protein subunits.

3.1.3. Pitrazepin. Pitrazepin (3-(piperazinyl-1)-9H-dibenz(c,f) triazolo(4,5-a)azepin) is a potent competitive inhibitor of $GABA_A$ receptors (Gähwiller *et al.*, 1984; Braestrup and Nielsen, 1985), 3–10 times more potent than bicuculline, depending on the test preparation (Johnston, 1991). Pitrazepin, however, is not specific for $GABA_A$ receptors since it inhibits the binding of the glycine antagonist, strychnine, at the same concentration as it inhibits $GABA_A$ receptors (Braestrup and Nielsen, 1985) and it blocks glycine neuronal inhibition *in vivo* (Curtis and Gynther, 1986).

In addition to pitrazepin, most N-aryl piperazines, including several clinically effective antidepressants (e.g., Amoxapine, Mianserin) and antipsychotics (e.g., Clothiapine, Loxapine, Metiapine, Clazapine, and Fluperlapine), are moderately to highly potent GABA antagonists (Squires and Saederup, 1993a).

3.1.4. Securinine. Securinine, from *Securinega suffructicosa*, and related convulsant indolizidine alkaloids antagonise $GABA_A$ receptors. Securinine is a selective $GABA_A$ antagonist in the cat spinal cord *in vivo* not influencing glycine receptors. In binding studies, it is a competitive antagonist some 7 times less potent than bicuculline (Beutler *et al.*, 1985).

3.1.5. RU5135. The aminidine steroid analogue RU5135 is the most potent competitive antagonist of $GABA_A$ receptors described

to date. It is some 500 times more potent than bicuculline in inhibiting GABA enhancement of diazepam binding (Hunt and Clements–Jewery, 1981). It is a very potent inhibitor of muscimol and bicuculline binding (Olsen, 1984). Its action, however, is not restricted to $GABA_A$ receptors, as it is even more effective as a glycine antagonist in the cat spinal cord *in vivo* (Curtis and Malik, 1985) and in the optic nerve (Simmonds and Turner, 1985).

3.1.6. Benzyl penicillin. The convulsant action of benzyl penicillin may result from its $GABA_A$ antagonist action (Davidoff, 1972; Curtis *et al.*, 1972). The effects of penicillin on GABA-activated chloride currents are complex (Katayama *et al.*, 1992). Penicillin is about one-hundredth as potent as bicuculline, while ampicillin is even less potent (Curtis and Johnston, 1974b). It shortens the lifetime of GABA-induced chloride channels (Chow and Mathers, 1986) by shortening the duration of channel openings while increasing the frequency of channel opening (Twyman *et al.*, 1992). Differences have been noted in the actions of penicillin and bicuculline as GABA antagonists (Pickles and Simmonds, 1980).

Penicillin antagonises glycine-activated chloride fluxes in a similar manner to the way it antagonises GABA-activated channels (Tokutami *et al.*, 1992).

3.1.7. (+)-Tubocurarine. The well known acetylcholine nicotinic antagonist (+)-tubocurarine is a relatively weak antagonist of $GABA_A$ receptors (Hill *et al.*, 1973). It also acts as a glycine receptor antagonist (Curtis and Johnston, 1974b). These observations suggest that (+)-tubocurarine may bind to sites on proteins of the nicotinic, $GABA_A$ and glycine ligand-gated receptor superfamily, which contain some common structural features (Siebler *et al.*, 1988).

3.2. *Noncompetitive $GABA_A$ Receptor Antagonists*

A wide range of compounds antagonise $GABA_A$ receptors in a noncompetitive manner. The structures of representative noncompetitive $GABA_A$ receptor antagonists are shown in Fig. 2. Of major interest are the so-called "cage" convulsants, such as picrotoxinin, which act at sites closely associated with the chloride ion channel of $GABA_A$ receptors. As ligands are known that can enhance GABA-mediated events by acting at these sites, the noncompetitive antagonists perhaps should be classified more correctly as negative allosteric modulators, even though they are traditionally considered to be antagonists. Their antagonist action is directed towards the $GABA_A$-activated chloride channel rather than the GABA recognition site on $GABA_A$ receptor complexes. As there is some evidence for an endogenous ligand for picrotoxinin binding sites (Olsen and Leeb–Lundberg, 1980), it may be that activation of these sites by such a ligand may be modulated by a range of substances acting allosterically in an analogous manner to the modulation of the activation of GABA recognition sites.

3.2.1. Picrotoxinin and related terpenoids. Picrotoxin is an equimolar mixture of picrotoxinin and picrotin isolated from *Anamirta cocculus* and related poisonous plants of the moonseed family. Picrotoxinin is a relatively potent convulsant and $GABA_A$ receptor antagonist, whereas picrotin is some 50 times less active than picrotoxinin (Curtis and Johnston, 1974b). Picrotoxinin is one of a number of structurally related convulsants of plant origin, including coriamyrtin and tutin, that act as $GABA_A$ receptor antagonists (Kerr and Ong, 1992). Interestingly, the structurally related alkaloid, dendrobine, is a glycine antagonist rather than a GABA antagonist (Curtis *et al.*, 1971). Most of the development of picrotoxinin-related compounds has been directed towards the discovery of new insecticides (Casida, 1993). Picrotoxinin has been

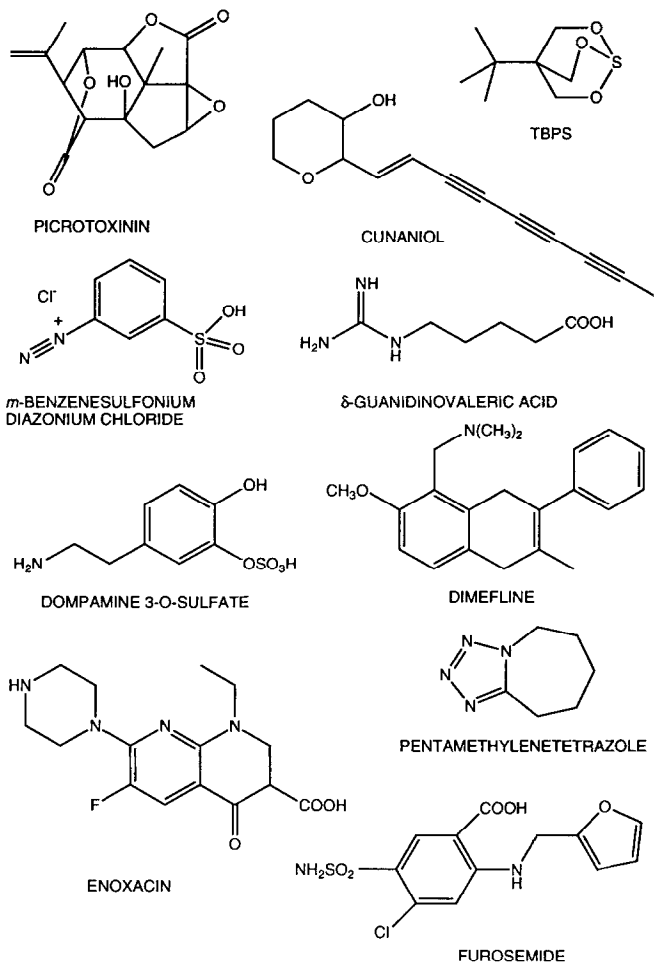

FIGURE 2. Some noncompetitive antagonists of $GABA_A$ receptors.

reported to antagonise the neuronal effects of 5-HT (Mayer and Straughan, 1981) and glycine (Curtis *et al.*, 1969) and the action of GABA on ϱ receptors (see Johnston, 1994).

Picrotoxinin does not inhibit the binding of $GABA_A$ agonists or benzodiazepines to $GABA_A$ receptors. Picrotoxinin binding sites, identified with [^{3}H]-dihydropicrotoxinin (DHP) or preferably with [^{35}S]*t*-butylbicyclophosphorothionate (TBPS), which gives a better signal-to-noise ratio than [^{3}H]-DHP, are closely associated with the chloride channel of $GABA_A$ receptor complexes. $GABA_A$ agonists and positive modulators, such as barbiturates, benzodiazepines, and steroids, allosterically inhibit TBPS binding by reducing its affinity. Some $GABA_A$ receptor negative modulators, such as convulsant β-carbolines and γ-butyrolactones, enhance TBPS binding affinity, suggesting that high affinity TBPS binding might be associated with a "closed" conformation of the chloride channel (Gee, 1988; Sieghart, 1992). A very wide range of compounds seems to bind to sites that influence picrotoxinin binding, sites that are clearly central to the activation of $GABA_A$ receptors (Kerr and Ong, 1992).

Unlike bicuculline, picrotoxinin can act as a GABA antagonist when administered intracellularly (Akaike *et al.*, 1985). It is clear that bicuculline and picrotoxinin act at different sites to antagonise GABA (Simmonds, 1980). The actual mechanisms by which picrotoxinin blocks GABA-induced chloride currents are complex (Newland and Cull-Candy, 1992; Yoon *et al.*, 1993). There is evidence that picrotoxinin can directly activate chloride channels

in the absence of GABA via the β_1 $GABA_A$ receptor subunit (Sigel *et al.*, 1989).

Recently, a new group of picrotoxane terpenoids, picrodendrins, have been described as potent inhibitors of TBPS binding (Ozoe *et al.*, 1994). Structure–activity studies indicate the importance of the spiro α-ethylidene γ-lactone moiety for the interaction of picrodendrins with picrotoxinin binding sites. As discussed in Section 5.4, relatively simple γ-lactones are ligands for picrotoxinin binding sites having positive, negative, and neutralising allosteric effects (Kerr and Ong, 1992).

3.2.2. Miscellaneous antagonists. δ-Guanidinovaleric acid may act as an endogenous antagonist of $GABA_A$ receptors, in that it is found in the brain in low concentrations and it can antagonise the inhibitory actions of muscimol and (−)-γ-amino-β-hydroxybutyric acid (GABOB) (Yokoi *et al.*, 1987).

m-Benzenesulfonic acid diazonium chloride (also known as *m*-sulfonate benzene diazonium chloride) was introduced as a compound capable of alkylating $GABA_A$ binding sites and, thus, acting as an irreversible affinity label (Bouchet *et al.*, 1992). It has been shown to be a noncompetitive $GABA_A$ receptor antagonist (IC_{50} 87 μM), as demonstrated on recombinant receptors expressed in *Xenopus* oocytes (Krishek *et al.*, 1994).

In addition to the substances mentioned above, a diverse range of compounds appear to act as GABA antagonists. Few of these have been investigated in any depth, which is unfortunate, as they may show selectivity between different subtypes of $GABA_A$ receptors (Kerr and Ong, 1992). These miscellaneous antagonists include the convulsant cunaniol (Quilliam and Stables, 1969), sulfated metabolites of dopamine (Buu *et al.*, 1984), and the analeptic dimefline (Kerr and Ong, 1992).

The convulsant side effects of quinolone antibiotics, such as enoxacin and norfloxacin, may be due to antagonism of $GABA_A$ receptors (Dodd *et al.*, 1989; Squires and Saederup, 1993b; Kawakami *et al.*, 1993; Halliwell *et al.*, 1993). These effects may be potentiated by nonsteroidal anti-inflammatory drugs, such as felbinac (Kawakami *et al.*, 1993), and their metabolites, such as biphenylacetic acid (Halliwell and Davey, 1994).

Some dihydroimidazoquinoxalines, including U-93631, cause a rapid decay in GABA-induced chloride currents by reversibly desensitising $GABA_A$ receptors (Dillon *et al.*, 1993). Structure–activity studies indicate that these compounds may interact with a unique site on $GABA_A$ receptors independent of the benzodiazepine sites activated by other imidazoquinoxalines.

The widely used convulsant pentylenetetrazole (1,5-pentamethylenetetrazole, metrazole) has relatively weak GABA antagonist properties, and other mechanisms are likely to contribute to its convulsant properties (De Deyn and Macdonald, 1989). Its action might be highly regionalised in the brain, where acute effects of pentylenetetrazole have been described on GABA, TBPS, and flunitrazepam binding only in the striatum (Ito *et al.*, 1986). Other tetrazoles have depressant activity and are discussed in Section 5.14.

Furosemide, a Cl^- transport blocker used as a diuretic, selectively antagonises recombinant receptors expressed in oocytes containing α_6-, $\beta_{2/3}$-, and γ_2-subunits typical of cerebellar granule cell $GABA_A$ receptors. Receptors made up of α_6-, β_1-, and γ_2- or α_1-, $\beta_{1/2/3}$-, and γ_2-subunits are not sensitive to furosemide and, thus, furosemide may be the first subtype-selective $GABA_A$ receptor antagonist (Korpi *et al.*, 1995). However, it appears to act via a novel recognition site that allosterically regulates the Cl^- ionophore. Thus, furosemide may be a negative allosteric modulator rather than an antagonist. As discussed in the next section, Zn^{2+} selectively inhibits $GABA_A$ receptors of particular subunit compositions, as do benzodiazepine negative allosteric modulators. Furosemide is known to inhibit the action of GABA in a variety of preparations, including ray superior cervical ganglia (Alkadhi *et al.*, 1993), mouse cultured oligodendrocytes (Hoppe and Kettenmann, 1989), guinea-pig ileum (Taniyama *et al.*, 1988), frog sensory neurons (Inomata *et al.*, 1988), and rat brain synaptoneurosomes (Luu *et al.*, 1987).

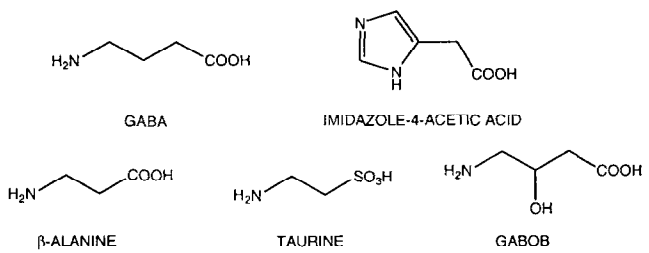

FIGURE 3. GABA and some other substances found in the brain that can act as $GABA_A$ receptor agonists.

4. $GABA_A$ RECEPTOR AGONISTS AND PARTIAL AGONISTS

There is considerable interest in $GABA_A$ agonists and partial agonists as targets for drug development (Allan and Johnston, 1983; Falch *et al.*, 1990; Johnston, 1992; Krogsgaard-Larsen *et al.*, 1994). The subunit composition of $GABA_A$ receptors greatly influences agonist and partial agonist efficacy (Ebert *et al.*, 1994). It is important to develop agonists and partial agonists showing selectivity for particular $GABA_A$ receptor isoforms.

4.1. *Endogenous Agonists*

A variety of substances are found in the brain that can act as $GABA_A$ agonists. Clearly, GABA itself is the most important endogenous agonist, but other agonists include imidazole-4-acetic acid, taurine, β-alanine, and GABOB. The structures of these compounds are shown in Fig. 3. A model of GABA binding to $GABA_A$ receptors based on hydrogen bonding and hydrophobic interactions "makes it seem unlikely that any other substance known to occur in nerve tissue would give rise to a high noise level at $GABA_A$ receptors" (Roberts and Sherman, 1993). Some other structurally related endogenous GABA analogues, including γ-aminobutyrylcholine, L-2,4-diaminobutyric acid, L-proline, and L-cystathionine, although having depressant actions on neuronal firing, do not appear to activate $GABA_A$ receptors *in vivo* (Curtis and Johnston, 1974a). Other endogenous agonists or modulators of $GABA_A$ receptors may exist, e.g., a small molecule (<2000 kDa) of unknown structure that has been isolated from brain inhibits the binding of the $GABA_A$ agonist muscimol and taurine, but does not influence benzodiazepine binding (Tang *et al.*, 1993). The procedures developed to study GABA binding to brain membranes involve the removal of substances that influence GABA binding (Johnston and Kennedy, 1978), and many endogenous substances interacting with $GABA_A$ receptors remain to be identified (Deplazas *et al.*, 1993). As noted in Section 3.2.2, δ-guanidinovaleric acid may be an endogenous $GABA_A$ antagonist (Yokoi *et al.*, 1987).

4.1.1. GABA. The first descriptions of the inhibitory effects of GABA were provided by Hayashi and Nagai (1956),* who showed

*Hayashi, T. and Nagai, K. (1956) Action of ω-amino acids on the motor cortex of higher animals, especially γ-amino-β-oxy-butyric acid as the real inhibitory principle in brain. In: 20th International Physiology Congress, Brussels, p. 410.

that topically applied GABA had an inhibitory effect on the electrical activity of the motor cortex. Subsequent workers provided evidence that GABA was the major constituent of Factor I, that GABA was an inhibitory transmitter in crustacea and, eventually, that GABA had an effect on CNS neurons similar to that produced by synaptic inhibition (Curtis and Johnston, 1974a).

The flexible structure of GABA means that it can adopt a variety of low energy conformations. It seems likely that different conformations of GABA are important in its interaction with different receptors, enzymes, and transporters that GABA encounters in its role as an inhibitory synaptic transmitter (Johnston *et al.*, 1979). For example, there is evidence that GABA interacts with $GABA_A$ receptors in relatively extended conformations and with $GABA_C$ receptors in relatively folded conformations (Johnston *et al.*, 1975; Johnston, 1994).

GABA mediates fast inhibitory synaptic transmission by activating $GABA_A$ receptors. These receptors are ligand-gated chloride ion channels that, on activation, open channels permeable to chloride ions. Normally, this means that chloride ions flow into neurons, producing a hyperpolarisation, but in some neurons, chloride ions flow out, producing a depolarisation. The ionic basis of $GABA_A$ receptor channel function has been reviewed in detail by Kaila (1994). The permeability of GABA-activated chloride channels, as assessed using large polyatomic anions, indicates an effective pore diameter of 0.56 nm, somewhat larger than the 0.52 nm pore diameter found for glycine-activated chloride channels (Bormann *et al.*, 1987). GABA-activated chloride channels are multiple conductance state channels (Bormann *et al.*, 1987) and display outward rectification (Gage and Chung, 1994). Most $GABA_A$ channels are open at the peak of the miniature inhibitory postsynaptic currents, and the subsynaptic receptors are virtually saturated by GABA released into the synaptic cleft (Edwards *et al.*, 1990). Desensitisation of $GABA_A$ receptors is voltage-dependent, while recovery from the desensitised state is distinct from the process of reactivation that is dependent on both the voltage and agonist. These observations suggest that the GABA-bound receptor has two alternate states: permissive (activated) and desensitised (Yoon, 1994). In addition to electrophysiological methods, faster and slower desensitising channels can be demonstrated using rapid reaction kinetics to measure $^{36}Cl^-$ fluxes in D_2O (Kardos, 1993). GABA is taken up by active transport processes that remove GABA from the synaptic environment and take it up into presynaptic terminals for rerelease and into neighbouring glial cells where it is metabolised (Johnston and Balcar, 1989). Diffusion from the synaptic environment, channel open time, and GABA uptake all influence the time course of GABA-mediated inhibitory postsynaptic potentials (ipsps). The time course of a single, brief ipsp is determined predominantly by postsynaptic channel kinetics and diffusion of GABA out of the synapse, whereas the inhibition produced by prolonged synaptic bursts or relatively long application of exogenous GABA can be markedly influenced by GABA uptake inhibitors that prolong the late phase of the ipsps (Dingledine and Korn, 1985).

While GABA does not pass the blood–brain barriers on systemic administration, GABA entrapped in liposomes can act on the brain on systemic administration, e.g., as an anticonvulsant (Loeb *et al.*, 1982). The nature of the lipid is important, with phosphatidylserine and lysophosphatidylserine being the most active in combination with GABA (Toffano *et al.*, 1984). Cyclic analogues of GABA, e.g., piracetam, pass the blood–brain barriers, and Banfi *et al.* (1984) have suggested that they are of interest for their effects on learning and memory.

4.1.2. Imidazole-4-acetic acid. The histamine metabolite imidazole-4-acetic acid (more correctly known as imidazole-4-ethanoic acid) structurally resembles both GABA and β-alanine. It has a bicuculline-sensitive inhibitory action on the firing of CNS neurons (Curtis *et al.*, 1971). It penetrates the blood–brain barriers on systemic administration and reduces blood pressure and heart rate by stimulating central $GABA_A$ receptors (Antonaccio and Snyder, 1981). Imidazole-4-acetic acid is a partial agonist at $GABA_C$ receptors (Kusama *et al.*, 1993).

4.1.3. Taurine and β-alanine. Taurine is a nonprotein sulfur containing amino acid, which occurs in varying concentrations throughout the CNS, being high during the period of rapid growth of the brain and falling during maturity. It is probably a neurotransmitter in its own right, but it may also activate GABA receptors. Taurine can act like other $GABA_A$ agonists in stimulating the development of low affinity $GABA_A$ binding sites in cultured cerebellar granule cells in a bicuculline-dependent manner (Abraham and Schousboe, 1989). Testosterone can enhance the action of both GABA and taurine in stimulating gonadotropin secretion (Trudeau *et al.*, 1993). Taurine acts as a partial $GABA_A$ agonist in modulating benzodiazepine binding (Quinn and Miller, 1992).

There is considerable evidence that β-alanine is able to activate receptors normally considered to be activated by GABA, whose carbon chain is one carbon longer than that of β-alanine, and receptors normally activated by the neurotransmitter glycine, whose carbon chain is one carbon shorter than that of β-alanine. Whether or not there are specific receptors for β-alanine itself is the subject of considerable debate, given that there is much in support of β-alanine acting as a neurotransmitter. The activation of $GABA_A$ receptors in culture neurons from chick spinal cord by β-alanine is enhanced by 5α-pregnan-3α-ol-20-one (Wu *et al.*, 1993). β-Alanine is about 230 times less potent than GABA in activating these $GABA_A$ receptors, but shows similar efficacy to GABA. It is a noncompetitive inhibitor of glycine binding to the strychnine-insensitive glycine binding site on the NMDA receptor (Saransaari and Oja, 1993).

β-Alanine and taurine have been shown to cross-desensitise both GABA and glycine responses in *Xenopus* oocytes injected with mouse brain mRNA (Horikoshi *et al.*, 1988).

