GABA RECEPTORS AND BENZODIAZEPINES

C. S. GOODCHILD

From an historical standpoint, the development of our understanding of basic mechanisms of the actions of gamma aminobutyric acid (GABA) and benzodiazepines began on separate paths. Classical pharmacology revealed similarity between the actions of GABA and benzodiazepines in the central nervous system. Furthermore, pharmacological experiments with selective agonists and antagonists indicated that there exists subtypes of both GABA and benzodiazepine receptors and that the actions of the benzodiazepines are produced by modulation of GABA effects. Thus the two paths of research on GABA and benzodiazepines had crossed in explaining the effects of benzodiazepines by an interaction with GABA_A receptors that were located on the same macromolecular complex. More recently, this interaction has been elucidated further by the cloning of subunits of GABA_A receptors: the interaction between GABA and benzodiazepines is now being explained at a molecular level.

GABA

Hayashi first proposed GABA as an inhibitory neurotransmitter in the mammalian central nervous system in 1954. This was based on the observation of the amino acid in the brain and anticonvulsant properties of the compound when injected into animals. Supporting evidence for the concept came when GABA was extracted from central nervous system, identified chemically shown to have inhibitory actions on the nervous system [3, 25]. In mammals, GABA is present in the central nervous system, although small amounts may be found in other areas such as autonomic ganglia. It is a simple amino acid molecule which is formed by decarboxylation of L-glutamic acid by the enzyme glutamic acid decarboxylase (GAD). This enzyme is found only in the cytoplasm of neurones containing GABA.

Although distributed widely in the central nervous system, the concentration of GABA varies in different regions with the greatest concentrations found in basal ganglia, hippocampus, cerebellum hypothalamus in the brain, and in the substantia gelatinosa of the dorsal horn of the spinal cord.

Large concentrations are found also in the cerebellum and retina. GABA in the retina is localized mostly in the horizontal cell layer where it is responsible for lateral inhibition.

Most GABAergic neurones are interneurones, with short axons and local connections. Generally the actions of GABA within the central nervous system are inhibitory. This effect is achieved by an increase in chloride conductance [4, 24, 30, 31]. Pre- and postsynaptic effects have been described. For example, GABA has long been known to produce presynaptic inhibition by hyperpolarization of afferent terminals in the spinal cord [16]. This effect is shown in figure 1. The GABAergic interneuron releases GABA onto a primary afferent fibre before it makes a synaptic contact. The effect of this is to cause hyperpolarization of the primary afferent fibre. This effect may be recorded from the cut central ends of these primary afferent fibres as a "dorsal root potential". The hyperpolarization decreases the central propagation of the action potential. Thus less excitatory primary afferent neurone transmitter is released onto the postsynaptic neurone.

KEY WORDS

Hypnotics: benzodiazepines, diazepam, midazolam. Receptors: GABA.

C. S. GOODCHILD, M.A., M.B., B.CHIR., PH.D., F.R.C.A., Monash Medical Centre, Clayton Road, Melbourne, Victoria 3168, Australia.

(Stim. DR VR PS AMP DRP REC)

FIG. 1. GABAergic presynaptic inhibition. The shaded interneuron releases GABA onto the presynaptic terminal (P). DRP = Dorsal root potential; VR = ventral root; PS = postsynaptic cell; Stim = stimulation; REC = recording electrodes.
Pharmacological studies have indicated functions for GABA within the central nervous system in the induction of sleep, control of neuronal excitability/epilepsy, anxiety, memory and hypnosis. Evidence for the involvement of GABA in these widely varying functions comes from the effects of the compound itself when injected into animals, and also from the effects of drugs that either alter the synthesis or metabolism of GABA or act at postsynaptic GABA receptors. Thus glutamic acid decarboxylase inhibitors lead to reduction in brain concentrations of GABA. This ultimately leads to convulsions. Drugs which block the reuptake of GABA from the subsynaptic cleft back into the presynaptic terminal, such as dianminobutyric acid, lead to increased concentrations of GABA. Such drugs possess anticonvulsant and sedative properties. Bicuculline and picrotoxin, which bind to the postsynaptic GABA receptor, are convulsants in large doses and in smaller doses alter memory.