4.1.4. GABOB. The pioneering work of Hayashi and Nagai (1956)* on the effects of ω-amino acids on the brain, which is often quoted as the first demonstration of the effects of GABA on brain function, in fact emphasised GABOB rather than GABA as the likely inhibitory neurotransmitter. GABOB can be formed in brain from 2-hydroxyputrescine (Noto *et al.*, 1988). GABOB is a partial agonist at $GABA_B$ receptors (Kerr and Ong, 1992). The *S*-(+)-enantiomer of GABOB is the more potent enantiomer, but the enantioselectivity is relatively low (Krogsgaard-Larsen *et al.*, 1985). In the ileum, the interactions between the enatiomers of GABA and $GABA_A$ and $GABA_B$ receptors is complex, with R-(−)-GABOB preferentially activating $GABA_B$ receptors (Kristiansen and Fjalland, 1991). Cyclic analogues of GABA, in particular oxiracetam (4-hydroxy-2-pyrrolidone), are of interest as memory-enhancing drugs (Banfi *et al.*, 1984).

*Hayashi, T. and Nagai, K. (1956) Action of ω-amino acids on the motor cortex of higher animals, especially γ-amino-β-oxy-butyric acid as the real inhibitory principle in brain. In: 20th International Physiology Congress, Brussels, p. 410.

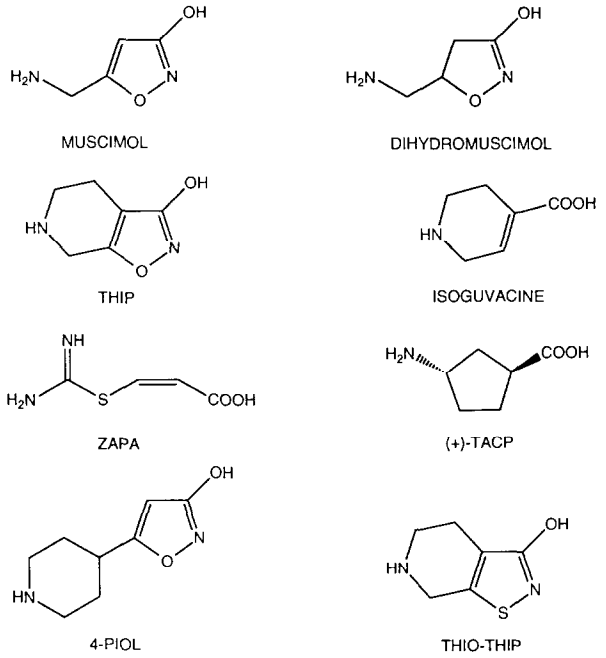

FIGURE 4. Some $GABA_A$ receptor agonists and partial agonists.

4.2. *Exogenous Agonists*

In defining the likely conformations of GABA that interact with $GABA_A$ receptors, GABA analogues of restricted conformation are particularly useful. For example, the isomers of 4-aminocrotonic acid show differing selectivity for GABA receptors, with the *trans*-isomer, an analogue of GABA in relatively folded conformations, showing selectivity for $GABA_A$ receptors, but nonetheless still active at $GABA_C$ receptors, whereas the *cis*-isomer, an analogue of GABA in relatively folded conformations, shows selectivity for $GABA_C$ receptors and is inactive at $GABA_A$ receptors (Johnston *et al.*, 1975; Johnston, 1994). The systematic study of conformationally restricted analogues of GABA has provided many examples of selective actions on aspects of the GABA transmitter system (Johnston *et al.*, 1979). A variety of photolabile derivatives of GABA have been prepared with a view to photoaffinity labelling GABA receptors (Wieboldt *et al.*, 1994; Kapfer *et al.*, 1995). The structures of the more important exogenous agonists are shown in Fig. 4.

4.2.1. Muscimol. One of the most widely used exogenous agonists of $GABA_A$ receptors is the naturally occurring isoxazole, muscimol, which is found in *Amanita muscaria* mushrooms, and may contribute to the psychoactive properties of these mushrooms (Johnston *et al.*, 1968). Muscimol became a prototype substance for the design and development of a range of isoxazoles with varying activities on GABA systems (Krogsgaard-Larsen *et al.*, 1975), including the selective GABA uptake inhibitor nipecotic acid (Krogsgaard-Larsen and Johnston, 1975). *S*-(+)-Dihydromuscimol is the most potent known $GABA_A$ agonist (Krogsgaard-Larsen *et al.*, 1985). Thiomuscimol is a moderately potent $GABA_A$ agonist that can act as a photoaffinity label for $GABA_A$ receptors (Nielsen *et al.*, 1995).

The 3-hydroxyisoxazole moiety of muscimol is a "masked carboxyl" group that is recognised as a carboxyl group equivalent by $GABA_A$ receptors, but not by $GABA_B$ receptors. The neuronal GABA uptake system recognises the 3-hydroxyisoxazole moiety in that muscimol is a weak inhibitor of GABA uptake (Johnston, 1971), but is neither an inhibitor nor a substrate for GABA aminotransferase, indicating that this enzyme does not recognise the 3-hydroxyisoxazole moiety (Beart and Johnston, 1973).

4.2.2. THIP and isoguvacine. 4,5,6,7-Tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) is a bicyclic isoxazole that represents a conformationally restricted analogue of muscimol. THIP has been described as a relatively rigid analogue of muscimol and thus, of GABA. Thus, it has more selective actions than either muscimol or GABA in acting as a selective $GABA_A$ receptor agonist (Krogsgaard-Larsen *et al.*, 1977). THIP is less potent than muscimol in inhibiting neuronal activity *in vivo* in the cat spinal cord, being approximately equipotent with GABA. The "reverse engineered" isoguvacine (1,2,3,6-tetrahydropyridine-4-carboxylic acid), in which the isoxazole moiety of THIP has been replaced by a carboxyl group, is equipotent with muscimol. THIP shows some selectivity for particular β-subunits of $GABA_A$ receptors (Bureau and Olsen, 1990).

Diazepam enhances the action of THIP on mouse spinal neurons in culture, but does not influence the binding of THIP to rat brain membranes under conditions where GABA binding is enhanced by diazepam (Skerritt and Johnston, 1983; Skerritt and Macdonald, 1984).

Neither THIP nor isoguvacine influence GABA uptake into brain slices or the activity of GABA transaminase (Krogsgaard-Larsen *et al.*, 1977). The selective actions of THIP and isoguvacine as $GABA_A$ agonists indicate that GABA interacts with $GABA_A$ receptors in a partially extended and almost planar conformation.

Isoguvacine has become the $GABA_A$ agonist of choice to define $GABA_A$ receptors and is used to occlude $GABA_A$ receptors in binding studies of $GABA_B$ (Hill and Bowery, 1981) and $GABA_C$ receptors (Johnston, 1994).

Like muscimol, THIP has CNS effects following systemic administration. THIP is somewhat weaker than muscimol as an anticonvulsant, but has a potent analgesic action (Hill *et al.*, 1981; Grognet *et al.*, 1983). Indeed, THIP is approximately equipotent to morphine as an analgesic, and, in contrast to morphine, does not produce respiratory depression. THIP is active clinically, for example, in patients with chronic pain of malignant origin at doses of 5–30 mg i.m. (Kjaer and Nielson, 1983). Other $GABA_A$ agonists show analgesic properties, e.g., kojic amine (Pelley and Vaught, 1987), and some stress-induced analgesias are associated with an apparent increase in $GABA_A$ receptors in the brain (Skerritt *et al.*, 1981).

THIP may act as a partial agonist of high efficacy at $GABA_A$ receptors (Krogsgaard-Larsen *et al.*, 1994). It inhibits its own analgesic action at higher doses, producing a bell-shaped dose-response curve (Zorn and Enna, 1987).

The analgesic action of THIP is not readily dissociated from its sedative or muscle relaxant properties and, thus, THIP is not a useful selective therapeutic agent (Grognet *et al.*, 1983). Further development of the "sons and daughters" of THIP may yield such agents. Such development is not confined to isoxazole analogues of GABA, and a variety of different approaches are being made to discover sufficiently selective agonists for subtypes of $GABA_A$ receptors (Johnston, 1991).

4.2.3. ZAPA. Z-3-[(aminoiminomethyl)thio]prop-2-enoic acid (ZAPA) is an isothiouronium analogue of GABA of restricted conformation due to the presence of a *cis*-double bond. It is a selective agonist for low affinity $GABA_A$ receptors that are modulated by benzodiazepines (Allan *et al.*, 1986). In addition, it is a substrate for the neuronal GABA transport system (Allan *et al.*, 1991). ZAPA does not cross the blood–brain barrier, and a suitable prodrug would have to be developed before a clinically useful CNS agent could emerge from compounds related to ZAPA. On the other hand,

the inability of ZAPA to cross the blood–brain barrier is advantageous, as ZAPA has a potent GABA-agonist action in nematodes and is regarded as an important lead compound for the design of novel anthelmintics (Holden–Dye and Walker, 1988).

4.2.4. (+)-TACP. (+)-*Trans*-(1S,3S)-3-aminocyclopentane-1-carboxylic acid ((+)-TACP) is one of the four possible stereoisomers of the cyclopentane analogue of GABA (Allan *et al.*, 1979). It is a potent $GABA_A$ agonist and does not act on GABA transport systems or enzymes. Ligand binding studies with [^{3}H]-(+)-TACP indicate that (+)-TACP binds to different $GABA_A$ receptors than does THIP (Dickenson *et al.*, 1990), and thus, these two conformationally restricted analogues may represent key ligands with which to further study the agonist profile of $GABA_A$ receptor subtypes.

Computer modelling studies on the structural similarities between $GABA_A$ agonists and the $GABA_A$ antagonist bicuculline suggest a limited range of active conformations for GABA at $GABA_A$ receptors (Andrews and Johnston, 1979). Within this range of conformations, THIP would adopt a conformation close to the "muscimol conformation," whereas (+)-TACP would adopt the "bicuculline conformation." These studies thus predicted the differences in THIP and (+)-TACP binding to $GABA_A$ receptors.

4.3. *Partial Agonists*

Partial agonists of $GABA_A$ receptors, particularly those of relatively low efficacy, show promise as therapeutic agents (Krogsgaard–Larsen *et al.*, 1994). The two most promising candidates are 5-(4-piperidyl)isoxazol-3-ol (4-PIOL) and thio-THIP. Piperidine-4-sulfonic acid and related compounds also show activity as partial agonists (Falch *et al.*, 1985). Different $GABA_A$ receptor subunit combinations influence the efficacy of partial agonists (Ebert *et al.*, 1994).

4.3.1. 4-PIOL. 4-PIOL, a "nonfused" analogue of THIP, is a low efficacy partial agonist at $GABA_A$ receptors (Kristiansen *et al.*, 1991). It is about 200 times less potent than isoguvacine as an agonist and about 30 times less potent than bicuculline methochloride as an antagonist. 4-PIOL does not pass the blood–brain barrier after systemic administration. 4-PIOL analogues, more potent than 4-PIOL and having different pharmacokinetic properties, are under development (Krogsgaard–Larsen *et al.*, 1994).

4.3.2. Thio-THIP. Although thio-THIP has $GABA_A$ agonist effects on cat spinal neurons (Krogsgaard–Larsen *et al.*, 1983), studies on human recombinant $GABA_A$ receptors show that thio-THIP is a low-efficacy partial agonist (Krogsgaard–Larsen *et al.*, 1994). The pK_a values of thio-THIP (6.1; 8.5) are such that a significant fraction of thio-THIP will contain the nonionised 3-hydroxyisothiazole group at physiological pH, and this may account for the very different efficacies of thio-THIP and THIP.

5. $GABA_A$ RECEPTOR ALLOSTERIC MODULATORS

The kinetics of GABA binding to rat brain membranes are dependent on the methods used to prepare the membranes, detergent extraction enhancing both the affinity of binding and the number of binding sites. This is due to the removal of substances that inhibit GABA binding and which are normally incorporated into the membranes (Johnston and Kennedy, 1978). The detergent treatment renders these inhibitors soluble, and their presence in the supernatant washes of such membrane preparations is readily demonstrated. A variety of such endogenous inhibitors of GABA binding exist and they have been called collectively GABARINS (GABA Receptor INhibitorS) (Johnston, 1981).

GABARINS appear to be involved in the modulation of $GABA_A$ receptor function. They may be involved in synaptic development and in synaptic changes in memory and learning. They could underlie phenomena such as denervation supersensitivity. Many drugs are likely to affect GABARINS, causing altered function of $GABA_A$ receptor complexes. GABARINS include GABA itself occluded in "cryptic" receptors (Elliott and Van Gelder, 1958), phospholipids (Johnston and Kennedy, 1978), GABA Receptor Inhibitory Factor (Yoneda and Kuriyama, 1980), purines (Ticku and Burch, 1980; Skerritt *et al.*, 1982a,b,c), peptides such as diazepam binding inhibitor (DBI) (Alho *et al.*, 1985), and steroids (Purdy *et al.*, 1991).

$GABA_A$ receptors possess a variety of sites independent of the agonist binding sites, activation of which, often bidirectionally, allosterically modulates the activity of agonists. The most widely investigated allosteric modulator sites are the benzodiazepine sites, where the clinically relevant antianxiety, anticonvulsant, sedative, and hypnotic properties of the benzodiazepines may result from the enhancement of the activation of some $GABA_A$ receptors by GABA. The discovery of some proanxiety, proconvulsant and stimulant benzodiazepines and β-carbolines that inhibit GABA-activation of $GABA_A$ receptors, led to the concept of "inverse agonists" that have the opposite effect on receptors to traditional benzodiazepine "agonists."

The key compound that led to the concept of benzodiazepine "inverse agonists" was methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-caboxylate (DMCM). The convulsant DMCM initially was described as having "negative efficacy" at benzodiazepine receptors (Braestrup *et al.*, 1983), but it was soon described as being an "inverse agonist" (Petersen *et al.*, 1983). Note that the actions of both the benzodiazepine "agonists" and "inverse agonists" are blocked by benzodiazepine "antagonists" such as Ro15-1788. More recently, the term "negative allosteric modulator" has been used for ligands such as DMCM (e.g., Puia *et al.*, 1991), and this appears to be a more accurate terminology. Benzodiazepines are not strictly "agonists" or "inverse agonist" at $GABA_A$ receptors, but allosteric modulators, since GABA is the real agonist at these receptors and benzodiazepines modulate the agonist action of GABA by acting at sites different from the agonist sites, i.e., allosteric sites. For these reasons, the terms "positive allosteric modulator," "negative allosteric modulator," and "neutralising allosteric modulator," i.e., an agent that neutralises the effects of positive and negative allosteric modulators, are preferable to the more widely used "agonist," "inverse agonist," and "antagonist" for agents that act allosterically on $GABA_A$ receptors to modulate agonist activation.

Furthermore, true inverse agonists have been described recently for β_2-adrenoceptors based on the two-state model of G-protein-coupled receptor activation (Bond *et al.*, 1995). In this model, the receptors are in equilibrium between the inactive conformation (R), and a spontaneously active conformation (R*) that can couple to G-protein in the absence of ligand. Classic agonists have a high affinity for R* and increase the concentration of R*, whereas inverse agonists have a high affinity for R and decrease the concentration of R*. Neutralising competitive antagonists have equal affinity for R and R* and do not displace the equilibrium, but can competitively antagonise the effects of both agonists and inverse agonists. Inverse agonists can switch off spontaneously active receptors in the absence of any agonist. It is not known if such a mechanism applies to $GABA_A$ receptors, which are likely to exist in a variety of different conformations and appear to be directly stimulated by agents such as barbiturates.

The mechanism of action of the three main classes of positive allosteric modulators has been investigated in patch-clamp studies

on mouse spinal neurons in tissue culture (reviewed in Rogers *et al.*, 1994). Barbiturates produce an increase in the mean open duration time of the $GABA_A$ chloride channels. Benzodiazepines produce an increase in the frequency of channel opening. Neuroactive steroids produce an increase in open duration time and in the frequency of channel opening, thus sharing aspects of the mechanisms of enhancement of both the barbiturates and the benzodiazepines. While most native $GABA_A$ receptors appear to be influenced by barbiturates and steroids, many native $GABA_A$ receptors are insensitive to benzodiazepines. It is known that benzodiazepine modulation depends on the presence of the γ_2-subunit (Pritchett *et al.*, 1989).

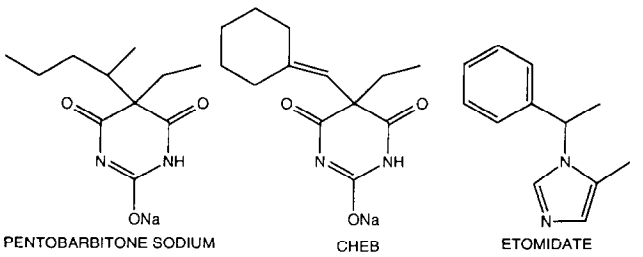

FIGURE 5. Structures of a sedative-hypnotic barbiturate, a convulsant barbiturate and an anaesthetic agent that act on $GABA_A$ receptors.

5.1. *Barbiturates*

Barbiturates have been used since the early 1900s as sedative-hypnotics, anticonvulsants, and anaesthetics. The first hint that they might act on GABA-mediated inhibition came from *in vivo* studies on presynaptic inhibition in the spinal cord (Eccles *et al.*, 1963). Barbiturates potentiated presynaptic inhibition. Furthermore, barbiturate anaesthesia could be reversed by picrotoxin and pentamethylenetetrazole, agents later found to be $GABA_A$ receptor antagonists. It is now known that barbiturates enhance the activation of $GABA_A$ receptors in a wide range of situations that may underlie their sedative-hypnotic and anaesthetic actions.

Barbiturates have a range of pharmacological actions in addition to enhancing $GABA_A$ receptor function, including antagonist effects on the activation of glutamate receptors and on glutamate release, effects likely to contribute to the anticonvulsant action of barbiturates (Willow and Johnston, 1983). It appears likely that the anaesthetic and anticonvulsant actions of barbiturates arise from different molecular actions (Skerritt *et al.*, 1983).

The enhancement of $GABA_A$ receptor function by barbiturates is related to an increase in the mean open duration time of the chloride channels (Macdonald *et al.*, 1989). This is achieved by the barbiturates producing a shift in the proportion of time spent in the two shorter open states to the longest open state. This is consistent with binding studies showing pentobarbitone slowing the rate of dissociation of GABA from its $GABA_A$ binding sites (Willow and Johnston, 1981a). Pentobarbitone has been shown to prolong the inhibitory action of GABA on spinal neurons *in vivo* without influencing the inhibitory action of glycine (Lodge and Curtis, 1978).

At higher concentrations than needed to enhance the action of GABA on $GABA_A$ receptors, some barbiturates directly activate $GABA_A$ receptors (Macdonald and Barker, 1979). Recombinant $GABA_A$ receptor studies indicate different domains on the β-subunits are required for activation by GABA and by pentobarbitone (Amin and Weiss, 1993).

There are marked similarities and differences between the actions of barbiturates, benzodiazepines, and steroids in enhancing the activation of $GABA_A$ receptors (Kerr and Ong, 1992; Rogers *et al.*, 1994). These probably reflect mutually interacting, but distinct, allosteric sites activated by barbiturates, benzodiazepines, and steroids on $GABA_A$ receptors. In general, the actions of the barbiturates and steroids on $GABA_A$ receptors are more widespread, while the actions of the benzodiazepines are more restricted to particular $GABA_A$ receptors. When the action of neurosteroids on $GABA_A$ receptors was discovered, it was thought that neurosteroids may represent endogenous ligands for the site on $GABA_A$ receptors activated by barbiturates (Majewska *et al.*, 1986). Subsequently, substantial differences were found between the actions of barbiturates and neurosteroids on $GABA_A$ receptors, e.g., barbiturates modulate $GABA_A$ autoreceptors, whereas steroids do not (Ennis and Minchin, 1993), and barbiturates and neurosteroids have different effects on the open time constants of $GABA_A$-activated chloride channels (Twyman and Macdonald, 1992).

Some barbiturates are convulsants. Indeed, the (+)-isomer of pentobarbitone is a convulsant directly depolarising neurons, whereas the sedative-hypnotic and anaesthetic properties reside in (−)-pentobarbitone (Mae Huang and Barker, 1980). The (+)-isomer is much weaker as an enhancer of the activation of $GABA_A$ receptors than is the (−)-isomer. The convulsant action of barbiturates does not appear to result from a negative allosteric modulation of $GABA_A$ receptors in the manner found for some other structural classes of allosteric modulators. Indeed, convulsant barbiturates enhance GABA binding to $GABA_A$ receptors in a similar manner to the anaesthetic barbiturates (Willow and Johnston, 1981b). The convulsant action of barbiturates appears to be associated with actions on calcium mechanisms. Thus, convulsant and anticonvulsant barbiturates have opposing effects on calcium-activated ATPase activity (Willow and Johnston, 1979) and on excitatory amino release (Willow *et al.*, 1980). The convulsant barbiturate 5-(-2-cyclohexylidine-ethyl)-5-ethyl barbituric acid (CHEB; see Fig. 5) has a direct excitatory action on neurons (Andrews *et al.*, 1981) and its depolarising action on dorsal root ganglion neurons results from opening a nonselective cation channel permeant to calcium ions (Pearce and Duchen, 1995). Glutarimides are structurally related to barbiturates and have either convulsant or depressant actions that do not seem to be related to any effects on $GABA_A$ receptors (Nicholson *et al.*, 1985).

The general anaesthetic etomidate (Fig. 5) has many properties similar to barbiturates, but it enhances GABA binding to $GABA_A$ receptors in a different way to the barbiturates (Willow, 1981). Its action on $GABA_A$ receptors appears to be dependent on the γ_2-subunit (Uchida *et al.*, 1995) and, thus, is similar to that of the benzodiazepines (Pritchett *et al.*, 1989).

5.2. *Benzodiazepines and Related Compounds*

Benzodiazepines were introduced as therapeutic agents in the early 1960s before GABA was considered to be a neurotransmitter. The enhancement of presynaptic inhibition by diazepam provided the first clue as to the mechanism of action of the benzodiazepines (Schmidt *et al.*, 1967), but it was not until much later that benzodiazepines were shown to enhance GABA-mediated synaptic inhibition (Polc and Haefely, 1976). Benzodiazepine binding sites were then described in rat brain (Squires and Braestrup, 1977), and such binding was found to be stimulated by GABA (Tallman *et al.*, 1978). The more pharmacologically relevant enhancement of GABA binding by benzodiazepines proved more difficult to demonstrate (Skerritt *et al.*, 1982d). GABA enhancement of diazepam binding

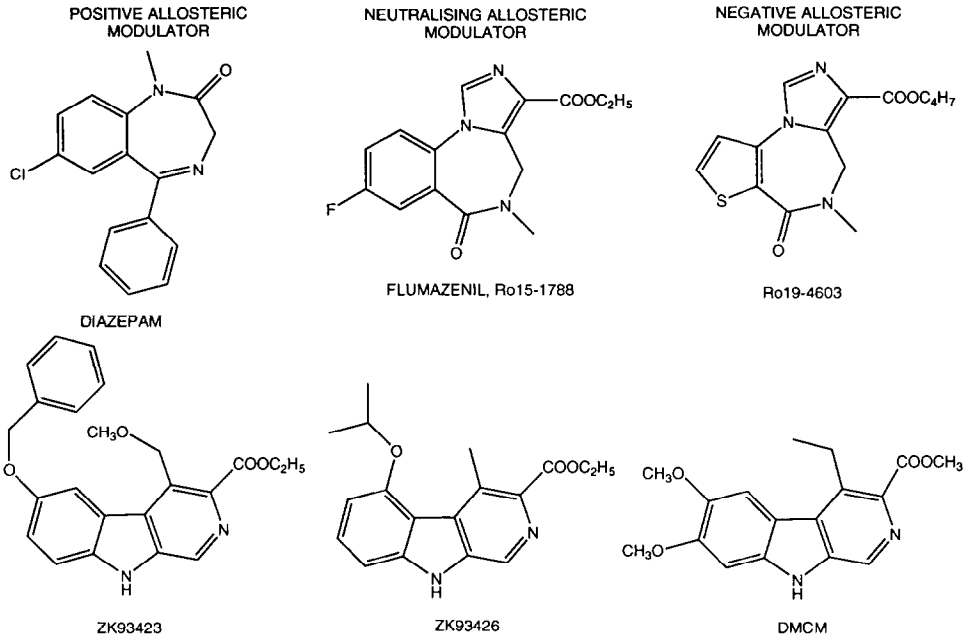

FIGURE 6. Examples of benzodiazepine and β-carboline positive, neutralising, and negative allosteric modulators of $GABA_A$ receptors.

is a relatively robust phenomenon, whereas diazepam enhancement of GABA binding is quite sensitive to membrane perturbations and may involve endogenous modulators (Skerritt *et al.*, 1982a). The discovery of flumazenil (Ro15-1788), an agent with no intrinsic activity at benzodiazepine receptors, but with the ability to block the activation of "central" (on $GABA_A$ receptors) rather than "peripheral" (independent of $GABA_A$ receptors) benzodiazepine receptors by other benzodiazepines, was an important advance (Polc *et al.*, 1981), as was the discovery of DBI, an endogenous peptide that inhibited diazepam binding, acting as a negative allosteric modulator (Alho *et al.*, 1985). A wide variety of ligands are now known to interact with benzodiazepine binding sites, and the field is being researched with increased vigour in an effort to produce better therapeutic agents that do not have the side effects of the traditional benzodiazepines (Gardner *et al.*, 1992). Unwanted side effects of benzodiazepines include dependence, potentiation of the effects of ethanol, mild ataxia, and mild amnesia.

Benzodiazepine receptors are subdivided into "central" and "peripheral" receptors, reflecting the predominate regional distribution of these receptors. Flumazenil only interacts with the "central" receptors, while Ro5-4868, the 4′-chloro derivative of diazepam, is a selective ligand for the "peripheral" receptors. Only the central receptors are associated with $GABA_A$ receptors. The "peripheral" receptors are found in mitochondria and have a variety of functions, including the control of steroidogenesis. The "peripheral" receptors are found in the CNS, and it might be more accurate to describe the two main benzodiazepine receptors types as "neuronal" and "non-neuronal" (Gardner *et al.*, 1992).

Central benzodiazepine receptors associated with $GABA_A$ receptors have been classified into subtypes on the basis of their pharmacology. Thus, BDZ_1 sites, which predominate in the cerebellum, show high affinity for the triazolopyridazine CL218872, zolpidem, and some β-carbolines (see Figs. 6 and 7), whereas BDZ_2 sites, which predominate in the spinal cord, show low affinity for these ligands and high affinity for flunitrazepam. The only functional difference between BDZ_1 and BDZ_2 sites appears to be a particularly strong sedative action induced by BDZ_1-preferring positive allosteric modulators (Gardner *et al.*, 1992). A further subtype, BDZ_3, which is found in cerebellar granule cells associated with the α_6 $GABA_A$-subunit, is selective for the negative allosteric modulator Ro15-4513 and is insensitive to diazepam. A natural mutant of the α_6 $GABA_A$-subunit has been described that yields receptors sensitive to diazepam (Korpi and Seeburg, 1993). The BZD_1 sites are seen in recombinant $\alpha_1\beta_2\gamma_2$ receptors, whereas recombinant receptors containing $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$ or $\alpha_5\beta_2\gamma_2$ largely match the BZD_2 sites (Kleingoor *et al.*, 1993). The nature of the β-subunit does not appear to significantly influence benzodiazepine pharmacology (Hadingham *et al.*, 1993).

The enhancement of $GABA_A$ receptor responses by diazepam requires the presence of the γ_2-subunit (Pritchett *et al.*, 1989). A single amino acid of the γ_2-subunit determines the effects of the benzodiazepines. Mutating threonine 142 to serine changes the action of flumazenil from a neutralising to a positive allosteric modulator and doubles the potencies of diazepam, flunitrazepam, and clonazepam as positive allosteric modulators (Mihic *et al.*, 1994). On the other hand, responses to the Type I benzodiazepine ligands, such as zolpidem and alpidem, were halved by this mutation, which did not influence GABA affinity or efficacy, or modulation by pentobarbitone or alphaxolone. As noted above, the α-subunits of the $GABA_A$ receptors are important in determining benzodiazepine pharmacology. Four amino acids in the α-subunits appear to be particularly important (Wieland and Lüddens, 1994). A glycine/glutamate exchange switches between BDZ_1 and BDZ_2 receptor subtypes. A histidine corresponding to position 100 in α_6 is essential for the binding of diazepam (Kleingoor *et al.*, 1993). A valine/isoleucine exchange in a α_6-derived mutant increases the affinity for diazepam and decreases the affinity for flumazenil. These $GABA_A$ receptor mutants may prove useful in modelling the benzodiazepine binding sites (e.g., Maddalena and Johnston, 1995).

The mechanism of action of positive and negative allosteric modulators of $GABA_A$ receptor function acting at benzodiazepine sites has been studied by patch clamping on mouse spinal neurones in culture (Rogers *et al.*, 1994). The results have been interpreted on a kinetic gating scheme for $GABA_A$ receptors involving a series open and closed states. Diazepam increased open frequency of channels without altering mean open duration or the relative occurrence of openings of the three open states. Diazepam increased the probability of channel opening without altering the kinetics

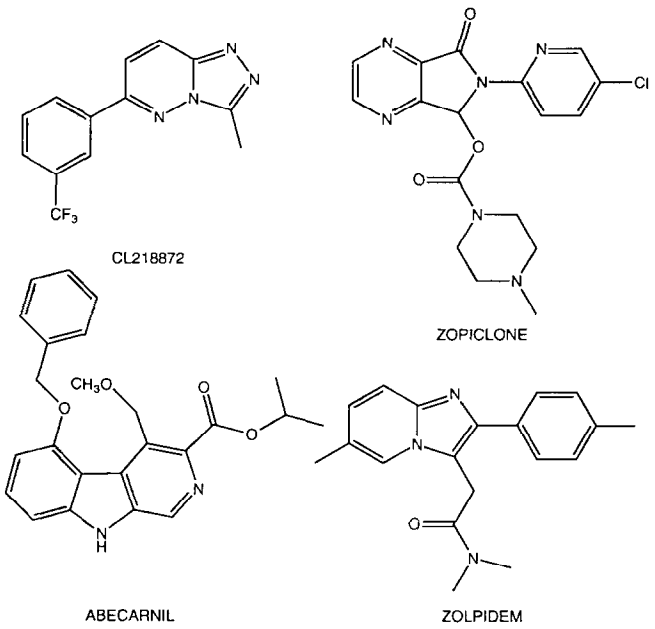

FIGURE 7. Some structurally diverse substances that act on benzodiazepine sites on $GABA_A$ receptors.

of channel closing or the relative proportion of time spent in any one of the open states once GABA is bound. The negative allosteric modulator, DMCM, a β-carboline, did the opposite of diazepam. It decreased the probability of channel opening without altering the kinetics of channel closing or the relative proportion of time spent in any one of the open states after GABA was bound. Since burst frequency, but not intraburst opening frequency, was altered, it is unlikely that channel opening rates were altered by diazepam or DMCM.

A number of endogenous ligands for benzodiazepine receptors have been found. Of most interest are peptides related to DBI (Alho *et al.*, 1985) and related "endozapines." Trace amounts of benzodiazepines have been found in brain tissue and their *in vivo* formation demonstrated (Medina *et al.*, 1993). A low molecular factor released from astroglia appears to act as a negative allosteric modular at benzodiazepine receptors (Rigo *et al.*, 1994).

DBI is a 10 kDa protein isolated independently in five different laboratories, based on its ability to (1) displace diazepam bound to brain membranes, (2) affect cell growth, (3) bind long-chain acyl-coenzyme A esters, (4) stimulate steroidogenesis in adrenal mitochondria, and (5) inhibit glucose-induced insulin secretion from the pancreas (Knudsen *et al.*, 1993). DBI acts as a relatively weak negative allosteric modulator of central benzodiazepine receptors and shows higher potency in interacting with peripheral benzodiazepine receptors where it regulates steroidogenesis (Whitehouse, 1992). Given that steroids capable of acting on $GABA_A$ receptors in the brain are produced in the adrenals, DBI provides a link between the nervous and endocrine systems that may represent an important site of drug action. DBI also provides a link between stress, anxiety, and the immune system (Ferrarese *et al.*, 1993).

5.3. *β-Carbolines and Related Compounds*

Proconvulsant β-carbolines were discovered in attempts to isolate endogenous ligands for benzodiazepine receptors, with the ethyl ester of β-carboline-3-carboxylate being isolated from an ethanol treatment of human urine at low pH (Nielsen *et al.*, 1979). This

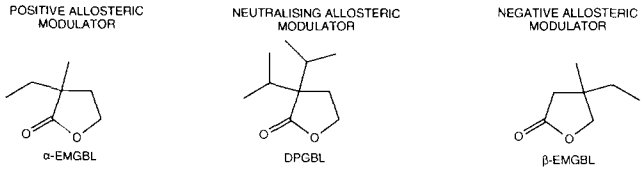

FIGURE 8. Examples of γ-butyrolactone positive, neutralising, and negative allosteric modulators of $GABA_A$ receptors.

probably arose as an artifact during the isolation by esterification with ethanol of β-carboline-3-carboxylic acid derived from tryptophan by ring closure (Kerr and Ong, 1992). Subsequently, the n-butyl ester of β-carboline-3-carboxylate has been isolated under conditions where esterification by n-butanol was unlikely and, thus, this ester may be a true endogenous β-carboline ligand for benzodiazepine receptors (De Robertis *et al.*, 1988). Some ^{18}F-labelled analogues of β-carboline esters show promise as PET imaging agents (Elder *et al.*, 1995).

The most potent β-carboline negative modulator is DMCM (see Fig. 7), methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (Braestrup *et al.*, 1982). β-Carbolines exhibit the full range of negative, neutralising, and positive allosteric modulators, with the negative allosteric modulators predominating. 6-Benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate ethyl ester (ZK93423) is a potent positive allosteric modulator (Hollinshead *et al.*, 1990). 5-Isopropyl-4-methyl-β-carboline-3-carboxylate ethyl ester (ZK93426) is a potent neutralising allosteric modulator neutralising the actions of DMCM and ZK93423 (Jensen and Petersen, 1983).

Abecarnil (Fig. 7) is a β-carboline that shows a behavioural profile of a partial positive allosteric modulator. It is anxioselective and may have differential effects on functionally different subtypes of benzodiazepine binding sites (Stephens *et al.*, 1990). Other selective agents that may act on different subtypes of benzodiazepine binding sites include Zolpidem, which is hypnoselective and is structurally related to Zopiclone (Langtry and Benfield, 1990).

5.4. *γ-Butyrolactones and Related Compounds*

The competitive $GABA_A$ receptor antagonist bicuculline and the $GABA_A$-activated chloride channel antagonist picrotoxinin both contain γ-lactone moieties that are essential to their activity. A variety of simple γ-butyrolactones (Fig. 8) appear to be able to modulate picrotoxinin sites on $GABA_A$ receptor complexes (Holland *et al.*, 1990, 1993), while thiolactones, dithiolactones, and spirolactones show analogous activity to γ-butyrolactones (Holland *et al.*, 1990; Peterson *et al.*, 1994).

Simple β-substituted γ-butyrolactones, e.g., β-ethyl-β-ethyl-γ-butyrolactone (β-EMGBL) are generally potent convulsants regardless of any other substitutions on the lactone ring, whereas γ-butyrolactones substituted on the α- or γ-positions are anticonvulsants, e.g., α-ethyl-α-ethyl-γ-butyrolactone (α-EMGBL). The convulsant effects of the β-substituted γ-butyrolactones can be reversed by the anticonvulsant γ-butyrolactones, and all of these γ-butyrolactones appear to act at the picrotoxinin sites on $GABA_A$ receptor complexes. Thus, γ-butyrolactones may act as positive or negative allosteric modulators of picrotoxinin sites. Furthermore α,α-di-isopropyl-γ-butyrolactone (DPGBL) appears to act as a neutralising allosteric modulator having no effect on GABA-induced responses, but neutralising the actions of both positive and negative allosteric modulators at picrotoxinin sites (Holland *et al.*, 1991).

The γ-butyrolactones exhibit a similar spread of actions on picro-

toxinin sites to that found for compounds acting on benzodiazepine sites on $GABA_A$ receptor complexes. Unlike benzodiazepine sites, picrotoxinin sites may be present on most, if not all, subunit proteins of $GABA_A$ receptor complexes.

Caprolactams may have similar effects to the γ-butyrolactones in that both convulsant and anticonvulsant caprolactams have been described, and these may act on picrotoxinin sites on $GABA_A$ receptor complexes (Kerr and Ong, 1992). For example, the convulsant 4,6,6-trimethylcaprolactam is a noncompetitive antagonist of $GABA_A$ responses in the guinea-pig ileum whose actions are reversed by pentobarbitone (Kerr *et al.*, 1986). Convulsant caprolactams block and anticonvulsant caprolactams enhance $GABA_A$ receptors in spinal cord neurons in culture and muscimol binding to rat brain membranes (Skerritt *et al.*, 1985).

5.5. *Ethanol and Related Compounds*

$GABA_A$ receptors may play important roles in the actions of ethanol and in alcoholism (Korpi, 1994). Ethanol has concentration-dependent enhancing effects on $GABA_A$ receptors, with the effects at relatively low concentrations (20 mM) considered to be related to sedative and motor-uncoordinating effects of ethanol, and the effects at higher concentrations (50–400 mM) considered to be related to the anaesthetic effects of ethanol. Ethanol differentially modulates $GABA_A$ receptor currents in different brain regions, reflecting multiple mechanisms of ethanol action on $GABA_A$ receptors (Soldo *et al.*, 1994). Ethanol may modulate most ligand-gated ion channels, including NMDA, $5\text{-}HT_3$, and ATP receptors (Li *et al.*, 1993; Grant, 1994).

The enhancement of $GABA_A$ responses induced by ethanol at relatively low concentrations (20 mM) is dependent on the presence of the alternatively spliced variant (γ_{2L}) of the γ_2-subunit containing an extra 8 amino acids in the region between M3 and M4, a proposed intracellular loop, which has been phosphorylated by protein kinase C (Wafford and Whiting, 1992). The relative sensitivity of neurons to these concentrations of ethanol in various regions of the brain might be explained by the expression of γ_{2S} rather than γ_{2L} $GABA_A$-subunits or, alternatively, the level of phosphorylation of the γ_{2L} subunits. The potentiation of GABA responses by ethanol has been shown to be modulated by protein kinase C (Weiner *et al.*, 1994). Individual differences in enzymes that can determine the phosphorylation state of $GABA_A$ receptor subunits may explain differences in behavioural sensitivity to ethanol.

Anaesthetic concentrations of ethanol (50–400 mM) and butanol (1–20 mM) enhance $GABA_A$-mediated currents in a variety of recombinant receptors that do not contain the γ_{2L}-subunit (Mihic *et al.*, 1994). Thus, these effects appear to be distinct from the effects of the low ethanol concentrations on $GABA_A$ receptors, suggesting different mechanisms of action on $GABA_A$ receptors for ethanol at subanaesthetic and anaesthetic concentrations.

Chronic ethanol alters the expression of various $GABA_A$ receptor subunits (Mhatre and Ticku, 1992). Of particular interest is the large increase in the level of the α_6-subunit in the cerebellum, which selectively encodes Ro15-4513 binding sites. The benzodiazepine receptor partial negative allosteric modulator Ro15-4513 is known to potently and specifically abolish ethanol intoxication (Suzdak *et al.*, 1988).

Trichloroethanol, the active metabolite of the general anaesthetic chloral hydrate, enhances GABA-activated chloride currents in the hippocampus at 0.2–10 mM (Lovinger *et al.*, 1993). The currents were both enhanced in amplitude and prolonged in time course in a manner similar to the action of barbiturates or steroid anaesthetics.

5.6. *Neurosteroids and Neuroactive Steroids*

The CNS depressant actions of steroids have been known since 1927 when Cashin and Moravek (1927) injected a colloidal suspension of cholesterol into cats, causing deep anaesthesia. Subsequently, cholesterol was found to potentiate the anaesthetic actions of pentobarbitone (Starkenstein and Weden, 1936), but it was not until the extensive investigations of Seyle (1942) that it became apparent that a wide range of natural and synthetic steroids have anaesthetic actions. These studies led to the development of steroid anaesthetic agents, such as alphaxolone (see Section 5.8). Electrophysiological studies showed that alphaxolone selectively enhanced the activation of $GABA_A$ receptors by GABA, thus providing a basis for the anaesthetic action of alphaxolone involving a specific receptor site (Harrison and Simmonds, 1984). Then came the discovery that steroid hormone metabolites that occur in the brain are "barbiturate-like modulators" of the $GABA_A$ receptor (Majewska *et al.*, 1986). This led to the concept that neurosteroids, produced in the brain, can directly modulate $GABA_A$ receptors on the cell surface rather than acting on receptors in the nucleus regulating gene expression (Baulieu, 1991). These neurosteroids are produced in glial cells, where, interestingly, their synthesis is controlled by the endogenous peptide, DBI, a ligand for the "peripheral" benzodiazepine binding sites, which are independent of $GABA_A$ binding sites (Costa *et al.*, 1994).

It may be important to distinguish between "neurosteroids" and "neuroactive steroids," the former being steroids synthesised in the brain, whereas the latter term refers to steroids active in the brain, but not necessarily synthesised in the brain. Studies on the synthesis of pregnenolone and metabolites such as allopregnanolone (3α-hydroxy-5α-pregnan-20-one; 3α-OH-DHP; Fig. 9) from cholesterol in brain tissue led to the term "neurosteroid" (Baulieu, 1991). On the other hand, allotetrahydrodeoxycorticosterone (3α,21-dihydroxy-5α-pregnan-20-one; 5α-THDOC; allotetrahydroDOC) is a "neuroactive steroid" because the sole source of this steroid appears to be the adrenals. Nonetheless, 5α-THDOC is found in the brain, where its concentration is increased during stress (Purdy *et al.*, 1991). 3α-OH-DHP and 5α-THDOC are among the most potent known steroid modulators of $GABA_A$ receptors. Steroids produced in the adrenals influence the expression of $GABA_A$ receptor subunits in the brain, as shown by adrenalectomy (Orchinik *et al.*, 1994).

Neuroactive steroids enhance the activation of $GABA_A$ receptors by increasing the average open time of the GABA-activated chloride channels. This is a result of increasing the proportion of the two longer open duration time constants at the expense of the shortest open duration time constant in mouse spinal cord neurons in culture (Twyman and Macdonald, 1992). In addition, the steroids increase opening frequency. At higher concentrations, some steroids inhibit the activation of $GABA_A$ receptors by decreasing the average open duration time. A bidirectional activity has been noted for many neuroactive steroids, enhancing the activation of $GABA_A$ receptors at lower concentrations and inhibiting the activation at higher concentrations, e.g., pregnenolone and its sulphate (Ong *et al.*, 1987b). Blockade of the enhancing effects of neuroactive steroids by epipregnanolone (3β-hydroxy-5β-pregnan-20-one) indicates the existence of more than one class of binding sites for steroids at $GABA_A$ receptors (Prince and Simmonds, 1993).

Neuroactive steroids appear to be able to modulate $GABA_A$ receptor activity only when applied extracellularly and are inactive on intracellular administration (Lambert *et al.*, 1990). There are regional differences in the sensitivity of $GABA_A$ receptors to

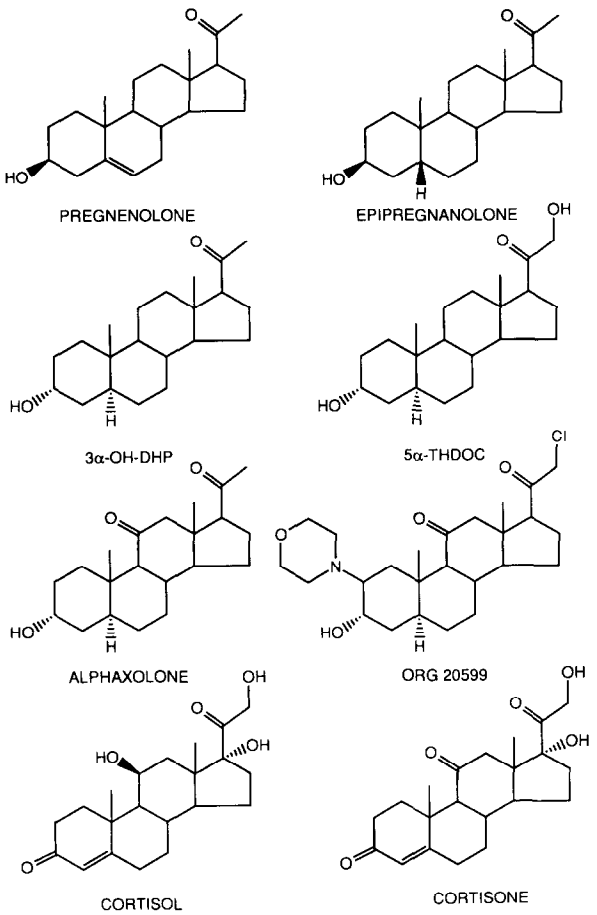

FIGURE 9. Neuroactive steroids that modulate $GABA_A$ receptor function.

modulation by neuroactive steroids (Jussofie, 1993), and the effects of neuroactive steroids are dependent on the subunit composition of the $GABA_A$ receptors (Puia *et al.*, 1993). Furthermore, the stage of the estrus cycle influences the potency of neuroactive steroids (Finn and Gee, 1993). Estrogens regulate $GABA_A$ receptor subunit mRNA expression in regions of the female rat brain known to contain estrogen receptors (Herbison and Fenelon, 1995).