**Hypnosis**

The sedative and hypnotic effects of GABA were the main properties of interest to early researchers. Sedation was found to be of anaesthetic interest. For example, Galindo [26] showed that halothane potentiates the inhibitory action of GABA in the cuneate nucleus. These observations led to one of the theories of anaesthetic action, that is potentiation of central nervous system depression by GABA. Other anaesthetic drugs are known to interact with GABA in this respect: propofol [50], barbiturates [1, 23, 69] and neurosteroids [52].

**Epilepsy**

The role of GABA in the prevention of epilepsy is now well established. Many of the conventional drugs used in the management of epilepsy are known to interact with GABA receptors (*vide infra* for benzodiazepines, barbiturates and neurosteroids). Sodium valproate is thought to produce its anticonvulsant action by reducing the metabolism of GABA. More recently introduced drugs include GABA analogues such as vigabatrin [32].

**Anxiety**

Anti-anxiety effects of GABA in animals are reduction in aggression and increase in social behaviour, thus allowing the animals to be handled more easily. This action of GABA is thought to be a result of interaction with 5-hydroxytryptamine (5-HT) in the limbic system. These interactions are known to occur at a number of sites within the nervous system [48] and also explain the anxiolytic effects of 5-HT\_3 receptor antagonists [14, 15]. It is interesting to note that GABA\_A receptor subunit mRNA (*vide infra*) are increased by social stress in animals [35]. This observation indicates that this is a real physiological role for GABA.

**Memory**

The first evidence for involvement of GABA mechanisms in memory storage and retention came from the observation that systemic picrotoxin, which is an antagonist at the postsynaptic GABA receptor, enhanced both these functions [9, 13, 41, 42]. Involvement of the GABA\_A subtype receptor in the laying down of memory has also been indicated by the observation that microinjections of the GABA\_A receptor agonist baclofen, impair memory [12].

Two subtypes of GABA receptors have been suggested by classical pharmacological experiments and these have been denoted GABA\_A and GABA\_B. They are characterized by different rank orders of potencies of available agonists for each of these two receptors and selective action of antagonists. GABA binds to both types of receptor. Baclofen is a potent GABA mimetic selective for the GABA\_B receptor; it is inactive at the GABA\_A receptor. Muscimol and isoguvacaine are potent GABA mimetics selective for the GABA\_A subtype. Although the GABA\_A subtype is of primary interest to this review because this is responsible for benzodiazepine actions, GABA\_B receptors will be considered briefly.

**GABA\_A receptors**

GABA\_A binding sites were described first in peripheral tissue preparations in which they were located presynaptically on autonomic nerve terminals [5]. However, further studies indicated a similar presynaptic location in central nervous system neurones, with functions being diminished release of amines, neuropeptides, hormones and excitatory amino acids, as well as GABA via autoreceptors (i.e. GABA\_A receptor binding presynaptically on a GABAergic neurone leads to diminished release of gamma aminobutyric acid from that neurone) [68].

GABA\_A and GABA\_B binding sites may be demonstrated in the central nervous system by autoradiography. The distribution of two receptor subtypes is quite different. GABA\_A receptors are located both presynaptically on nerve terminals and postsynaptically in many brain regions, as well as in the dorsal horn of the spinal cord. In the spinal cord dorsal horn, 50% of GABA\_A binding sites have been reported to be associated with small diameter primary afferent fibres [56].

The functional role of GABA\_A receptors in the central nervous system is still understood poorly. The wide distribution in the nervous system suggests they are likely to have many effects. One therapeutic benefit of GABA\_A agonists is control of spasticity. Baclofen (GABA\_A agonist) given orally or intrathecally, reduces spasticity and prevents flexor spasms in a number of different neurological disorders. This is thought to be a result of interruption of mono- and polysynaptic spinal reflexes [17].

**GABA\_B receptors**

GABA\_B receptors are stimulated by GABA, muscimol and isoguvacaine. Molecular biological studies performed in the past decade indicate that the A subtype of GABA receptor may be a group of different receptors rather than a single entity. Four types of subunits have been described: \( \alpha, \beta, \gamma \) and \( \delta \) [2, 28, 75]. Combinations of these subunits produce macromolecular complexes which include a chloride ion channel. It has been noted that there are many similarities with the nicotinic acetylcholine receptor.
It has been proposed that the GABA$_A$ receptor has a similar structure (i.e. a pentameric form). Combination of GABA and a GABA$_A$ subtype receptor leads to an increase in chloride conductance and subsequent hyperpolarization of the postsynaptic membrane.