Although many of the actions of neuroactive steroids are similar to those of barbiturates on $GABA_A$ receptors, steroids and barbiturates interact with different sites on $GABA_A$ receptors (Kerr and Ong, 1992). GABA autoreceptors are modulated by barbiturates, but not by steroids (Ennis and Minchin, 1993). Insect GABA receptors are only weakly influenced by neuroactive steroids (Rauth *et al.*, 1993).

There is considerable interest in the development of drugs to act on the neuroactive steroid sites of $GABA_A$ receptors, particularly analogues of steroids that lack the traditional properties of steroids, but retain the ability to modulate $GABA_A$ receptors (e.g., Burden *et al.*, 1991; Hu *et al.*, 1993). A model of the interaction of pregnenolone sulfate binding site on $GABA_A$ receptors has been published (Roberts, 1995).

Other actions of neuroactive steroids include the positive and negative allosteric modulation of NMDA receptors (Bowlby, 1993; Park-Chung *et al.*, 1994), and the negative modulation of calcium currents via a pertussis-toxin-sensitive G-protein-coupled mechanism (ffrench-Mullen *et al.*, 1994).

5.7. *Corticosteroids*

Cortisol is a potent bidirectional modulator of $GABA_A$ receptors in the guinea-pig ileum, enhancing at low (1–10 pM) concentrations and inhibiting at higher (10–1000 nM) concentrations (Ong *et al.*, 1987a). Cortisone is a potent noncompetitive inhibitor of these $GABA_A$ receptors acting at concentrations as low as 1 pM (Ong *et al.*, 1990). Thus, these corticosteroids are the most potent agents acting on $GABA_A$ receptors. The actions of cortisol may be restricted to particular $GABA_A$ receptors, since cortisol has little effect on $GABA_A$ responses in the rat cuneate nucleus (Andres-Trelles *et al.*, 1989), although there well may be species differences regarding the effects of corticosteroid since rats do not employ 17α-hydroxy-corticosteroids, whereas guinea-pigs do (Kerr *et al.*, 1990). Biphasic effects of corticosteroids have been described on TBPS binding to rat brain membranes, low (nanomolar) concentrations enhancing binding and higher (micromolar) concentrations inhibiting, the effect of nanomolar concentrations indicative of an antagonist action as observed at these concentrations on GABA responses in the guinea-pig ileum (Majewska, 1987).

The rapid and readily reversible modulation of certain $GABA_A$ receptors by corticosteroids is indicative of actions at the level of the receptor complexes on neuronal membranes rather than a delayed action via cytosolic receptors. Given the relationships between stress and the release of corticosteroid hormones (Munck and Guyre, 1986) and the rapid changes induced by stress in $GABA_A$ receptor properties, it may be that stress-induced steroid release from the adrenals represents an important mechanism whereby the intestine and other tissues respond to stress via changes in GABA receptor function (Kerr *et al.*, 1990).

The very potent actions of corticosteroids on some GABA receptors indicate the possibility of physiological regulation of GABA-mediated mechanisms by endogenous corticosteroids not only in the intestine, but also in the CNS where cortisol has been shown to modulate hypothalamic neurons (Mandelbrod *et al.*, 1981), possibly by modifying GABA-mediated synaptic inhibition. Modulation of GABA-mediated inhibition may underlie the specific corticosteroid-induced reversal of the depressed, withdrawn and apathetic mood in the hypocortisolism of Addison's disease (Mason, 1968). Benzodiazepines are known to suppress cortisol secretion possibly by inhibiting the hypothalamic release of corticotropin releasing factor (Gram and Christensen, 1986). Cortisol and cortisone are unlikely to be synthesised in the brain as neurosteroids due to the lack of key enzymes for their synthesis in the brain (Mellon and Deschepper, 1993), and adrenalectomy abolishes the effects of swim stress on $GABA_A$ receptors in rat brain (Schwartz *et al.*, 1987).

The very potent, but opposing, effects of picomolar concentrations of cortisol and cortisone on GABA receptors in the intestine, with cortisol enhancing and cortisone reducing GABA responses, is very interesting both from a structure–activity and a physiological viewpoint. Cortisol and cortisone differ in structure only by the level of oxidation at carbon 11, cortisol being the 11β-hydroxy compound and cortisone the 11-oxo compound (Fig. 9). A single enzyme interconverts cortisol and cortisone, an 11β-hydroxysteroid dehydrogenase, in the CNS and other tissues (Funder, 1994). The potency and selectivity of corticosteroid actions on particular GABA receptors are indicative of specific receptors that could be sites of drug action.

Corticosteroids also influence the expression of $GABA_A$ receptor subtypes in the brain, presumably via genomic receptors, as shown by the effects of short-term adrenalectomy and corticosterone replacement in female rats (Orchinik *et al.*, 1994).

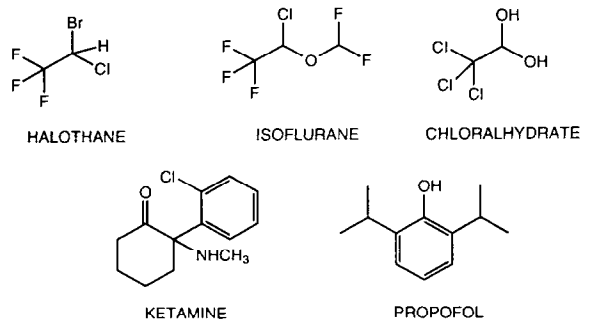

FIGURE 10. Anaesthetic agents that modulate $GABA_A$ receptor function.

5.8. *Anaesthetic Agents*

Enhancement of $GABA_A$ receptor responses may be a common action for anaesthetic agents. A wide range of general anaesthetics, including inhalation (halothane, diethylether, enflurane, and isoflurane), i.v. (alphaxolone, ketamine, and propofol) and alcohol (pentanol) anaesthetics, enhance the action of GABA on $GABA_A$ receptors expressed in *Xenopus* oocytes in a calcium-independent manner at clinically relevant doses (Lin *et al.*, 1992). The structures of some of these agents are shown in Fig. 10. The effects of the anaesthetics on $GABA_A$ responses were dependent on GABA concentrations, enhancement being most marked at low GABA concentrations and decreasing exponentially as the GABA concentration increased. The effectiveness of enflurane enhancement of GABA responses is dependent on the subunit composition of the recombinant receptors with the order of sensitivity being $\alpha_1\beta_1$ > $\alpha_1\beta_1\gamma_{2S}$ > $\alpha_1\beta_1\gamma_{2L}$ > total mRNA (Lin *et al.*, 1993).

The enhancement of $GABA_A$ responses by inhalation anaesthetics is not restricted to GABA-activated chloride channels since isoflurane enhances glycine-activated chloride channels (Harrison *et al.*, 1993). Isoflurane, however, is inactive on homomeric ϱ_1 recombinant receptors.

The volatile anaesthetic halothane increased the binding to mouse brain membranes of the $GABA_A$ agonist muscimol and decreased the binding of the $GABA_A$ antagonist SR 95531 via changes in the apparent B_{max} of the two ligands (Harris *et al.*, 1994), suggesting that halothane may have altered the equilibrium between agonist and antagonist states of the $GABA_A$ receptors. Moreover, in similar experiments, the volatile anaesthetic isoflurane showed the appropriate stereoselectivity in that the (+)-isomer was approximately twice as potent as the (−)-isomer in enhancing muscimol binding. These studies are consistent with the existence of specific recognition sites on $GABA_A$ receptors for inhalation anaesthetics.

The general anaesthetic action of chloral hydrate is likely to be due to the metabolite trichloroethanol, which enhances $GABA_A$-mediated synaptic transmission, as noted in Section 5.5 (Lovinger *et al.*, 1993).

The steroid anaesthetic alphaxolone (3α-hydroxy-5α-dihydropregnane-11,20-dione) is no longer used in human medicine due to reports of allergic reactions (Clarke *et al.*, 1975), which may have been due to other components in the commercial preparation Althesin, a 3:1 mixture of alphaxolone and alphadolone acetate (5α-pregnane-3α,21-diol-11,20-dione 21 acetate) solubilised in water with Cremophor EL, a polyethoxylated castor oil. In fact, the Cremophor EL may have been the actual problem since it has been shown to cause similar allergic responses in dogs (Phillipps, 1975). Althesin is currently in use as a veterinary sedative/anaesthetic for monkeys and cats. Structure–activity studies on alphaxolone, involving over 1000 compounds, produced the aminosteroid minaxalone, which showed great promise as a clinically useful steroidal anaesthetic (Phillipps, 1975; Phillipps *et al.*, 1979). The search for water-soluble steroid anaesthetic agents continues with a new agent, (2β,3α,5α)-21-chloro-3-hydroxy-2-(4-morpholinyl)pregnan-20-one methanesulphonate (ORG 20599), recently described (Hill-Venning *et al.*, 1994). This agent is a potent positive allosteric modulator of $GABA_A$ receptor function.

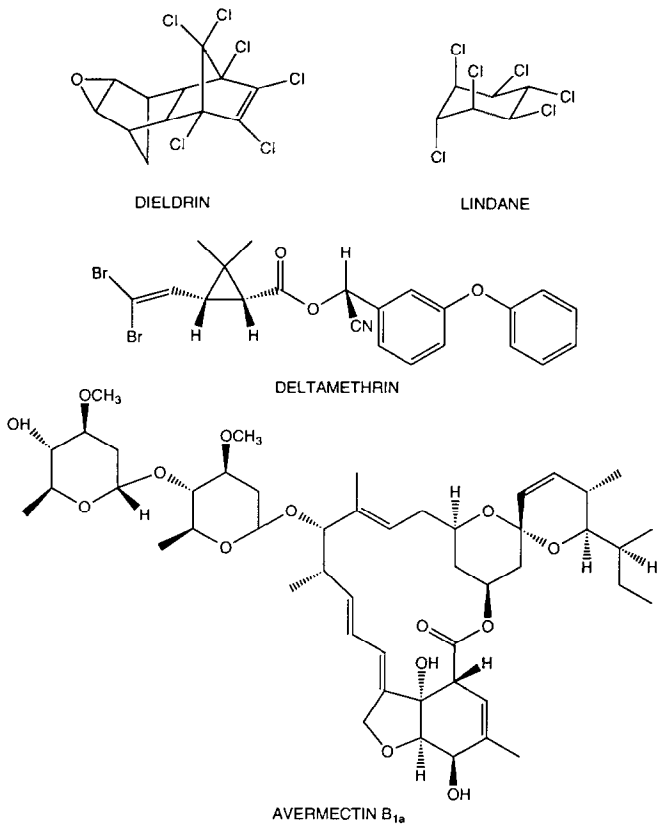

FIGURE 11. Insecticides that act on $GABA_A$ receptors.

5.9. *Insecticides*

A variety of insecticides are known to influence mammalian $GABA_A$ receptors, probably by interacting with the picrotoxinin binding site (Casida, 1993). The structures of some of these insecticides are shown in Fig. 11. Molecular modelling studies have shown a close structural resemblance between picrotoxin and representative compounds from five classes of insecticides: 1-phenyltrioxabicyclooctanes, dithianes, silatranes, lindane and isomers, and cyclodienes such as dieldrin (Calder *et al.*, 1993).

The insecticides dieldrin and lindane have been shown to both enhance and inhibit GABA-induced chloride currents in mammalian preparations (Nagata *et al.*, 1994). The interactions are complex, with dieldrin enhancing at higher concentrations and two components being involved in the inhibition seen at lower concentrations. Convulsant cyclodiene and hexachlorocyclohexane insecticides inhibit $GABA_A$ receptor function, whereas depressant hexachlorohexanes have bidirectional actions (Pomés *et al.*, 1994).

Analogues of DDT have been shown to enhance benzodiazepine binding, under conditions where DDT itself and pyrethroids such as deltamethrin inhibit benzodiazepine binding (Lummis *et al.*, 1987). GABA-activated chloride channels are a likely target

for pyrethroids, in addition to their well-known action on sodium channels (Narahashi *et al.*, 1992).

Avermectin B_{1a}, a macrocyclic lactone insecticide and anthelmintic, modulates $GABA_A$ receptor function. It has been shown to enhance or inhibit GABA and flunitrazepam binding, depending on the concentrations and conditions used, while avermectin binding is modulated by GABA agonists and antagonists in a chloride-independent manner (Drexler and Sieghart, 1984). Avermectin appears to directly activate chloride channels in mammalian central neurons, which resemble the channels activated by GABA and glycine, but are clearly distinguishable from them (Payne and Soderlund, 1991; Schönrock and Bormann, 1993b). It has been suggested that avermectin opens $GABA_A$-receptor channels by binding to the GABA recognition site and acting as a partial agonist, in addition to opening voltage-dependent chloride channels, which are totally insensitive to GABA, but are very sensitive to 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (Abalis *et al.*, 1986). Other workers have suggested that overall, the effects of avermectin are unique and require the presence of another separate drug receptor site on $GABA_A$ receptor complexes (Olsen and Snowman, 1985).

5.10. *Simple Cations*

Protons (H^+) facilitate $GABA_A$ receptor-mediated responses in that decreases in extracellular pH increase GABA responses, while more alkaline pH values decrease GABA responses (reviewed by Kaila, 1994). The facilitation by extracellular protons may be due to an increase in mean channel lifetime, whereas the decrease at alkaline pH values may be the result of increased desensitisation. In functional terms, the responses of $GABA_A$ receptors to changes in extracellular pH is qualitatively opposite to the responses of the NMDA subtype of glutamate receptors (Tang *et al.*, 1990). The sensitivity of $GABA_A$ and NMDA receptors to changes in extracellular pH might play a protective role in conditions such as anoxia and ischaemia, which are known to be associated with large acid shifts in extracellular fluids.

Ammonium ions (NH_4^+) enhance the action of GABA on $GABA_A$ receptors in dissociated rat cortical neurons, an effect independent of benzodiazepine receptors, in that it is insensitive to Ro15-1788 (Takahashi *et al.*, 1993). This action of ammonium ions on $GABA_A$ receptors could contribute to the symptoms of hepatic encephalopathy, which are characterised by large increases in ammonium ion concentrations and in GABA-mediated inhibition in the brain.

Divalent cations have been reported to exert a bidirectional modulation of GABA-gated chloride fluxes in synaptoneurosomes consistent with their ability to permeate and block Ca^{2+} channels (Schwartz *et al.*, 1994). The order of potency for inhibitory effects on the action of GABA was $Ca^{2+} > Sr^{2+} > Ba^{2+}$, similar to the order of potency for permeation of Ca^{2+} channels in neurons. The order of potency for enhancement of GABA action was $Cd^{2+} > Mn^{2+} > Mg^{2+}$, similar to the order for blockade of Ca^{2+} channels in neurons.

Changes in intracellular calcium ion concentration (Ca^{2+}) exert significant modulatory action on $GABA_A$ receptors. There appears to be a bell-shaped dependence of $GABA_A$ receptor activity on internal calcium ion concentration, with a maximum around 0.1 μM (Taleb *et al.*, 1987). The effects of intracellular calcium ions on $GABA_A$ receptor function may be mediated by modulation of phosphorylation of sites on the intracellular loops of particular protein subunits (see Section 5.12). A calcium-dependent suppression of GABA responses might be important in epileptogenesis and could play a role in synaptic plasticity.

Zinc ions (Zn^{2+}) noncompetitively inhibit the action of GABA on some $GABA_A$ receptors. Studies on recombinant receptors suggest that the presence of a γ-subunit leads to an insensitivity to zinc ions (Smart *et al.*, 1991), although there are $GABA_A$ receptor subtypes that do not contain a γ-subunit that are insensitive to zinc ions (Smart, 1992). The α-subunits influence the effects of Zn^{2+}, with inhibition being greater in receptors containing α_2- and α_3-subunits than those containing α_1-subunits (White and Gurley, 1995). The inhibition of GABA responses by zinc ions appears to result from a decrease in the frequency of channel opening via a binding site that is independent of the sites of action of barbiturates, benzodiazepines, steroids, and picrotoxin. Zinc ions may bind to a site located on the extracellular part of the $GABA_A$ receptor complex (Kilic *et al.*, 1993). Zinc and copper (Cu^{2+}) ions may share binding sites, since copper ions have a very similar action to zinc ions on $GABA_A$ receptor responses in terms of potency and efficacy (Yan Ma and Narahashi, 1993). Since certain CNS neurons contain zinc in their presynaptic boutons, the modulation of $GABA_A$ (and NMDA and ATP) receptors by zinc ions may have physiological relevance.

Lanthanum ions (La^{3+}) stimulate GABA currents in $\alpha_1\beta_2\gamma_2$ receptors expressed in human kidney cells (Im *et al.*, 1992). Studies on TBPS binding indicate that the lanthanum site on $GABA_A$ receptors appears to be distinct from the Zn^{2+} site and from other monovalent and divalent cation recognition sites (Im and Pregenzer, 1993). La^{3+}-induced enhancement of $GABA_A$ responses in rat dorsal root ganglia did not appear to compete with benzodiazepines, barbiturates, or picrotoxin for binding sites and acted independently of the sites activated by Cu^{2+} and Zn^{2+} (Yan Ma and Narahashi, 1993). These studies indicate that the La^{3+}, Cu^{2+}, and Zn^{2+} binding sites are likely to be located at or near the external orifice of the chloride channel of $GABA_A$ receptors.

5.11. *Simple Anions*

Chloride ions are clearly intimately involved in $GABA_A$ receptor-mediated synaptic inhibition, and this means that it is very difficult to assess if chloride ions have any direct modulatory role on the function of $GABA_A$ receptors (Kaila, 1994). Extensive studies have been carried out on the anion permeability of $GABA_A$ receptor channels.

The antiepileptic effect of bromide ions might result from the potentiation of $GABA_A$ receptor-mediated inhibition. Bromide potentiated GABA-activated currents in cultured neurons from rat cerebral cortex at the therapeutic concentrations of 10–20 mM (Suzuki *et al.*, 1994).

5.12. *Agents Acting on cAMP-Dependent Protein Kinase Activity*

The intracellular loop of the β-subunit of $GABA_A$ receptor complexes contains consensus sequence sites for phosphorylation by cAMP-dependent protein kinase (Schofield *et al.*, 1987). Such phosphorylation directly modulates the function of $GABA_A$ receptors, suggesting that agents that regulate intracellular cAMP levels may modulate the responses of neurons to GABA and, thus, have profound effects on synaptic excitability. The functional modulation of a variety of $GABA_A$ receptors has been demonstrated using the adenylate cyclase activator forskolin, which decreased GABA-mediated effects. In addition, site-specific mutagenesis of the key serine on the β-subunit in recombinant $GABA_A$ receptors abolished the phosphorylation-induced decreased amplitude of the GABA responses and reduced the extent of rapid desensitisation

of the GABA responses (Moss *et al.*, 1992). Interestingly, potentiation of GABA-mediated currents by cAMP-dependent protein kinase has been reported in cerebellar Purkinje cells following treatment with forskolin or 8-bromo-cAMP, rather than the inhibition found in other tissue preparations (Kano and Konnerth, 1992).

There is also evidence that intracellular cGMP may modulate $GABA_A$ receptor activation via a cGMP-dependent protein kinase (Bradshaw and Simmonds, 1995).

5.13. *Phospholipids*

Phospholipids appear to be endogenous modulators of $GABA_A$ receptors. GABA binding to rat brain membranes is increased by incubation with phospholipase C, which splits off the polar head groups of endogenous phospholipids (Giambalvo and Rosenberg, 1976; Toffano *et al.*, 1981). Phospholipids are liberated from membranes in the freeze-thaw and detergent extraction procedures used to maximise GABA binding. The addition of these phospholipids back to the incubation mixture inhibits GABA binding, with phosphatidylethanolamine being more potent than phosphatidylcholine or phosphatidylserine in inhibiting GABA binding (Johnston and Kennedy, 1978). The structural similarities between the polar head group of phosphatidylethanolamine and GABA have been noted by Watkins (1965), thus providing a molecular basis for the modulation of GABA receptors by this class of phospholipid. In addition, phosphatidylserine has been shown to influence $GABA_A$ receptor function (Hammond and Martin, 1987; Rapallino *et al.*, 1990). Benzodiazepine binding sites are modulated by endogenous phospholipids susceptible to treatment with phospholipase C and phospholipase A_2 (Ueno and Kuriyama, 1981). Some of the actions of phospholipids on GABA, barbiturate, and benzodiazepine sites may be mediated via activation of calcium and phospholipid-dependent protein kinases (Leidenheimer *et al.*, 1993).

There is evidence for a phospholipid defect in $GABA_A$ receptors in Huntington's disease (Lloyd *et al.*, 1979). Phospholipid methylation is involved in the regulation of GABA and benzodiazepine receptors (Di Perri *et al.*, 1983), and benzodiazepines are known to stimulate phospholipid methylation (Strittmatter *et al.*, 1979). Phospholipid methylation is considered to play an important role in the regulation of β-adrenoceptors (Hirata and Axelrod, 1980). The interaction of phospholipids with GABA receptors may have therapeutic implications which deserve further investigation.

5.14. *Miscellaneous Substances*

Two endogenous substances have been found to modulate $GABA_A$ receptor function in brain, interleukin-1 (Miller *et al.*, 1991) and arachidonic acid (Samochocki and Strosznajder, 1993). These interactions may represent targets for drug action. The accumulation of arachidonic acid in the brain during ischaemia may result in dysfunction of $GABA_A$ receptors. Arachidonic acid also inhibits glutamate release (Herrero *et al.*, 1991) and enhances NMDA action (Miller *et al.*, 1992).

Several endogenous purines, including adenosine, hyoxanthine, and inosine, are noncompetitive inhibitors of GABA binding (Ticku and Burch, 1980). Purines have complex effects on GABA-benzodiazepine interactions (Skerritt *et al.*, 1982a) and interact with central rather than peripheral benzodiazepine receptors (Skerritt *et al.*, 1982b).

While pentylenetetrazole is a potent convulsant, 1,5-dialkyltetrazoles have either analeptic or depressant effects (Kerr and Ong, 1992), recalling the bidirectional actions of the γ-butyrolactones and caprolactams discussed in Section 5.4. A range of tetrazoles, glutarimides, succinimides, and benzodiazepines appear to be able to modulate picrotoxinin receptor sites. Various tetrazoles show agonist and antagonist properties at glutamate receptors (Lunn *et al.*, 1992; Monn *et al.*, 1993).

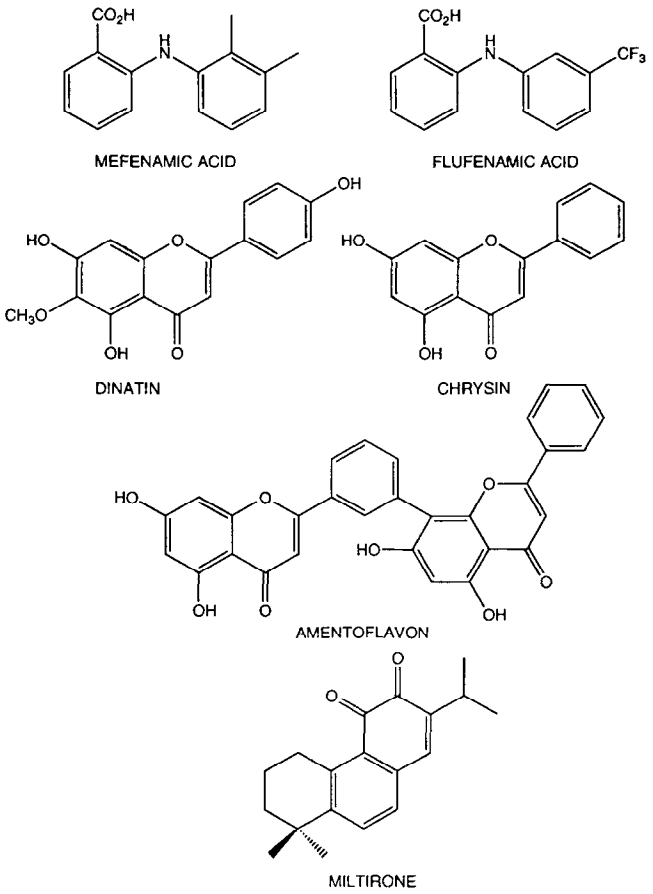

FIGURE 12. Miscellaneous agents that act on $GABA_A$ receptors.

Fenamates, such as mefenamic and flufenamic acid (Fig. 12), and related nonsteroidal anti-inflammatory drugs that inhibit prostaglandin synthesis have a dual effect on recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes (Woodward *et al.*, 1994). These drugs enhanced the currents produced by low concentrations of GABA and inhibited currents produced by high concentrations of GABA. The effects are not specific to $GABA_A$ receptors since the drugs also influence a variety of ion channels and transporters.

Indomethacin, ibuprofen, felbinac, and related anti-inflammatory drugs and their metabolites have been reported to potentiate the antagonist actions of quinolone antibacterial drugs, such as enoxacin and norfloxacin, on $GABA_A$ receptors in binding studies (Squires and Saederup, 1993b; Kawakami *et al.*, 1993; Halliwell and Davey, 1994).

Several 5-HT_3 receptor antagonists with effects on anxiety and the behavioural action of ethanol also have effects on $GABA_A$ receptors (Klein *et al.*, 1994). ICS 205-930, MDL 72222 and LY 278584 inhibited GABA currents in oocytes at micromolar concentrations. ICS 205-930 differed from the other two 5-HT_3 receptor antagonists in that its effects were biphasic, enhancing GABA currents at low concentrations (0.1–5 μM) and inhibiting at higher concentrations (50–100 μM). The enhancing effect of ICS 205-930 could be blocked by the benzodiazepine antagonist Ro15-1788.

Amitriptyline has been shown to modulate GABA-stimulated chloride fluxes producing enhancement in tissue from dominant rats and inhibition in tissue from submissive rats consistent with an involvement of $GABA_A$ receptors in aspects of depression (Malatynska *et al.*, 1995). Oxidised metabolites of Δ^8-tetrahydrocannabinol potentiate diazepam-induced narcosis and interact with benzodiazepine binding sites in mouse brain membranes (Yamamoto *et al.*, 1992).

Microtubule depolymerizing agents, such as colchicine, nocodazole, vinblastine, and taxol, inhibit GABA receptor function by disrupting the interaction of $GABA_A$ receptors with microtubules proposed to anchor receptor clusters at postsynaptic membranes (Whatley *et al.*, 1994).

A variety of flavonoids with anticonvulsant, anxiolytic, and sedative properties modulates the binding of benzodiazepines to $GABA_A$ receptor complexes (Fig. 12). Structure–activity studies indicate that the most potent flavonoids are dinatin (4',5,7-trihydroxy-6-methoxyflavone), chrysin (5,7-dihydroxyflavone), and its 5,7-dimethoxy derivative (Medina *et al.*, 1990; Shen *et al.*, 1994; Häberlein *et al.*, 1994). The activity of infusions made from *Tilia* species, traditional medicinal plants widely used in Latin America as sedatives and tranquillisers, and of other plant species may be due to flavonoids (Viola *et al.*, 1994; Häberlein *et al.*, 1994). The biflavonoid, amentoflavon, is a potent noncompetitive inhibitor of benzodiazepine binding (Nielsen *et al.*, 1988). Miltirone is the most potent of a series of diterpene quinones from the Chinese medicinal herb *Salvia miltiorrhiza* that show activity as positive allosteric modulators acting on benzodiazepine sites (Lee *et al.*, 1991). Structure–activity studies reveal more potent analogues of miltirone and a possible neutralising or negative allosteric modulator (Chang *et al.*, 1991).

6. STRESS AND SEX DIFFERENCES

$GABA_A$ receptors are influenced by stress, and this may affect pharmacological responses. Furthermore, there are major sex differences in the response of $GABA_A$ receptors to a variety of pharmacological agents.

Stress has been termed the neglected variable in experimental pharmacology (Vogel, 1987), and it is important to note that various stressors have profound effects on $GABA_A$ receptors. Foot shock causes a rapid decrease in handling-habituated rats (Biggio *et al.*, 1981), an effect blocked by Ro15-1788 (Corda *et al.*, 1985). A simple warm swim stress of female mice in only 3 min substantially increases the apparent number of cortical $GABA_A$ receptors (Skerritt *et al.*, 1981; Akinci and Johnston, 1993). These experiments show that $GABA_A$ receptors are rapidly regulated in the brain. Handling male rats and giving them an i.p. injection of saline once a day for 3 days results in a large increase in the apparent number of cortical $GABA_A$ receptors compared with nonhandled, noninjected animals (Maddison *et al.*, 1987). These experiments were carried out to examine the possible role of $GABA_A$ receptors in hepatic encephalopathy in which animals were injected with thioacetamide once a day for 3 days to destroy liver function, and produced experimental hepatic encephalopathy. The thioacetamide-injected animals had very high levels of cortical $GABA_A$ receptors compared with noninjected animals but, in fact, did not differ from those injected with saline. Handling of neonatal rats produces changes in cortical $GABA_A$ receptors that can be detected 100 days later (Bolden *et al.*, 1990). Furthermore, $GABA_A$ receptors can change to compensate for changes in other receptor populations. For example, in calves with an inherited disorder of strychnine-sensitive glycine receptors, $GABA_A$ receptor numbers are increased to compensate for the lack of glycine-mediated synaptic inhibition (Lummis *et al.*, 1990). Other aspects of the GABA system in addition to $GABA_A$ receptors can be influenced by stress, e.g., building construction noise alters GABA release and uptake processes in rat brain (Fernandes and File, 1993).

Sex differences are also important to $GABA_A$ receptor function in that female animals show greater changes in $GABA_A$ receptor function than do male animals in response to a variety of drugs that influence $GABA_A$-mediated synaptic transmission. Female rats are much more sensitive to the GABA antagonist picrotoxin than are male rats with respect to induction of convulsions and elevation of plasma corticosterone (Pericic *et al.*, 1985). Gonadectomised male and female rats are equally susceptible to picrotoxin-induced seizures. Benzodiazepines decrease plasma corticosterone in female, but not in male, rats (Pericic *et al.*, 1985), and stress-induced increases in plasma corticosterone are much more apparent in female than in male mice (Akinci and Johnston, 1993). A much higher dose of the GABA-enhancing steroid anaesthetic alphaxolone is required to produce surgical anaesthesia in male than in female rats (Fink *et al.*, 1982). There are sex differences in the binding of the $GABA_A$ agonist muscimol in different parts of the rat brain (Jüptner and Hiemke, 1990). There are sex differences in $GABA_A$ receptor binding after chronic ethanol drinking in mice (Unwin and Taberner, 1980). Ovarian steroids modulate muscimol binding to $GABA_A$ receptors in rat spinal cord (McCarthy *et al.*, 1991), and there are functional changes in $GABA_A$ receptor stimulation during the oestrus cycle in the rat (Westerling *et al.*, 1991).

7. CONCLUSION

This review clearly shows the variety of agents that interact with $GABA_A$ receptors. The number of different sites on $GABA_A$ receptors for such a structurally diverse range of agents, however, is far from clear.

Likely sites include:

(1) Agonist recognition sites, which are also the sites of action of competitive antagonists such as bicuculline and of partial agonists such as 4-PIOL. These sites can exist in a number of different conformations;
(2) Picrotoxinin sites, which appear to be associated with the chloride ion channels. These site are accessible from inside neurons. Other agents likely to interact with these sites, or overlapping sites, include the γ-butyrolactones, caprolactams, some insecticides, and possibly some anaesthetic agents;
(3) The sedative-hypnotic barbiturate sites, which interact with both the agonist recognition sites and the picrotoxinin sites;
(4) Neuroactive steroid sites, activation of which appears to influence chloride channel kinetics in a different way to activation of barbiturate sites. The steroid sites may be in a hydrophobic environment, e.g., the interfaces between receptor proteins and membrane lipids and, surprisingly, accessible only from the extracellular surface of the neurons. It is likely that there are subtypes of neuroactive steroid sites;
(5) Benzodiazepine sites, which are dependent on the presence of a γ_2-subunit in the $GABA_A$ receptor complex. β-Carbolines and a range of structurally diverse substances, such as flavonoids, may act at these sites. Positive, neutralising, and negative allosteric modulation of the agonist activation can result from interaction with these benzodiazepine sites. The enhancement of $GABA_A$ responses induced by benzodiazepine positive allosteric modulators results from

different effects on chloride channel kinetics to that resulting from activation of barbiturate or neuroactive steroid sites. There are certainly subtypes of benzodiazepine sites;

(6) Ethanol sites, which appear to be dependent on the presence of a phosphorylated γ_{2L}-subunit in the $GABA_A$ receptor complex;

(7) Stereoselective sites for inhalation anaesthetics, such as isoflurane, which may be found in a hydrophobic environment;

(8) Sites for furosemide associated with the chloride channels of some $GABA_A$ receptors containing α_6-subunits;

(9) Sites for Zn^{2+}, which are found in some $GABA_A$ receptor complexes that do not contain γ_2-subunits;

(10) Sites for a variety of divalent cations, such as Ca^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Mn^{2+}, and Mg^{2+}, that may modulate $GABA_A$ receptor function by acting on the chloride ion channels. Separate intracellular sites may exist for Ca^{2+} associated with the phosphorylation of intracellular loops of receptor protein subunits; and

(11) Sites for La^{3+}, which are distinct from the Zn^{2+} sites and the other divalent cation sites.

In addition, there are possibly sites associated with (a) phospholipids interacting with $GABA_A$ receptor protein subunits, (b) cyclic nucleotide protein kinase activity involved phosphorylation of the intracellular loop of some $GABA_A$ receptor protein subunits, and (c) the interaction of $GABA_A$ receptors and microtubules that may anchor receptor clusters at postsynaptic membranes.

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References

Abalis, I. M., Eldefrawi, A. T. and Eldefrawi, M. E. (1986) Actions of avermectin B_{1b} on the gamma-aminobutyric $acid_A$ receptor and chloride channels in rat brain. J. Biochem. Toxicol. 1: 69–82.

Abraham, J. H. and Schousboe, A. (1989) Effects of taurine on cell morphology and expression of low-affinity GABA receptors in cultured cerebellar granule cells. Neurochem. Res. 14: 1031–1038.

Akaike, N., Hattori, K., Oomura, Y. and Carpenter, D. O. (1985) Bicuculline and picrotoxin block γ-aminobutyric acid-gated Cl^- conductance by different mechanisms. Experientia 41: 70–71.

Akinci, M. K. and Johnston, G. A. R. (1993) Sex differences in the effects of acute swim stress on binding to $GABA_A$ receptors in mouse brain. J. Neurochem. 60: 2212–2216.

Alho, H., Costa, E., Ferrero, P., Fujimoto, M., Cosenza-Murphy, D. and Guidotti, A. (1985) Diazepam-binding inhibitor: a neuropeptide located in selected neuronal populations of rat brain. Science 229: 179–182.

Alkadhi, K. A., Salgado, D., Davis, C. A. and Udofia, E. I. (1993) Effects of γ-aminobutyric acid on the compound action potential of the rat superior cervical ganglion. Arch. Int. Pharmacodyn. Ther. 322: 66–79.

Allan, R. D. and Apostopoulos, C. (1990) Synthesis of substituted (+)-bicuculline derivatives through chloromethylation. Aust. J. Chem. 43: 1259–1268.

Allan, R. D. and Johnston, G. A. R. (1983) Synthetic analogs for the study of GABA as a neurotransmitter. Med. Res. Rev. 3: 91–118.

Allan, R. D., Johnston, G. A. R. and Twitchin, B. (1979) Synthesis of analogues of GABA. III. All four stereoisomers of 3-aminocyclopentane carboxylic acid and a stereochemical correlation with amidinomycin. Aust. J. Chem. 32: 2517–2521.

Allan, R. D., Evans, R. H. and Johnston, G. A. R. (1980) γ-Aminobutyric acid agonists: an *in vitro* comparison between depression of spinal synaptic activity and depolarization of spinal root fibres in the rat. Br. J. Pharmacol. 70: 609–615.

Allan, R. D., Dickenson, H. W., Hiern, B. P., Johnston, G. A. R. and Kazlauskas, R. (1986) Isothiouronium compounds as γ-aminobutyric acid agonists. Br. J. Pharmacol. 88: 379–387.

Allan, R. D., Apostopoulos, C. and Richardson, J. A. (1990) 2-Imino-1,3,4-thiadiazole derivatives of GABA as $GABA_A$ antagonists. Aust. J. Chem. 43: 1767–1772.

Allan, R. D., Dickenson, H. W., Duke, R. K. and Johnston, G. A. R. (1991) ZAPA, a substrate for the neuronal high affinity GABA uptake system in rat brain slices. Neurochem. Int. 18: 63–67.

Amin, J. and Weiss, D. S. (1993) $GABA_A$ receptor needs two homologous domains of the β-subunit for activation by GABA but not by pentobarbital. Nature 366: 565–569.

Andres-Trelles, F., Bibby, V., Lustman, S. and Simmonds, M. A. (1989) Effects of cortisol on $GABA_A$ receptor-mediated responses compared in the guinea-pig ileum and rat cuneate nucleus. Neuropharmacology 28: 705–708.

Andrews, P. R. and Johnston, G. A. R. (1979) GABA agonists and antagonists. Biochem. Pharmacol. 28: 2697–2702.

Andrews, P. R., Evans, R. H., Johnston, G. A. R. and Willow, M. (1981) Direct excitant action of convulsant barbiturates. Experientia 37: 172–174.

Antonaccio, M. J. and Snyder, D. W. (1981) Reductions in blood pressure, heart rate and renal sympathetic nervous discharge after imidazole-4-acetic acid: mediation through central γ-aminobutyric acid (GABA) receptor stimulation. J. Pharmacol. Exp. Ther. 218: 200–205.

Aprison, M. H. and Lipkovitz, K. B. (1989) On the $GABA_A$ receptor: a molecular modelling approach. J. Neurosci. Res. 23: 129–135.

Backus, K. H., Arigoni, M., Drescher, U., Scheurer, L., Malherbe, P., Möhler, H. and Benson, J. A. (1993) Stoichiometry of a recombinant $GABA_A$ receptor deduced from mutation-induced rectification. Neuroreport 5: 285–288.

Banfi, S., Fonio, W., Allieve, E., Pinza, M. and Dorigotti, L. (1984) Cyclic GABA-GABOB analogues. IV. Activity on learning and memory. Farm. Ed. Sci. 39: 16–22.

Baulieu, E. E. (1991) Neurosteroids: a new function in the brain. Biol. Cell 71: 3–10.

Beart, P. M. and Johnston, G. A. R. (1973) Transamination of analogues of γ-aminobutyric acid by extracts of rat brain mitochondria. Brain Res. 49: 459–46.

Beutler, J. A., Karbon, E. W., Brubaker, A. N., Malik, R., Curtis, D. R. and Enna, S. J. (1985) Securinine alkaloids: a new class of GABA receptor antagonist. Brain Res. 330: 135–140.

Biggio, G., Corda, M. G., Concas, A., Demontis, G., Rossetti, Z. and Gessa G. L. (1981) Rapid changes in GABA binding induced by stress in different areas of the rat brain. Brain Res. 229: 363–369.

Bolden, S. W., Hambley, J. W., Johnston, G. A. R. and Rogers, L. J. (1990) Neonatal stress and long-term modulation of GABA receptors in rat brain. Neurosci. Lett. 111: 258–262.

Bond, R., Leff, P., Johnson, T. D., Milano, C. A., Rockman, H. A., McMinn, T. R., Apparsundaram, S., Hyek, M. F., Kenakin, T. P., Allen, L. F. and Lefkovitz, R. J. (1995) Physiological effects of inverse agonists in transgenic mice with myocardial overexpression of the β_2-adrenoceptor. Nature 374: 272–276.

Bormann, J., Hamill, O. P. and Sakmann, B. (1987) Mechanism of anion permeation through channels gated by glycine and γ-aminobutyric acid in mouse cultured spinal neurons. J. Physiol. 385: 243–286.

Bouchet, M.-J., Jacques, P., Ilien, B., Goeldner, M. and Hirth, C. (1992) m-Sulfonate benzene diazonium chloride: a powerful affinity label for the γ-aminobutyric acid binding site from rat brain. J. Neurochem. 59: 1405–1413.

Bowlby, M. R. (1993) Pregnenolone sulfate potentiation of N-methyl-D-aspartate receptor channels in hippocampal neurons. Mol. Pharmacol. 43: 813–819.

Bradshaw, D. J. and Simmonds, M. A. (1995) γ-Aminobutyric acid receptor function is modulated by cyclic GMP. Brain Res. Bull. 37: 67–72.

Braestrup, C. and Nielsen, M. (1985) Interaction of pitrazepin with the GABA/benzodiazepine receptor complex and with glycine receptors. Eur. J. Pharmacol. 118: 115–121.

Braestrup, C., Schmiechen, R., Neef, G., Nielsen, M. and Petersen, E. N. (1982) Interaction of convulsive ligands with benzodiazepine receptors. Science 216: 1241–1243.

Braestrup, C., Nielsen, M. and Honoré, T. (1983) Benzodiazepine receptor ligands with positive and negative efficacy. Adv. Biochem. Psychopharmacol. 37: 237–245.

Breuker, E. and Johnston, G. A. R. (1975) Inhibition of acetylcholinesterase by bicuculline and related alkaloids. J. Neurochem. 25: 903–904.

Burden, P. M., Capper, H. R., Allan, R. D. and Johnston, G. A. R. (1991) The synthesis of 1,8-disubstituted 10,11-dihydrodibenz-[*b*,*f*]oxepin-10-ones. Analogues of anaesthetic steroids. J. Chem. Soc. Perkin Trans. 1: 3291–3294.

Bureau, M. and Olsen, R. W. (1990) Multiple distinct subunits of the γ-aminobutryic $acid_A$ receptor protein show different ligand binding properties. Mol. Pharmacol. 37: 497–502.

Buu, N. T., Duhaime, J. and Kuchel, O. (1984) The bicuculline-like properties of dopamine sulfate in rat brain. Life Sci. 35: 1083–1090.

Calder, J. A., Wyatt, J. A., Frenkel, D. A. and Casida, J. E. (1993) CoMFA validation of the superposition of six classes of compounds which block GABA receptors non-competitively. J. Comput. Aided Mol. Des. 7: 45–60.

Cashin, M. F. and Moravek, V. (1927) The physiological action of cholesterol. Am. J. Physiol. 82: 294–298.

Casida, J. E. (1993) Insecticide action at the GABA-gated chloride channel: recognition, progress, and prospects. Arch. Insect Biochem. Physiol. 22: 13–23.

Chang, H. M., Chui, K. Y., Tan, F. W., Yang, Y., Zhong, Z. P., Lee, C. M., Sham, H. L. and Wong, H. N. (1991) Structure–activity relationship of miltirone, an active central benzodiazepine receptor ligand isolated from *Salvia miltiorrhiza* Bunge (Danshen). J. Med. Chem. 34: 1675–1692.

Chow, P. and Mathers, D. (1986) Convulsant doses of penicillin shorten the lifetime of GABA-induced channels in cultured central neurones. Br. J. Pharmacol. 88: 541–547.

Clarke, R. S., Dundee, J. W., Garrett, F. T., McArdle, G. K. and Sutton J. A. (1975) Adverse reactions to intravenous anaesthetics. Br. J. Anaesth. 47: 575–85.

Corda, M. G., Concas, A. and Biggio, G. (1985) Selective blockade of benzodiazepine receptors by Ro 15-1788 prevents foot shock-induced decrease of low affinity γ-aminobutyric acid receptors. Neurosci. Lett. 56: 265–269.

Costa, E., Cheney, D. L., Grayson, D. R., Korneyev, A., Longone, P., Pani, L., Romeo, E., Zivkovich, E. and Guidotti, A. (1994) Pharmacology of neurosteroid biosynthesis. Role of the mitochondrial DBI receptor (MDR) complex. Ann. NY Acad. Sci. 746: 223–242.