Multiple variants exist within each of these classes: six $\alpha$, three $\beta$, two $\gamma$ and one $\delta$ [36, 44, 58, 73]. Analysis of GABA receptors within the retina have yielded two other subunits, $\rho$1 and $\rho$2. Each of the subunits contains an N-terminal segment of approximately 200 amino acids and four hydrophobic membrane-spanning segments. Each of these four segments is connected to the others by short hydrophilic segments of amino acids, except for the third and fourth which have a longer segment that may be phosphorylated by intracellular cyclic AMP-dependent protein kinase (protein kinase A). This opens up the possibility for a large number of interactions with other drugs that may alter intracellular cyclic AMP levels, such as: opioids ($\mu$ and $\delta$); purinceptors; alpha$_2$ adrenoceptors (subtypes A and B); beta adrenoceptors; dopamine D1 and D2; GABA$_B$, 5-HT (subtypes 1A, 1B and 1D); muscarinic; cholinergic (M2); prostanoid DP receptors; and neurophysin and oxytocin receptors (subtype V$_2$) [47].

Co-expression studies using cloned GABA$_A$ subunit variants in mammalian cells have led to combinations of subunit that respond to GABA, and this response may be modulated by benzodiazepines [43, 57]. The presence of a $\gamma$ subunit confers benzodiazepine sensitivity [37]. Less well defined actions at GABA$_A$ receptors have also been described for barbiturates and neurosteroids (e.g. alphaxalone, alphadolone, both constituents of Althesin, and pregnanolone, currently in phase I trials as an i.v. anaesthetic) [11, 21, 33, 55, 59, 74]. Although it is possible to make a number of different combinations of these subunits, apart from the alteration in benzodiazepine specificity and sensitivity (vide infra), the functional significance of this diversity has yet to be elucidated [49].

**BENZODIAZEPINES**

The first clinically useful benzodiazepine, chlor-diazepoxide hydrochloride, was discovered in 1957 after a search for compounds with sedative and anxiolytic properties related to 4,5-benzo-hept-1,2,6-oxdiazines, compounds known since 1891 [66]. These chemicals lend themselves readily to modification by organic chemists so that a large number of related compounds were synthesized. Three basic types of structure exit: basic 1–4 benzodiazepines, such as diazepam and lorazepam (see fig. 2); heterocyclic 1–4 benzodiazepines, such as triazolam (fig. 2); 1–5 benzodiazepines, such as clobazam (fig. 2).

All of these compounds possess similar properties, that is reduction of anxiety, sedation and anti-convulsant properties. However, the 1–5 benzodiazepines have been found to be less sedative. These drugs also have significant muscle relaxant properties, without any effect on coordination. This latter effect may be because of an antianxiety action, but is probably mediated also by potentiation of the effects of GABA on spinal and supraspinal motor reflexes. As the dose of benzodiazepine is increased, anxiolytic effects are produced first followed by anticonvulsant effects and then a reduction in muscle tone, followed by sedation and hypnosis. The similarity between these effects and those of GABA was obvious. It was logical to look to GABA to explain the therapeutic effects of this group of compounds.

**GABA–BENZODIAZEPINE INTERACTION**

Before Davidoff’s suggestion of a role for GABA in mediation of presynaptic inhibition [16], Schmidt, Vogel and Zimmerman [62] showed that diazepam increased the amplitude of the dorsal root potential, and enhanced monosynaptic ventral root responses. Polc and colleagues [53] investigated this further and showed an interaction between diazepam and GABA. Benzodiazepines alone lack GABA mimetic effects. However, they have been shown to enhance the effects of GABA in numerous regions in the central nervous system, for example spinal cord, cerebellum, hippocampus, cuneate nucleus, cerebral cortex, hypothalamus, amygdala and brain stem raphé nuclei [34, 69].

**Benzodiazepine receptors**

Specific high affinity benzodiazepine binding sites were demonstrated in the central nervous system in 1977 [7, 46]. These specific binding sites are located on neurones within the central nervous system, but also on non-neuronal tissues, perhaps glia. The function of these is unknown [20]. Binding studies with agonists and antagonists promoted the concept of these recognition sites as functional receptors. Drugs acting stereospecifically with this receptor may be classified as selective agonists (e.g. flunitrazepam) that potentiate GABA effects, inverse agonists, such as DMCM or Ro194603 that produce exactly opposite effects (proconvulsant) and an-
A strong correlation between binding and pharmacological activity has been reported. Haefely suggested that a GABA₆ receptor, benzodiazepine receptor and chloride ionophore formed one macromolecular complex in order to explain experimental observations that benzodiazepines were not GABA mimetic per se, but potentiated the effects of binding with GABA₆ receptors in operating a chloride channel [24, 29, 63]. Not all GABA receptors are coupled to benzodiazepine receptors; this macromolecular complex forms a subset of GABA₆ receptors. This subspecialization of GABA₆ receptors with respect to the benzodiazepines goes further. The first indications of this came from the pharmacological profile of agonists at the benzodiazepine receptor.