Curtis, D. R. and Gynther, B. D. (1986) Pitrazepin: a central glycine and GABA antagonist. Eur. J. Pharmacol. 131: 311–313.

Curtis, D. R. and Johnston, G. A. R. (1974a) Amino acid transmitters in the mammalian central nervous system. Rev. Physiol. 69: 97–188.

Curtis, D. R. and Johnston, G. A. R. (1974b) Convulsant alkaloids. In: Neuropoisons, Their Pathophysiological Actions, Vol. 2, Poisons of Plant Origin, pp. 207–248, Simpson, L. L. and Curtis, D. R. (eds.) Plenum Press, New York.

Curtis, D. R. and Malik, R. (1985) Glycine antagonism by RU5135. Eur. J. Pharmacol. 110: 383–384.

Curtis, D. R., Hösli, L., Johnston, G. A. R. and Johnston, I. H. (1967) Glycine and spinal inhibition. Brain Res. 5: 112–114.

Curtis, D. R., Duggan, A. W. and Johnston, G. A. R. (1969) Glycine, strychnine, picrotoxin and spinal inhibition. Brain Res. 14: 759–62

Curtis, D. R., Duggan, A.W., Felix, D. and Johnston, G. A. R. (1970) GABA, bicuculline and central inhibition. Nature 226: 1222–1224.

Curtis, D. R., Duggan, A. W., Felix, D. and Johnston, G. A. R. (1971) Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord. Brain Res. 32: 69–96.

Curtis, D. R., Game, C. J. A., Johnston, G. A. R., McCulloch, R. M. and Maclachlan, R. M. (1972) Convulsive action of penicillin. Brain Res. 43: 242–245.

Davidoff, R. A. (1972) Penicillin and inhibition in the cat spinal cord. Brain Res. 45: 638–642.

De Deyn, P. P. and Macdonald, R. L. (1989) Effects of antiepileptic drugs on GABA responses and on reduction of GABA responses by PTZ and DMCM on mouse neurons in cell culture. Epilepsia 30: 17–25.

Dekoninck, Y. and Mody, I. (1994) Noise analysis of miniature ipscs in adult rat brain slices—properties and modulation of synaptic $GABA_A$ receptor channels. J. Neurophysiol. 71: 1318–1335.

Deplazas, S. F., Gravielle, M. C., Denovara, A. M. and Flores, V. (1993) Methods for removing endogenous factors from CNS membrane preparations—differences in [^{3}H] GABA binding parameters and developmental-related effects. Neurochem. Res. 18: 385–391.

De Robertis, E., Peña, C., Paladini, A. C. and Medina, J. H. (1988) New developments on the search for the endogenous ligand(s) of central benzodiazepine receptors. Neurochem. Int. 13: 1–11.

Dickenson, H. W., Duke, R. K., Balcar, V. J., Allan, R. D. and Johnston, G. A. R. (1990) Binding to rat brain membranes of (+)-*trans*-(1S,3S)-3-aminocyclopentane-1-carboxylic acid, (+)-TACP, a selective $GABA_A$ receptor agonist. Mol. Neuropharmacol. 1: 1–6.

Dillon, G. H., Im, H. I., Hamilton, B. J., Carter, D. B., Gammill, R. B., Judge, T. M. and Im, W. B. (1993) U-93631 causes rapid decay of γ-aminobutyric acid-induced chloride currents in recombinant rat γ-aminobutyric acid type A receptors. Mol. Pharmacol. 44: 860–864.

Dingledine, R. and Korn, S. J. (1985) γ-Aminobutyric acid uptake and the termination of inhibitory synaptic potentials in the rat hippocampal slice. J. Physiol. 366: 387–409.

Di Perri, B., Calderini, G., Battistella, A., Raciti, R. and Toffano, G. (1983) Phospholipid methylation increases [^{3}H]diazepam and [^{3}H]GABA binding in membrane preparations of rat cerebellum. J. Neurochem. 41: 302–308.

Dodd, P. R., Davies, L. P., Watson, W. E. J., Nielsen, B., Dyer, J. A., Wong, L. S. and Johnston, G. A. R. (1989) Neurochemical studies on quinolone antibiotics: effects on glutamate, GABA and adenosine systems in mammalian CNS. Pharmacol. Toxicol. 64: 404–411.

Drexler, G. and Sieghart, W. (1984) Evidence for association of a high affinity avermectin binding site with the benzodiazepine receptor. Eur. J. Pharmacol. 101: 201–207.

Ebert, B., Wafford, K. A., Whiting, P. J., Krogsgaard-Larsen, P. and Kemp, J. A. (1994) Molecular pharmacology of γ-aminobutyric acid type A receptor agonists and partial agonists in oocytes injected with different alpha, beta, and gamma receptor subunit combinations. Mol. Pharmacol. 46: 957–963.

Eccles, J. C., Schmidt, R. F. and Willis, W. D. (1963) Pharmacological studies on presynaptic inhibition. J. Physiol. 185: 298–322.

Edwards, F. A., Konnerth, A. and Sakmann, B. (1990) Quantal analysis of inhibitory synaptic transmission in the dentate gyrus of rat hippocampal slices: a patch-clamp study. J. Physiol. 430: 213–249.

Elder, S. T., Mach, R. H., Nowak, P. A., Moroney, D. A., Rao, A. V. and Ehrenkaufer, R. L. E. (1995) Esters of 6-(4′-fluorobenzylamino)-β-carboline-3-carboxylic acid as potential benzodiazepine imaging agents for P.E.T. J. Lab. Comp. Radiopharmaceut. 36: 205–211.

Elliott, K. A. C. and Van Gelder, N. M. (1958) Occlusion and metabolism of gamma-aminobutyric acid by brain tissue. J. Neurochem. 3: 28–40.

Endo, S. and Olsen, R. W. (1993) Antibodies specific for α-subunit subtypes of $GABA_A$ receptors reveal brain regional heterogeneity. J. Neurochem. 60: 1388–1398.

Ennis, C. and Minchin, M. C. W. (1993) Modulation of the $GABA_A$-like autoreceptor by barbiturates but not by steroids. Neuropharmacology 32: 355–257.

Falch, E., Jacobsen, P., Krogsgaard-Larsen, P. and Curtis, D. R. (1985) GABA-mimetic activity and effects on diazepam binding of aminosulphonic acids structurally related to piperidine-4-sulphonic acid. J. Neurochem. 44: 68–75.

Falch, E., Larsson, O. M., Schousboe, A. and Krogsgaard-Larsen, P. (1990) $GABA_A$ agonists and GABA uptake inhibitors. Drug Dev. Res. 21: 169–188.

Fernandes, C. and File, S. E. (1993) Beware the builders: construction noise changes [^{14}C]GABA release and uptake from amygdaloid and hippocampal slices in the rat. Neuropharmacology 32: 1333–1336.

Ferrarese, C., Appollonio, I., Bianchi, G., Frigo, M., Marzorati, C., Pecora, N., Perego, M., Pierpaoli, C. and Frattola, F. (1993) Benzodiazepine receptors and diazepam binding inhibitor: a possible link between stress, anxiety and the immune system. Psychoendrocrinology 18: 3–22.

ffrench-Mullen, J. M., Danks, P. and Spence, K. T. (1994) Neurosteroids modulate calcium currents in hippocampal CA1 neurons via a pertussis toxin-sensitive G-protein-coupled mechanism. J. Neurosci. 14: 1963–1977.

Fink, G., Sarkar, D. K., Dow, R. C., Dick, H., Borthwick, N., Malnick, S. and Twine, M. (1982) Sex differences in response to alfaxalone anaesthesia may be oestrogen dependent. Nature 298: 270–272.

Finn, D. A. and Gee, K. W. (1993) The influence of estrus cycle on neurosteroid potency at the γ-aminobutyric $acid_A$ receptor complex. J. Pharmacol. Exp. Ther. 265: 1374–1379.

Funder, J. W. (1994) Corticosteroid receptors and the central nervous system. J. Steroid Biochem. Mol. Biol. 49: 381–384.

Gage, P. W. and Chung, S.-H. (1994) Influence of membrane potential on conductance sublevels of chloride channels activated by GABA. Proc. R. Soc. Lond. B 255: 167–172.

Gähwiller, B. H., Maurer, R. and Wüthrich, H. J. (1984) Pitrazepin, a novel $GABA_A$ antagonist. Neurosci. Lett. 45: 311–316.

Galzi, J.-L. and Changeux, J. P. (1994) Neurotransmitter-gated ion channels as unconventional allosteric proteins. Curr. Opin. Struct. Biol. 4: 554–565.

Gardner, C. R., Tully, W. R. and Hedgecock, C. J. R. (1992) The rapidly expanding range of neuronal benzodiazepine receptor ligands. Prog. Neurobiol. 40: 1–61.

Gee, K. W. (1988) Steroid modulation of the GABA/benzodiazepine receptor-linked chloride ionophore. Mol. Neurobiol. 2: 291–317.

Giambalvo, C. and Rosenberg, P. (1976) The effect of phospholipase and proteases on the binding of γ-aminobutyric acid to junctional complexes of rat cerebellum. Biochim. Biophys. Acta 436: 741–756.

Gram, L. F. and Christensen, P. (1986) Benzodiazepine suppression of cortisol secretion: a measure of anxiolytic activity? Pharmacopsychiatry 19: 19–22.

Grant, K. A. (1994) Emerging neurochemical concepts in the actions of ethanol at ligand-gated ion channels. Behav. Pharmacol. 5: 383–404.

Grognet, A., Hertz, F. and De Feudis, F. V. (1983) Comparison of the analgesic actions of THIP and morphine. Gen. Pharmacol. 14: 585–589.

Gynther, B. D. and Curtis, D. R. (1986) Pyridazinyl-GABA derivatives as GABA and glycine antagonists in the spinal cord of the cat. Neurosci. Lett. 68: 211–215.

Häberlein, H., Tschiersch, K.-P. and Schäfer, H. L. (1994) Flavonoids from *Leptospermum scoparium* with affinity to the benzodiazepine receptor characterized by structure activity relationships and *in vivo* studies of a plant extract. Pharmazie 49: 912–921.

Hadingham, K. L., Wingrove, P. B., Wafford, K. A., Bain, C., Kemp, J. A., Palmer, K. J., Wilson, A. W., Wilcox, A. S., Sikela, J. M., Ragan, C. I. and Whiting, P. J. (1993) Role of the beat-subunit determining the pharmacology of human γ-aminobutyric acid type-A receptors. Mol. Pharmacol. 44: 1211–1218.

Halliwell, R. F. and Davey, P. G. (1994) Biphenyl acetic acid potentiates the antagonist effects of fluoroquinolones at the $GABA_A$ receptor of the rat isolated vagus nerve. Br. J. Pharmacol. 111: 181P.

Halliwell, R. F., Davey, P. G. and Lambert, J. J. (1993) Antagonism of $GABA_A$ receptors by 4-quinolones. J. Antimicrob. Chemother. 31: 457–462.

Hammond, J. R. and Martin, I. L. (1987) Modulation of [^{3}H]flunitrazepam binding to rat cerebellar benzodiazepine receptors by phosphatidylserine. Eur. J. Pharmacol. 137: 49–58.

Harris, B. D., Moody, E. J., Basile, A. S. and Skolnick, P. (1994) Volatile anesthetics bidirectionally and stereospecifically modulate ligand binding to GABA receptors. Eur. J. Pharmacol. 267: 269–274.

Harrison, N. L. and Simmonds, M. A. (1984) Modulation of the GABA receptor complex by a steroid anaesthetic. Brain Res. 323: 287–292.

Harrison, N. L., Kugler, J. L., Jones, M. V., Greenblatt, E. P. and Pritchett, D. B. (1993) Positive modulation of human γ-aminobutryic acid type-A and glycine receptors by the inhalation anesthetic isoflurane. Mol. Pharmacol. 44: 628–632.

Heaulme, M., Chambon, J.-P., Leyris, R., Molimard, J.-C., Wermuth, C. G. and Biziere, K. (1986) Biochemical characterization of the interaction of three pyridazinyl-GABA derivatives with the $GABA_A$ receptor site. Brain Res. 384: 224–231.

Herbison, A. E. and Fenelon, V. S. (1995) Estrogen regulation of $GABA_A$ receptor subunit mRNA expression in preoptic area and bed nucleus of the stria terminalis of female rat brain. J. Neurosci. 15: 2328–2337.

Herrero, I., Miras-Portugal, M. T. and Sanchez-Prieto, J. (1991) Inhibition of glutamate release by arachidonic acid in rat cerebrocortical synaptosomes. J. Neurochem. 57: 718–721.

Hill, D. R. and Bowery, N. G. (1981) ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline insensitive GABA sites in rat brain. Nature 290: 149–152.

Hill, R. G., Simmonds, M. A. and Straughan, D. W. (1973) Amino acid antagonists and the depression of cuneate neurones by γ-aminobutyric acid (GABA) and glycine. Br. J. Pharmacol. 47: 642P–643P.

Hill, R. G., Maurer, R., Buscher, H. H. and Römer, D. (1981) Analgesic properties of the GABA-mimetic THIP. Eur. J. Pharmacol. 69: 221–224.

Hill-Venning, C., Callachan, H., Peters, J. A., Lambert, J. J., Gemmell, D. K. and Campbell, A. C. (1994) Modulation of the $GABA_A$ receptor by ORG 20599: a water-soluble pregnane steroid. Br. J. Pharmacol. 111: 183P.

Hirata, F. and Axelrod, J. (1980) Phospholipid methylation and biological signal transmission. Science 209: 1082–1090.

Holden-Dye, L. and Walker, R. J. (1988) ZAPA, (Z)-3-[aminoiminomethyl) thio]-2-propenoic acid hydrochloride, a potent agonist at GABA-receptors on the Ascaris muscle cell. Br. J. Pharmacol. 95: 3–5.

Holland, K. D., McKeon, A. C., Covey, D. F. and Ferrendelli, J. A. (1990) Binding interactions of convulsant and anticonvulsant γ-butyrolactones and γ-thiobutyrolactones with the picrotoxin receptor. J. Pharmacol. Exp. Ther. 254: 578–583.

Holland, K. D., Yoon, K. W., Ferrendelli, J. A., Covey, D. F. and Rothman, S. M. (1991) γ-Butyrolactone antagonism of the picrotoxin receptor: comparison of a pure antagonist and a mixed antagonist/inverse agonist. Mol. Pharmacol. 39: 79–84.

Holland, K. D., Bouley, M. G., Covey, D. F. and Ferrendelli, J. A. (1993) Alkyl-substituted γ-butyrolactones act at a distinct site allosterically linked to the TBPS/picrotoxin site on the $GABA_A$ receptor complex. Brain Res. 615: 170–174.

Hollinshead, S. P., Trudell, M. L, Skolnick, P. and Cook, J. M. (1990) Structural requirements for agonist actions at the benzodiazepine receptor: studies with analogues of 6-(benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic acid ethyl ester. J. Med. Chem. 33: 1062–1069.

Hoppe, D. and Kettenmann, H. (1989) GABA triggers a Cl^- efflux from cultured mouse oligodendrocytes. Neurosci. Lett. 97: 334–339.

Horikoshi, T., Asanuma, A., Yanagisawa, K., Anzai, K. and Goto, S. (1988) Taurine and β-alanine act on both GABA and glycine receptors in *Xenopus* oocyctes injected with mouse brain messenger RNA. Mol. Brain Res. 4: 97–105.

Hu, Y., Zorumsky, C. F. and Covey, D. F. (1993) Neurosteroid analogues: structure–activity studies of benz[e]indene modulators of $GABA_A$ receptor function. 1. The effect of 6-methyl substitution on the electrophysiological activity of 7-substituted benz[e]indene-3-carbonitriles. J. Med. Chem. 36: 3956–3967.

Huang, J. H. and Johnston, G. A. R. (1990) (+)-Hydrastine, a potent competitive antagonist at mammalian $GABA_A$ receptors Br. J. Pharmacol. 99: 727–730.

Hunt, P. and Clements-Jewery, S. (1981) A steroid derivative, R 5135, antagonises the GABA/benzodiazepine receptor interaction. Neuropharmacology 20: 357–361.

Im, M. S., Hamilton, B., Carter, D. B. and Im, W. B. (1992) Selective potentiation of GABA-mediated Cl^- current by lanthanum ion in subtypes of cloned $GABA_A$ receptors. Neurosci. Lett. 144: 165–168.

Im, W. B. and Pregenzer, J. F. (1993) Interaction of La^{3+} with $GABA_A$ receptors in rat cerebrocortical membranes as detected with [^{35}S]-t-butylbicyclophosphorthionate binding. Eur. J. Pharmacol. 245: 111 117.

Inomata, N., Ishiohara, T. and Akaike, N. (1988) Effects of diuretics on GABA-gated chloride current in frog isolated sensory neurones. Br. J. Pharmacol. 93: 679–683.

Ito, M., Chiu, T. H. and Rosenberg, H. C. (1986) Effects of pentylenetetrazol on $GABA_A$/benzodiazepine/picrotoxinin receptor complexes in rat brain regions. Neurochem. Res. 11: 637–646.

Jensen, L. H. and Petersen, E. N. (1983) Bidirectional effects of benzodiazepine receptor ligands against picrotoxin- and pentylenetetrazol-induced seizures. J. Neural Transm. 58: 183–191.

Johnston, G. A. R. (1971) Muscimol and the uptake of γ-aminobutyric acid by rat brain slices. Psychopharmacology 22: 230–233.

Johnston, G. A. R. (1978) Neuropharmacology of amino acid inhibitory transmitters. Annu. Rev. Pharmacol. Toxicol. 18: 269–289.

Johnston, G. A. R. (1981) GABA receptors. In: The Role of Peptides and Amino Acids as Neurotransmitters, pp. 1–17, Lombardini, J. B. and Kenny, A. D. (eds.) Alan Liss, New York.

Johnston, G. A. R. (1985) First Sino-Australian workshop in pharmacology. Trends Pharmacol. Sci. 4: 159.

Johnston, G. A. R. (1991) $GABA_A$ antagonists. Semin. Neurosci. 3: 205–210.

Johnston, G. A. R. (1992) $GABA_A$ agonists as targets for drug development. Clin. Exp. Pharmacol. Physiol. 19: 73 78.

Johnston, G. A. R. (1994) GABA receptors—as complex as ABC? Clin. Exp. Pharmacol. Physiol. 21: 521–526.

Johnston, G. A. R. and Balcar, V. J. (1989) GABA enzymes and transport systems. In: GABA: Basic Research to Clinical Implications, pp. 1–23, Bowery, N. G. and Nistico, G. (eds.) Pythagora Press, Rome.

Johnston, G. A. R. and Kennedy, S. M. E. (1978) GABA receptors and phospholipids. In: Amino Acids as Chemical Transmitters, pp. 507–516, Fonnum, F. (ed.) Plenum Press, New York.

Johnston, G. A. R., Curtis, D. R., de Groat, W. C. and Duggan, A. W. (1968) Central actions of ibotenic acid and muscimol. Biochem. Pharmacol. 17: 2488–2489.

Johnston, G. A. R., Beart, P. M., Curtis, D. R., Game, C. J. A., McCulloch, R. M. and Maclachlan, R. M. (1972) Bicuculline methochloride as a GABA antagonist. Nature New Biol. 240: 219–220.

Johnston, G. A. R., Curtis, D. R., Beart, P. M., Game, C. J. A., McCulloch, R. M. and Twitchin, B. (1975) Cis and trans-4-aminocrotonic acid as GABA analogues of restricted conformation. J. Neurochem. 24: 157–160.

Johnston, G. A. R., Allan, R. D., Kennedy, S. M. E. and Twitchin, B. (1979) Systematic study of GABA analogues of restricted conformation. In: GABA-Neurotransmitters, pp. 149–164, Krogsgaard-Larsen, P., Scheel-Krüger, J. and Kofod, H. (eds.) Munksgaard, Copenhagen.

Jüptner, M. and Hiemke, C. (1990) Sex differences in $GABA_A$ receptor binding in rat brain measured by an improved in vitro binding assay. Exp. Brain Res. 81: 297–302.

Jussofie, A. (1993) Brain region-specific effects of neuroactive steroids on the affinity and density of the GABA-binding site. Biol. Chem. Hoppe-Seyler 374: 265–270.

Kaila, K. (1994) Ionic basis of $GABA_A$ receptor channel function in the nervous system. Prog. Neurobiol. 42: 489–537.

Kano, M. and Konnerth, A. (1992) Potentiation of GABA-mediated currents by cAMP-dependent protein kinase. Neuroreport 3: 563–566.

Kapfer, I., Jacques, P., Toubal, H. and Goeldner, M. P. (1995) Comparative photoaffinity labeling study between azidophenyl, difluorazidophenyl, and tetrafluorazidophenyl derivatives for the GABA-gated chloride channels. Bioconjug. Chem. 6: 109–114.

Kardos, J. (1993) The $GABA_A$ receptor channel mediated chloride ion translocation through the plasma membrane: new insights from $^{36}Cl^-$ ion flux measurements. Synapse 13: 74–93.

Katayama, N., Tokutomi, N., Nabekura, J. and Akaike, N. (1992) Penicillin-induced triphasic modulation of $GABA_A$ receptor-operated chloride current in frog sensory neuron. Brain Res. 595: 249–255.

Kawakami, J., Shimokawa, M., Yamamoto, K., Sawada, Y., Asanuma, A., Yangisawa, K. and Iga, T. (1993) Inhibition of $GABA_A$ receptor-mediated current responses by enoxacin (new quinolone) and felbinac (non-steroidal anti-inflammatory drug) in Xenopus oocytes injected with mouse-brain messenger RNA. Biol. Pharm. Bull. 16: 726–728.

Kerr, D. I. B. and Ong, J. (1992) GABA agonists and antagonists. Med. Res. Rev. 12: 593–636.

Kerr, D. I. B., Ong, J., Prager, R. H. and Ward, D. A. (1986) Caprolactam–barbiturate interaction at the $GABA_A$ receptor complex in the guinea-pig intestine. Eur. J. Pharmacol. 124: 203–206.

Kerr, D. I. B., Ong, J. and Johnston, G. A. R. (1990) Stress and cortisol modulation of GABA receptors. Stress Anxiety 13: 209–213.

Kilic, G., Moran, O. and Cherubini, E. (1993) Currents activated by GABA and their modulation by ZN^{2+} in cerebellar granule cells in culture. Eur. J. Neurosci. 5: 65–72.

Kjaer, M. and Nielson, H. (1983) The analgesic effects of the GABA-agonist THIP in patients with chronic pain of malignant origin. A phase I-2 study. Br. J. Clin. Pharmacol. 16: 447–485.

Klein, R. L., Sanna, E., McQuilkin, S. J., Whiting, P. J. and Harris, R. A. (1994) Effects of $5\text{-}HT_3$ receptor antagonists on binding and function of mouse and human $GABA_A$ receptors. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 15: 237–246.

Kleingoor, C., Wieland, H., Korpi, E. R., Seeburg, P. H. and Kettenmann, H. (1993) Current potentiation by diazepam but not by GABA is determined by a single histidine residue. Neuroreport 4: 187–190.

Knudsen, J., Mandrup, S., Rasmussen, J. T., Andreasen, P. H., Poulsen, F. and Kristiansen, K. (1993) The function of acyl-CoA-binding protein (ACBP)/diazepam binding inhibitor (DBI). Mol. Cell. Biochem. 123: 129–138.

Korpi, E. R. (1994) Role of $GABA_A$ receptors in the actions of alcohol and in alcoholism: recent advances. Alcohol Alcohol. 29: 115–129.

Korpi, E. R. and Seeburg, P. H. (1993) Natural mutation of $GABA_A$ receptor $\alpha 6$ subunit alters benzodiazepine affinity but not allosteric GABA effects. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 247: 23–27.

Korpi, E. R., Kuner, T., Seeburg, P. H. and Luddens, H. (1995) Selective antagonist for the cerebellar granule cell-specific γ-aminobutyric acid type A receptor. Mol. Pharmacol. 47: 283–289.

Krebs, M.-O., Kemel, M.-L., Gauchy, C., Desban, M. and Glowinski, J. (1994) Does bicuculline antagonize NMDA receptors? Further evidence in the rat striatum. Brain Res. 634: 345–348.

Krishek, B. J., Xie, X., Bouchet, M. J. and Smart, T. G. (1994) m-Sulphonate benzene diazonium chloride: a novel $GABA_A$ receptor antagonist. Neuropharmacology 33: 1125–1130.

Kristiansen, U. and Fjalland, B. (1991) Ligand structural specificity of $GABA_A$ receptors in guinea pig ileum. Pharmacol. Toxicol. 68: 332–339.

Kristiansen, U., Lambert, J. D. C., Schousboe, A. and Krogsgaard-Larsen, P. (1991) Electrophysiological studies of the $GABA_A$ receptor ligand 4-PIOL, on cultured hippocampal neurones. Br. J. Pharmacol. 104: 85–90.

Krogsgaard-Larsen, P. and Johnston, G. A. R. (1975) Inhibition of GABA uptake in rat brain slices by nipecotic acid, various isoxazoles and related compounds. J. Neurochem. 25: 797–802.

Krogsgaard-Larsen, P., Johnston, G. A. R., Curtis, D. R., Game, C. J. A. and McCulloch, R. M. (1975) Structure and biological activity of a series of conformationally restricted analogues of GABA. J. Neurochem. 25: 803–809.

Krogsgaard-Larsen, P., Johnston, G. A. R., Lodge, D. and Curtis, D. R. (1977) A new class of GABA agonist. Nature 268: 53–55.

Krogsgaard-Larsen, P., Mikkelsen, H., Jacobsen, P., Falch, E., Curtis, D. R., Peet, M. J. and Leah, J. (1983) 4,5,6,7-Tetrahydroisothiazolo[5,4-*c*]pyridin-3-ol and related analogues of THIP. Synthesis and biological activity. J. Med. Chem. 26: 895–900.

Krogsgaard-Larsen, P., Nielsen, L., Falch, E. and Curtis, D. R. (1985) GABA agonists. Resolution, absolute stereochemistry, and enantioselectivity of S-(+)- and R-(−)-dihydromuscimol. J. Med. Chem. 28: 1612–1617.

Krogsgaard-Larsen, P., Frølund, B., Jørgensen, F. S. and Schousboe, A. (1994) $GABA_A$ receptor agonists, partial agonists and antagonists. Design and therapeutic prospects. J. Med. Chem. 37: 2489–2505.

Kusama, T., Wang, T. L., Guggino, W. B., Cutting, G. R. and Uhl, G. R. (1993) GABA rho 2 receptor pharmacological profile: GABA recognition site similarities to rho 1. Eur. J. Pharmacol. 245: 83–84.

Lambert, J. J., Peters, J. A., Sturgess, N. C. and Hales, T. G. (1990) Steroid modulation of the $GABA_A$ receptor complex: electrophysiological studies. In: Steroids and Neuronal Activity, pp. 56–82, Chadwick, D. and Widdows, K. (eds.) Wiley, Chichester.

Langtry, H. D. and Benfield, P. (1990) Zolpidem. A review of its pharmacodynamic and pharmacokinetic properties. Drugs 40: 291–313.

Lee, C. M., Wong, H. N., Chui, K. Y., Choang, T. F., Hon, P. M. and Chang, H. M. (1991) Miltirone, a central benzodiazepine receptor partial agonist from a Chinese medicinal herb Salvia miltiorrhiza. Neurosci. Lett. 127: 237–241.

Leidenheimer, N. J., Whiting, P. J. and Harris, R. A. (1993) Activation of calcium-phospholipid-dependent protein kinase enhances benzodiazepine and barbiturate potentiation of the $GABA_A$ receptor. J. Neurochem. 60: 1972–1975.

Li, C., Aguayo, L., Peoples, R. W. and Weight, F. F. (1993) Ethanol inhibits a neuronal ATP-gated ion channel. Mol. Pharmacol. 44: 871–875.

Lin, L.-H., Chen, L. L., Zirrolli, J. A. and Harris, R. A. (1992) General anesthetics potentiate γ-aminobutyric actions on γ-aminobutyric $acid_A$ receptors expressed by *Xenopus* oocytes: lack of involvement of intracellular calcium. J. Pharmacol. Exp. Ther. 263: 569–578.

Lin, L.-H., Whiting, P. and Harris, R. A. (1993) Molecular determinants of general anesthetic action: role of $GABA_A$ receptor structure. J. Neurochem. 60: 1548–1553.

Lloyd, K. G., Dreksler, S., Shemen, L. and Davidson, L. (1979) Sodium-independent, high-affinity binding of [^{3}H]gamma-aminobutyric acid in human neurological disorders. Adv. Exp. Med. Biol. 123: 399–418.

Lodge, D. and Curtis, D. R. (1978) Time course of GABA and glycine actions on cat spinal neurones: effect of pentobarbitone. Neurosci. Lett. 8: 125–129.

Loeb, C., Benassi, E., Besio, G., Maffini, M. and Tanganelli, P. (1982) Liposome-entrapped GABA modifies behavioral and electrographic changes of penicillin-induced epileptic activity. Neurology 32: 1234–1238.

Lovinger, D. M., Zimmerman, S. A., Levitin, M., Jones, M. V. and Harrison, N. L. (1993) Trichloroethanol potentiates synaptic transmission mediated by γ-aminobutyric $acid_A$ receptors in hippocampal neurons. J. Pharmacol. Exp. Ther. 264: 1097–1103.

Lummis, S. C. R., Chen Chow, S., Holan, G. and Johnston, G. A. R. (1987) γ-Aminobutyric acid receptor ionophore complexes: differential effects of deltamethrin, DDT and some novel insecticides in a rat brain membrane preparation. J. Neurochem. 48: 689–694.

Lummis, S. C. R., Gundlach, A. L., Johnston, G. A. R., Harper, P. A. W. and Dodd, P. R. (1990) Increased GABA receptor function in cerebral cortex of calves with an inherited deficit of spinal glycine/strychnine receptors. J. Neurochem. 55: 421–426.

Lunn, W. H. W., Schoepp, D. D., Calligaro, D. O., Vasileff, R. T., Heinz, L. J., Salhoff, C. R. and O'Malley, P. J. (1992) DL-Tetrazol-5-ylglycine, a highly potent NMDA agonist: its synthesis and NMDA receptor efficacy. J. Med. Chem. 35: 4608–4612.

Luque, J. M., Erat, R., Kettler, R., Cesura, A., Da Prada, M. and Richards, J. G. (1994) Radioautographic evidence that the $GABA_A$ receptor antagonist SR 95531 is a substrate inhibitor of MAO-A in the rat and human locus coeruleus. Eur. J. Neurosci. 6: 1038–1049.

Luu, M. D., Morrow, A. L., Paul, S. M. and Schwartz, R. D. (1987) Characterization of $GABA_A$ receptor-mediated ^{36}chloride uptake in rat brain synaptoneurosomes. Life Sci. 41: 1277–1287.

Macdonald, R. L. and Barker, J. L. (1979) Anticonvulsant and anesthetic barbiturates: different postsynaptic actions in cultured mammalian neurons. Neurology 29: 432–447.

Macdonald, R. L. and Olsen, R. W. (1994) $GABA_A$ receptor channels. Annu. Rev. Neurosci. 17: 569–602.

Macdonald, R. L., Rogers, C. J. and Twyman, R. E. (1989) Barbiturate regulation of kinetic properties of the $GABA_A$ receptor channel of mouse spinal neurones in culture. J. Physiol. 417: 483–500.

Maddalena, D. J. and Johnston, G. A. R. (1995) Prediction of receptor properties and binding affinity of ligands to benzodiazepine/GABA-A receptors using artificial neural networks. J. Med. Chem. 8: 715–724.

Maddison, J. E., Dodd, P. R., Johnston, G. A. R. and Farrell, G. C. (1987) Brain GABA receptor binding is normal in rats with thioacetamide-induced hepatic encephalopathy despite elevated plasma GABA-like activity. Gastroenterology 93: 1062–1068.

Mae Huang, L.-Y. and Barker, J. L. (1980) Pentobarbital: stereospecific actions of (+) and (−) isomers revealed on cultured mammalian neurones. Science 207: 195–197.

Majewska, M. D. (1987) Antagonist-type interaction of glucocorticoids with the GABA-receptor coupled chloride channel. Brain Res. 418: 377–382.

Majewska, M. D., Harrison, N. L., Schwartz, R. D., Barker, J. L. and Paul, S. M. (1986) Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232: 1004–1007.

Maksay, G. (1994) Thermodynamics of γ-aminobutyric acid type A receptor binding differentiate agonists from antagonists. Mol. Pharmacol. 46: 386–390.

Maksay, G. and Ticku, M. K. (1984) Diazotization and thiocyanate differentiate agonists from antagonists for the high- and low-affinity receptors of γ-aminobutryic acid. J. Neurochem. 43: 261–268.

Malatynska, E., Deleon, I., Allen, D. and Yamamura, H. I. (1995) Effects of amitriptyline on GABA-stimulated $Cl\text{-}36^-$ uptake in relation to a behavioural model of depression. Brain Res. Bull. 37: 53–59.

Mandelbrod, I., Feldman, S. and Werman, R. (1981) Modification of responses to sensory and hippocampal stimuli in neurons of the rat mediobasal hypothalamus in the presence of iontophoretically applied cortisol. Brain Res. 218: 115–130.

Martin, R. J., Sitamze, J.-M., Duittoz, A. H. and Wermuth, C. G. (1995) Novel arylaminopyridazine-GABA receptor antagonists examined electrophysiologically in *Ascaris suum*. Eur. J. Pharmacol. 276: 9–19.

Mason, J. W. (1968) A review of psychoendocrine research on the pituitary–adrenal cortical system. Psychosom. Med. 30: 576–607.

Mayer, M. L. and Straughan, D. W. (1981) Effects of 5-hydroxytryptamine on central neurones antagonized by bicuculline and picrotoxin. Neuropharmacology 20: 347–350.

McCarthy, M. M., Coirini, H., Schumacher, M., Pfaff, D. W., McEwen, B. S. and Schwartz-Giblin, S. (1991) Ovarian steroid modulation of [^{3}H]muscimol binding in the spinal cord of the rat. Brain Res. 556: 321–323.

Medina, J. H., Paladini, A. C., Wolfman, C., Levi de Stein, M., Calvo, D., Diaz, L. E. and Pena, C. (1990) Chrysin (5,7-di-OH-flavone), a naturally occurring ligand for benzodiazepine receptors, with anticonvulsant properties. Biochem. Pharmacol. 40: 2227–2231.

Medina, J. H., Levi de Stein, M., Wolfman, C., Wasowski, C., De Blas, A. and Paladini, A. C. (1993) *In vivo* formation of benzodiazepine-like molecules in mammalian brain. Biochem. Biophys. Res. Commun. 195: 1111–1118.

Mellon, S. H. and Deschepper, C. F. (1993) Neurosteroid biosynthesis: genes for adrenal steroidogenic enzymes are expressed in the brain. Brain Res. 629: 283–292.

Mhatre, M. C. and Ticku, M. K. (1992) Chronic ethanol administration alters γ-aminobutyric acidA receptor gene expression. Mol. Pharmacol. 42: 415–422.

Michaud, J. C., Mienville, J. M., Chambon, J. P. and Biziere, K. (1986) Interactions between three pyridazinyl-GABA derivatives and central GABA and glycine receptors in the rat, an *in vitro* microiontophoretic study. Neuropharmacology 25: 1197–1203.

Mihic, S. J., Whiting, P. J. and Harris, R. A. (1994) Anaesthetic concentrations of alcohols potentiate $GABA_A$ receptor-mediated currents: lack of subunit specificity. Eur. J. Pharmacol. 268: 209–214.

Miller, B., Sarantis, M., Traynelis, S. F. and Attwell, D. (1992) Potentiation of NMDA receptor currents by arachidonic acid. Nature 355: 722–725.

Miller, L. G., Galpern, W. R., Dunlap, K., Dinarello, C. A. and Turner, T. J. (1991) Interleukin-1 augments γ-aminobutryic $acid_A$ receptor function in brain. Mol. Pharmacol. 39: 105–108.

Minchin, M. S. W., Ennis, C., Lattimer, N., White, J. F., White, A. C. and Lloyd, G. K. (1992) A novel $GABA_A$-like autoreceptor modulates GABA release. Mol. Neuropharmacol. 2: 137–139.

Möhler, H. and Okada, T. (1978) Properties of γ-aminobutryic acid receptor binding with (+)-[^{3}H]bicuculline methiodide in rat cerebellum. Mol. Pharmacol. 14: 256–265.

Monn, J. A., Valli, M. J., True, R. A., Schoepp, D. D., Leander, J. D. and Lodge, D. (1993) Synthesis and pharmacological characterization of L-trans-4-tetrazolylproline (LY300030): a novel systemically active AMPA receptor agonist. Bioorg. Med. Chem. Lett. 3: 95–98.

Moss, S. J., Smart, T. G., Blackstone, C. D. and Huganir, R. L. (1992) Functional modulation of $GABA_A$ receptors by cAMP-dependent protein phosphorylation. Science 257: 661–665.

Munck, A. and Guyre, P. M. (1986) Glucocorticoid physiology, pharmacology and stress. Adv. Exp. Med. Biol. 196: 81–96.

Nagata, K., Hamilton, B. J., Carter, D. B. and Narahashi, T. (1994) Selective effects of dieldrin on the $GABA_A$ receptor-channel subunits expressed in human embryonic kidney cells. Brain Res. 645: 19–26.

Narahashi, T., Frey, J. M., Ginsberg, K. S. and Roy, M. L. (1992) Sodium and GABA-activated channels as the targets of pyrethroids and cyclodienes. Toxicol. Lett. 64/65: 429–436.

Nayeem, N., Green, T. P., Martin, I. L. and Barnard, E. A. (1994) Quaternary structure of the native $GABA_A$ receptor determined by electron microscopic image analysis. J. Neurochem. 62: 815–818.

Newland, C. F. and Cull-Candy, S. G. (1992) On the mechanism of action of picrotoxin on GABA receptor channels in dissociated sympathetic neurones of the rat. J. Physiol. 445: 97–127.

Nicholson, G. M., Spence, I. and Johnston, G. A. R. (1985) Effects of a depressant/convulsant pair of glutarimides on neuronal activity in the isolated spinal cord of the immature rat. Neuropharmacology 24: 461–464.

Nielsen, M., Gredal, O. and Braestrup, C. (1979) Some properties of ^{3}H-diazepam displacing activity from human urine. Life Sci. 25: 679–686.

Nielsen, M., Frokjaer, S. and Braestrup, C. (1988) High affinity of the naturally-occurring biflavonoid, amentoflavon, to brain benzodiazepine receptors *in vitro*. Biochem. Pharmacol. 37: 3285–3287.

Nielsen, M., Witt, M. R., Ebert, B. and Krogsgaard-Larsen, P. (1995) Thiomuscimol, a new photoaffinity label for the $GABA_A$ receptor. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 289: 109–112.

Noto, T., Hasegawa, T., Nakao, J., Kamimura, H., Harada, H. and Nakajima, T. (1988) Formation of γ-amino-β-hydroxybutyric acid from 2-hydroxyputrescine in rat brain. J. Neurochem. 51: 548–551.

Olsen, R. W. (1984) γ-Aminobutyric acid receptor binding antagonism by the amidine steroid RU5135. Eur. J. Pharmacol. 103: 333–337.

Olsen, R. W. and Leeb-Lundberg, F. (1980) Endogenous inhibitors of picrotoxinin convulsant binding sites in rat brain. Eur. J. Pharmacol. 65: 101–104.

Olsen, R. W. and Snowman, A. M. (1983) [^{3}H]Bicuculline methochloride binding to low-affinity gamma-aminobutyric acid receptor sites. J. Neurochem. 41: 1653–1663.

Olsen, R. W. and Snowman, A. M. (1985) Avermectin B_{1a} modulation of gamma-aminobutyric acid/benzodiazepine receptor binding in mammalian brain. J. Neurochem. 44: 1074–1082.

Olsen, R. W., Ban, M., Miller, T. and Johnston, G. A. R. (1975) Chemical instability of the GABA antagonist bicuculline under physiological conditions. Brain Res. 98: 383–387.

Ong, J., Kerr, D. I. B. and Johnston, G. A. R. (1987a) Cortisol: a potent biphasic modulator at $GABA_A$-receptor-complexes in the guinea-pig isolated ileum. Neurosci. Lett. 82: 101–106.

Ong, J., Kerr, D. I. B. and Johnston, G. A. R. (1987b) Pregnenolone and its sulphate modulate $GABA_A$-receptor-mediated contractile responses in the guinea-pig isolated ileum. Eur. J. Pharmacol. 142: 461–464.

Ong, J., Kerr, D. I. B., Capper, H. R. and Johnston, G. A. R. (1990) Cortisone, a potent $GABA_A$ antagonist in the guinea-pig isolated ileum. J. Pharm. Pharmacol. 42: 662–664.

Orchinik, M., Weiland, N. G. and McEwen, B. S. (1994) Adrenalectomy selectively regulates $GABA_A$ receptor subunit expression in the hippocampus. Mol. Cell. Neurosci. 5: 451–458.

Ortells, M. O. and Lunt, G. G. (1995) Evolutionary history of the ligand-gated ion-channel superfamily of receptors. Trends Neurosci. 18: 121–127.

Ozoe, Y., Hasegawa, H., Mochida, K., Koike, K., Suzuki, Y., Nagahisa, M. and Ohmoto, T. (1994) Picrodendrins, a new group of picrotoxane terpenoids: structure–activity profile of action at the $GABA_A$ receptor-coupled picrotoxinin binding site in rat brain. Biosci. Biotech. Biochem. 58: 1506–1507.

Park-Chung, M., Wu, F. S. and Farb, D. H. (1994) 3-α-Hydroxy-5β-pregnan-20-one sulfate: a negative modulator of the NMDA-induced current in cultured neurons. Mol. Pharmacol. 46: 146–150.

Payne, G. T. and Soderlund, D. M. (1991) Activation of γ-aminobutyric acid insensitive chloride channels in mouse brain synaptic vesicles by avermectin B1. J. Biochem. Toxicol. 6: 283–292.

Pearce, R. A. (1993) Physiological evidence for two distinct $GABA_A$ responses in rat hippocampus. Neuron 10: 189–200.

Pearce, R. J. and Duchen, M. R. (1995) Electrophysiological and metabolic effects of a convulsant barbiturate on dissociated mouse primary sensory neurons. J. Physiol. 483: 407–420.

Pelley, K. A. and Vaught, J. L. (1987) An antinociceptive profile of kojic amine: an analogue of γ-aminobutyric acid (GABA). Neuropharmacology 26: 301–307.

Perezvelazquez, J. L. and Angelides, K. J. (1993) Assembly of $GABA_A$ receptor subunits determines sorting and localization in polarized cells. Nature 361: 457–460.

Pericic, D., Manev, H. and Lakic, N. (1985) Sex differences in the response to drugs affecting GABAergic transmission. Life Sci. 36: 541–547.

Petersen, E. N., Jensen, L. H., Honoré, T. and Braestrup, C. (1983) Differential pharmacological effects of benzodiazepine receptor inverse agonists. Adv. Biochem. Psychopharmacol. 38: 57–64.

Peterson, E. M., Xu, K., Holland, K. D., McKeon, A. C., Rothman, S. M., Ferrendelli, J. A. and Covey, D. F. (1994) α-Spirocyclopentyl- and α-spriocyclopropyl-γ-butyrolactones: conformationally constrained derivatives of anticonvulsant and convulsant α,α,-disubstituted γ-butyrolactones. J. Med. Chem. 37: 275–286.

Phillipps, G. H. (1975) Structure–activity relationships in steroidal anaesthetics. J. Steroid Biochem. 6: 607–613.

Phillipps, G. H., Ayers, B. E., Bailey, E. J., Ewan, G. B., Looker, B. and May, P. J. (1979) Water soluble steroid anaesthetics. J. Steroid Biochem. 11: 79–86.

Pickles, H. G. and Simmonds, M. A. (1980) Antagonism by penicillin of gamma-aminobutyric acid depolarizations at presynaptic sites in rat olfactory cortex and cuneate nucleus *in vitro*. Neuropharmacology 19: 35–38.

Polc, P. and Haefely, W. (1976) Effects of two benzodiazepines, phenobarbitone, and baclofen on synaptic transmission in the cat cuneate nucleus. Naunyn Schmiedebergs Arch. Pharmacol. 294: 121–131.

Polc, P., Laurent, J. P., Scherschlicht, R. and Haefely, W. (1981) Electrophysiological studies on the specific benzodiazepine antagonist Ro15-1788. Naunyn Schmiedebergs Arch. Pharmacol. 316: 317–325.

Pomés, A., Rodríguez, E. and Suñol, C. (1994) Disruption of GABA-dependent chloride flux by cyclodienes and hexachlorocyclohexanes in primary cultures of cortical neurons. J. Pharmacol. Exp. Ther. 271: 1616–1623.

Pooler, G. W. and Steward, E. G. (1988) Structural factors governing agonist and antagonist activity in the $GABA_A$ system. Biochem. Pharmacol. 37: 943–945.

Prince, R. J. and Simmonds, M. A. (1993) Differential antagonism by epipregnanolone of alphaxalone and pregnanolone potentiation of [^{3}H]flunitrazepam binding suggests more than one class of binding site for steroids at $GABA_A$ receptors. Neuropharmacology 32: 59–63.

Pritchett, D. B., Sontheimer, H., Shivers, B. D., Ymer, S., Kettenmann, H., Schofield, P. R. and Seeburg, P. H. (1989) Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. Nature 338: 582–585.

Puia, G., Vicini, S., Seeburg, P. H. and Costa, E. (1991) Influence of recombinant gamma-aminobutyric acid-A receptor subunit composition on the action of allosteric modulators of gamma-aminobutyric acid-gated Cl^- currents. Mol. Pharmacol. 39: 691–696.

Puia, G., Ducic, I., Vicini, S. and Costa, E. (1993) Does neurosteroid modulatory efficacy depend on $GABA_A$ receptor subunit composition? Recept. Channels 1: 135–142.

Puia, G., Costa, E. and Vicini, S. (1994) Functional diversity of GABA-activated Cl^- currents in Purkinje versus granule neurons in rat cerebellar slices. Neuron 12: 117–126.

Purdy, R. H., Morrow, A. L., Moore, P. H. and Paul, S. M. (1991) Stress-induced elevations of γ-aminobutyric acid type A receptor-active steroids in the rat brain. Proc. Natl. Acad. Sci. USA 88: 4553–4557.

Quilliam, J. P. and Stables, R. (1969) Convulsant effects of cunaniol, a polyacetylenic alcohol isolated from the plant *Clibadium sylvestre*, on frogs and mice. Pharmacol. Res. Commun. 1: 7–11.

Quinn, M. R. and Miller, C. L. (1992) Taurine allosterically modulates flunitrazepam binding to synaptic membranes. J. Neurosci. Res. 33: 136–141.

Rapallino, M. V., Cupello, A., Mainardi, P., Besio, G. and Loeb, C. W. (1990) Effect of phosphatidylserine on the basal and GABA-activated Cl^- permeation across single nerve membranes from rabbit Deiters' neurons. Neurochem. Res. 15: 593–596.

Rauth, J. J., Vassallo, J. G., Lummis, S. C. R., Wafford, K. A. and Sattelle, D. B. (1993) Steroids reveal differences between GABA-operated chloride channels of insects and vertebrates. Mol. Neuropharmacol. 3: 1–9.

Rigo, J. M., Belachew, S., Lefebvre, P. P., Leprince, P., Malgrange, B., Register, B., Kettenman, H. and Moonen, G. (1994) Astroglia-released factor shows similar effects as benzodiazepine inverse agonists. J. Neurosci. Res. 39: 364–376.

Roberts, E. (1995) Pregnenolone–from Seyle to Alzheimer and a model of the pregnenolone sulfate binding site on the $GABA_A$ receptor. Biochem. Pharmacol. 49: 1–16.

Roberts, E. and Sherman, M. A. (1993) GABA–the quintessential neurotransmitter: electroneutrality, fidelity, specificity, and a model for the ligand binding site of $GABA_A$ receptors. Neurochem. Res. 18: 365–376.

Rogers, C. J., Twyman, R. E. and Macdonald, R. L. (1994) Benzodiazepine and β-carboline regulation of single $GABA_A$ receptor channels of mouse spinal neurones in culture. J. Physiol. 475: 69–82.

Rognan, D., Boulanger, T., Hoffmann, R., Vercauteren, D. P., Andre, J. M., Durant, F. and Wermuth, C. G. (1992) Structure and molecular modelling of $GABA_A$ receptor antagonists. J. Med. Chem. 35: 1969–1977.

Samochocki, M. and Strosznajder, J. (1993) Modulatory action of arachidonic acid on $GABA_A$/chloride channel receptor function in adult and aged brain cortex membranes. Neurochem. Int. 23: 261–267.

Saransaari, P. and Oja, S. S. (1993) Strychnine-insensitive glycine binding to cerebral cortical membranes in developing and ageing mice. Mech. Ageing Dev. 72: 57–66.

Schmidt, R. F., Vogel, M. E. and Zimmerman, M. (1967) Die Wirkung von Diazepam auf die prasynaptische Hemmung und anderen Ruckenmarksreflexe. Naunyn Schmiedebergs Arch. Pharmacol. 258: 69–82.

Schofield, P. R., Darlison, M. G., Fujita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., Rhee, L. M., Ramachandran, J., Reale, V., Glencorse, T. A., Seeburg, P. H. and Barnard, E. A. (1987) Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor superfamily. Nature 328: 221–227.

Schönrock, B. and Bormann, J. (1993a) Functional heterogeneity of hippocampal $GABA_A$ receptors. Eur. J. Neurosci. 5: 1042–1049.

Schönrock, B. and Bormann, J. (1993b) Activation of Cl^- channels by avermectin in rat cultured hippocampal neurons. Naunyn Schmiedebergs Arch. Pharmacol. 348: 628–632.

Schwartz, R. D., Wess, M. J., Labarca, R., Skolnick, P. and Paul, S. M. (1987) Acute stress enhances the activity of GABA receptor-gated chloride channels in brain. Brain Res. 411: 151–155.

Schwartz, R. D., Wagner, J. P., Yu, X. and Martin, D. (1994) Bidirectional modulation of GABA-gated chloride channels by divalent cations: inhibition by Ca^{2+} and enhancement by Mg^{2+}. J. Neurochem. 62: 916–922.

Seyle, H. (1942) Correlations between the chemical structure and the pharmacological actions of the steroids. Endocrinology 30: 437–453.

Shen, X.-L., Nielsen, M., Witt, M. R., Sterner, O., Bergendorff, O. and Khayyal, M. (1994) Inhibition of [methyl-^{3}H]diazepam binding to rat brain membranes *in vitro* by dinatin and skrofulein. Acta Pharmacol. Sin. 15: 385–388.

Siebler, M., Koller, H., Schamallenbach, C. and Muller, H. W. (1988) GABA activated chloride currents in cultured rat hippocampal and septal region neurons can be inhibited by curare and atropine. Neurosci. Lett. 93: 220–224.

Sieghart, W. (1992) $GABA_A$ receptors: ligand-gated Cl^- ion channels modulated by multiple drug-binding sites. Trends Pharmacol. Sci. 13: 446–450.

Sigel, E., Baur, R., Malherbe, P. and Möhler, H. (1989) The rat β_1-subunit of the $GABA_A$ receptor forms a picrotoxin-sensitive anion channel open in the absence of GABA. FEBS Lett. 257: 377–379.

Sigel, E., Baur, R., Kellenberger, S. and Malherbe, P. (1992) Point mutations affecting antagonist affinity and agonist dependent gating of $GABA_A$ receptor channels. EMBO J. 11: 2017–2023.

Simmonds, M. A. (1980) Evidence that bicuculline and picrotoxin act at separate sites to antagonize γ-aminobutyric acid in rat cuneate nucleus. Neuropharmacology 19: 39–45.

Simmonds, M. A. and Turner, J. P. (1985) Antagonism of inhibitory amino acids by the steroid derivative RU5135. Br. J. Pharmacol. 84: 631–635.

Skerritt, J. H. and Johnston, G. A. R. (1983) Diazepam stimulates the binding of GABA and muscimol but not THIP to rat brain membranes. Neurosci. Lett. 38: 315–320.

Skerritt, J. H. and Macdonald, R. L. (1984) Diazepam enhances the action but not the binding of the GABA analog, THIP. Brain Res. 297: 181–186.

Skerritt, J. H., Trisdikoon, P. and Johnston, G. A. R. (1981) Increased GABA binding in mouse brain following acute swim stress. Brain Res. 215: 398–403.

Skerritt, J. H., Chen Chow, S. and Johnston, G. A. R. (1982a) Differences in the interactions between GABA and benzodiazepine binding sites. Neurosci. Lett. 33: 173–187.

Skerritt, J. H., Chen Chow, S., Johnston, G. A. R. and Davies, L. P. (1982b) Purines interact with "central" but not "peripheral" benzodiazepine binding sites. Neurosci. Lett. 34: 63–68.

Skerritt, J. H., Davies, L. P., Chen Chow, S. and Johnston, G. A. R. (1982c) Contrasting regulation by GABA of the displacement of benzodiazepine antagonist binding by benzodiazepine agonists and purines. Neurosci. Lett. 32: 169–174.

Skerritt, J. H., Willow, M. and Johnston, G. A. R. (1982d) Diazepam enhancement of low affinity GABA binding to rat brain membranes. Neurosci. Lett. 29: 63–66.

Skerritt, J. H., Johnston, G. A. R., Katsikas, T., Tabar, J., Nicholson, G. M. and Andrews, P. R. (1983) Actions of pentobarbitone and derivatives with modified 5-butyl substituents on GABA and diazepam binding to rat brain synaptosomal membranes. Neurochem. Res. 8: 1337–1350.

Skerritt, J. H., Johnston, G. A. R., Chen Chow, S., Macdonald, R. L., Prager, R. H. and Ward, A. D. (1985) Differential modulation of GABA receptors by caprolactam derivatives with CNS depressant or convulsant activity. Brain Res. 331: 225–233.

Smart, T. G. (1992) A novel modulatory binding site for zinc on the $GABA_A$ receptor complex in cultured rat neurons. J. Physiol., Lond. 447: 587–625.

Smart, T. G., Moss, S. J., Xie, X. and Huganir, R. L. (1991) $GABA_A$ receptors are differentially sensitive to zinc: dependence on subunit composition. Br. J. Pharmacol. 99: 643–654.

Soldo, B. L., Proctor, W. R. and Dunwiddie, T. V. (1994) Ethanol differentially modulates $GABA_A$ receptor-mediated chloride currents in hippocampal, cortical, and septal neurons in rat brain slices. Synapse 18: 94–103.

Squires, R. F. and Braestrup, C. (1977) Benzodiazepine receptors in rat brain. Nature 266: 732–734.

Squires, R. F. and Saederup, E. (1993a) Mono N-aryl ethylenediamine and piperazine are GABA(A) receptor blockers – implications for psychiatry. Neurochem. Res. 18: 787–793.

Squires, R. F. and Saederup, E. (1993b) Indomethacin/ibuprofen-like anti-inflammatory agents selectively potentiate the γ-aminobutyric acid-antagonistic effects of several norfloxacin-like quinolone antibacterial agents on [^{35}S]-t-butylbicyclophosphorothionate binding. Mol. Pharmacol. 43: 795–800.

Starkenstein, E. and Weden, H. (1936) Zur Physiologie und Pharmakologie der Sterine: der Einfluss des Cholesterins auf die Wirkung der Hypnotica und Narcotica. Arch. Exp. Pathol. Pharmakol. 182: 700–714.

Stephanson, F. A. (1995) Understanding the molecular heterogeneity of the $GABA_A$ receptors. J. Neurochem. 64: S29.

Stephens, D. N., Schneider, H. H., Kehr, W., Andrews, J. S., Rettig, K.-J., Turski, L., Schmiechen, R., Turner, J. D., Jensen, J. H., Petersen, E. N., Honoré, T. and Bondo Hansen, J. (1990) Abecarnil, a metabolically stable, anxioselective β-carboline acting at benzodiazepine receptors. J. Pharmacol. Exp. Ther. 253: 334–343.

Strittmatter, W. J., Hirata, F., Axelrod, J., Mallorga, P., Tallman, J. F. and Henneberry, R. C. (1979) Benzodiazepine and β-adrenergic receptor ligands independently stimulate phospholipid methylation. Nature 282: 857–859.

Suzdak, P. D., Paul, S. M. and Crawley, J. N. (1988) Effects of RO15-4513 and other benzodiazepine receptor inverse agonists on alcohol-induced intoxication in the rat. J. Pharmacol. Exp. Ther. 245: 880–886.

Suzuki, S., Kawakami, K., Nakamura, F., Nishimura, S., Yagi, K. and Seino, M. (1994) Bromide, in the therapeutic concentration, enhances GABA-activated currents in cultured neurons of rat cerebral cortex. Epilepsy Res. 19: 89–97.

Takahashi, K., Kameda, H., Kataoka, M., Sanjou, K., Harata, N. and Akaike, N. (1993) Ammonia potentiates $GABA_A$ response in dissociated rat cortical neurons. Neurosci. Lett. 151: 51–54.

Taleb, O., Trouslard, J., Demeneix, B. A., Feltz, P., Bossu, J. L., Dupont, J. L. and Feltz, A. (1987) Spontaneous and GABA evoked chloride channels on pituitary intermediate lobe cells and their internal Ca requirements. Pflügers Arch. 409: 620–631.

Tallman, J., Thomas, J. W. and Gallagher, D. (1978) GABAergic modulation of benzodiazepine binding site sensitivity. Nature 274: 383–385.

Tang, C.-M., Dichter, M. and Morad, M. (1990) Modulation of the N-methyl-D-aspartate channel by extracellular H^+. Proc. Natl. Acad. Sci. USA 87: 6445–6449.

Tang, X. W., Yarom, M., Carlson, R. G., Velde, D. V., Huang, P. Y., Lee, Y.-H., Seah, E.-C., Deupree, D. and Wu, J.-Y. (1993) Isolation of endogenous modulators for the $GABA_A$ and taurine receptors. Neurochem. Int. 23: 485–493.

Taniyama, K., Hashimoto, S., Hanada, S. and Tanaka, C. (1988) Benzodiazepines and barbiturates potentiate the pre- and postsynaptic γ-aminobutyric acid $(GABA)_A$ receptor mediated response in the enteric nervous system of guinea pig small intestine. J. Pharmacol. Exp. Ther. 245: 250–256.

Ticku, M. K. and Burch, T. (1980) Purine inhibition of [^{3}H]-gamma-aminobutyric acid receptor binding to rat brain membranes. Biochem. Pharmacol. 29: 1217–1220.

Toffano, G., Aldinio, C., Balzano, M., Leon, A. and Savoini, G. (1981) Regulation of GABA receptor binding to synaptic plasma membrane of rat cerebral cortex: the role of endogenous phospholipids. Brain Res. 222: 95–102.

Toffano, G., Mazzari, S., Zanotti, A. and Bruni, A. (1984) Synergistic effect of phosphatidylserine with gamma-aminobutyric acid in antagonizing the isoniazid-induced convulsions in mice. Neurochem. Res. 9: 1065–1073.

Tokutami, N., Agopyan, N. and Akaike, N. (1992) Penicillin-induced potentiation of glycine receptor-operated chloride current in rat ventro-medial hypothalamic neurones. Br. J. Pharmacol. 106: 73–78.

Trudeau, V. L., Sloley, B. D. and Peter, R. E. (1993) Testosterone enhances GABA and taurine but not N-methyl-D,L-aspartate stimulation of gonadotropin secretion in the goldfish: possible sex steroid feedback mechanisms. J. Neuroendocrinol. 5: 129–136.

Twyman, R. E. and Macdonald, R. L. (1992) Neurosteroid regulation of $GABA_A$ receptor single-channel kinetic properties of mouse spinal cord neurons in culture. J. Physiol. 456: 215–245.

Twyman, R. E., Green, R. M., and Macdonald, R. L. (1992) Kinetics of open channel block by penicillin of single $GABA_A$ receptor channels from mouse spinal cord neurones in culture. J. Physiol. 445: 97–127.

Uchida, I., Kamatchi, G., Burt, D. and Yang, J. (1995) Etomidate potentiation of $GABA_A$ receptor gated current depends on the subunit composition. Neurosci Lett. 185: 203–206.

Ueno, E. and Kuriyama, K. (1981) Phospholipids and benzodiazepine recognition sites of brain synaptic membranes. Neuropharmacology 20: 1169–1176.

Unwin, J. W. and Taberner, P. V. (1980) Sex and strain differences in GABA receptor binding after chronic ethanol drinking in mice. Neuropharmacology 19: 1257–1259.

Viola, H., Wolfman, C., Levi de Stein, M., Wasowski, C., Pena, C., Medina, J. H. and Paladini, A. C. (1994) Isolation of pharmacologically active benzodiazepine receptor ligands from *Tilia tomentosa* (Tiliaceae). J. Ethnopharmacol. 44: 47–53.

Vogel, W. H. (1987) Stress – the neglected variable in experimental pharmacology and toxicology. Trends Pharmacol. Sci. 8: 35–38.

Wafford, K. A. and Whiting, P. J. (1992) Ethanol potentiation of $GABA_A$ receptors requires phosphorylation of the alternatively spliced variant of the γ_2 subunit. FEBS Lett. 313: 113–117.

Watkins, J. C. (1965) Pharmacological receptors and general permeability phenomena of cell membranes. J. Theor. Biol. 9: 37–50.

Weiner, J. L., Zhang, L. and Carlen, P. L. (1994) Potentiation of $GABA_A$-mediated synaptic current by ethanol in hippocampal CA1 neurons: possible role of protein kinase C. J. Pharmacol. Exp. Ther. 268: 1388–1395.

Wermuth, C. G., Bourhuignon, J.-J., Schlewer, G., Gies, J.-P., Schoenfelder, A., Melikian, A., Bouchet, M.-J., Chantreux, D., Molimard, J.-C., Heaulme, M., Chambon, J.-P. and Biziere, K. (1987) Synthesis and structure–activity relationships of a series of aminopyridazine derivatives of γ-aminobutyric acid acting as selective $GABA_A$ antagonists. J. Med. Chem. 30: 239–249.

Westerling, P., Lindgren, S. and Meyerson, B. (1991) Functional changes in $GABA_A$ receptor stimulation during the oestrus cycle of the rat. Br. J. Pharmacol. 103: 1580–1584.

Whatley, V. J., Mihic, S. J., Allan, A. M., McQuilkin, S. J. and Harris, R. A. (1994) γ-Aminobutyric $acid_A$ receptor function is inhibited by microtubule depolymerization. J. Biol. Chem. 269: 19546–19552.

White, G. and Gurley, D. A. (1995) α Subunits influence Zn block of γ_2 containing $GABA_A$ receptor currents. Neuroreport 6: 461–464.

Whitehouse, B. J. (1992) Benzodiazepine and steroidogensis. J. Endocrinol. 134: 1–3.

Wieboldt, R., Ramesh, D., Carpenter, B. K. and Hess, G. P. (1994) Synthesis and photolabile derivatives of γ-aminobutyric acid receptor in the millisecond time region. Biochemistry 33: 1526–1533.

Wieland, H. A. and Lüddens, H. (1994) Four amino acid exchanges convert a diazepam-insensitive, inverse agonist-preferring $GABA_A$ receptor into a diazepam-preferring $GABA_A$ receptor. J. Med. Chem. 37: 4576–4580.

Willow, M. (1981) A comparison of the actions of pentobarbitone and etomidate on [^{3}H]GABA binding to crude synaptosomal rat brain membranes. Brain Res. 220: 427–431.

Willow, M. and Johnston, G. A. R. (1979) Barbiturates and calcium-activated adenosine triphosphatase. Neurosci. Lett. 14: 361–364.

Willow, M. and Johnston, G. A. R. (1981a) Pentobarbitone slows the dissociation of GABA from rat brain synaptosomal binding sites. Neurosci. Lett. 23: 71–74.

Willow, M. and Johnston, G. A. R. (1981b) Enhancement by anesthetic and convulsant barbiturates of GABA binding to rat brain synaptosomal membranes. J. Neurosci. 1: 364–367.

Willow, M. and Johnston, G. A. R. (1983) Pharmacology of barbiturates: electrophysiological and neurochemical studies. Int. Rev. Neurobiol. 24: 15–49.

Willow, M., Bornstein, J. C. and Johnston, G. A. R. (1980) The effects of anaesthetic and convulsant barbiturates on the efflux of [^{3}H]-D-aspartate from brain minislices. Neurosci. Lett. 18: 185–190.

Woodward, R. M., Polenzani, L. and Miledi, R. (1994) Effects of fenamates and other nonsteroidal anti-inflammatory drugs on rat brain $GABA_A$ receptors expressed in *Xenopus* oocytes. J. Pharmacol. Exp. Ther. 268: 806–817.

Wu, F.-S., Gibbs, T. T. and Farb, D. H. (1993) Dual activation of $GABA_A$ and glycine receptors by β-alanine: inverse modulation by progesterone and 5α-pregnan-3α-ol-20-one. Eur. J. Pharmacol. 246: 239–246.

Yamamoto, I., Kimura, T., Yoshida, H., Watanabe, K. and Yoshimura, H. (1992) Cannabinoid metabolite interacts with benzodiazepine receptor. Res. Commun. Subst. Abuse 13: 299–313.

Yan Ma, J. and Narahashi, T. (1993) Differential modulation of $GABA_A$ receptor-channel complex by polyvalent cations in rat dorsal root ganglion neurons. Brain Res. 607: 222–232.

Yokoi, I., Tsurata, K., Shiraga, H., Mori, A. and Shiraga, H. (1987) δ-Guanidinovaleric acid as an endogenous and specific GABA-receptor antagonist: electroencephalographic study. Epilepsy Res. 1: 114–120.

Yoneda, Y. and Kuriyama, K. (1980) Presence of a low molecular weight endogenous inhibitor of ^{3}H-muscimol binding in synaptic membranes. Nature 285: 670–673.

Yoon, K.-W. (1994) Voltage-dependent modulation of $GABA_A$ receptor channels in rat hippocampal neurons. J. Neurophysiol. 71: 2151–2160.

Yoon, K.-W., Covey, D. F. and Rothman, S. M. (1993) Multiple mechanisms of picrotoxin block of GABA-induced currents in rat hippocampal neurons. J. Physiol. 464: 423–430.

Zhang, Z.-W. and Feltz, P. (1991) Bicuculline blocks nicotinic acetylcholine response in isolated intermediate lobe cells of the pig. Br. J. Pharmacol. 102: 19–22.

Zorn, S. H. and Enna, S. J. (1987) The GABA agonist THIP attenuates antinociception in the mouse by modifying central cholinergic transmission. Neuropharmacology 26: 433–437.