Two subtypes of benzodiazepine receptor were suggested originally. The benzodiazepine, subtype (high affinity for triazolopyridazines) is found throughout the brain with large concentrations in the cerebellum. The benzodiazepine₁ subtype was found principally in the cerebral cortex, spinal cord and hippocampus. These distributions were derived from the differential binding of the triazolopyridazine CL 218772, leading to displacement of [³H]benzodiazepine binding, particularly in the hippocampus. Beta carbolines tended to react with benzodiazepine receptors in the cerebellum [54]. In addition, CL 218772 appeared to possess anxiolytic effects with only mild sedation. Therefore, anxiolysis was ascribed to the benzodiazepine, site [39].

Finally, multiple subunits associated with the receptor labelled with [³H]flunitrazepam were demonstrated [64]. These four proteins had different molecular masses of 51, 53, 55 and 59 kDa. The smaller molecular mass protein had a greater affinity for CL 218872 and this had a different region distribution to the others, a distribution which coincided with the benzodiazepine. Cloning experiments have subsequently elucidated further on this heterogeneity of benzodiazepine-GABA₆ receptor complexes.

Molecular biology experiments

Experimental studies using specific antibodies raised against subunit variants of GABA₆ receptors have shown that the 51 kDa protein, labelled with radiolabelled benzodiazepine, is the α₁ subunit: the 53 kDa protein is the α₂ subunit and the 59 kDa protein corresponds to the α₃ subunit [10, 65]. Pritchett, Luddens and Seeburg [57] analysed the pharmacological characteristics of GABA₆ receptors produced by recombining subunits, each moiety containing a single α subunit isoform. They combined different α subunits with β₁ and γ₂ in isolated human kidney cells. They then evaluated the binding characteristics of the subsequently formed GABA₆ receptors. Receptors containing the α₁ subunit behaved like benzodiazepine receptors, that is they had high affinity for CL 218872 and the beta carbolines. Those GABA₆ receptors which contained α₂ or α₃ subunits behaved like benzodiazepine receptors.

Further experiments with other α subunits combined with β₂ and γ₂ along similar lines revealed a multiplicity of GABA₆ receptors and benzodiazepine affinities. These are summarized in figure 3. The largest circle contains α subunits that produce GABA₆ receptors which bind muscimol. Polygon 2 contains all those that confer high affinity for diazepam while the circle labelled 3 contains those that have a low affinity for diazepam. The α₁ subunit (circle labelled 4) confers benzodiazepine, pharmacology (i.e. high affinity for CL 218872 and zolpidem). Ellipse 5 contains receptors with benzodiazepine₁ pharmacology. Those receptors containing the α₅ subunit also behaved like benzodiazepine receptors, but were different from the benzodiazepine₁ receptors containing α₂ and α₃ subunits because these recombinant receptors had a different rank order of affinity for zolpidem and CL 218872 [53]. The α₆ isoform of the GABA₆ receptor has an extremely small binding affinity for benzodiazepines. It is found in the cerebellar granule cell layer [40]. This subtype may be involved in the pharmacological effects of ethanol.

Although this new molecular biological evidence has brought together the pharmacology of GABA and the benzodiazepines, the obvious question to ask concerns the functional significance in vivo of these in vitro studies. The messenger RNA for the six different α subunits are known and have been mapped within the mammalian central nervous system. It has been shown that the subunits have different topographical distributions [38, 71, 72]. The differences in these distributions may indicate different types of GABA receptor associated with different functions. As yet, this remains to be realized in the development of a drug specially selected for anxiety, lacking sedative and muscle relaxant effects.

Recent pharmacological experiments with the GABA₆ antagonist bicuculline indicated separate

IT IS STILL UNCERTAIN IF THESE ENDOZEPINES ARE NATURALLY OCCURRING NEUROTRANSMITTERS. THESE SUBSTANCES HAVE BEEN ISOLATED ALSO FROM COMMON FOODSTUFFS [67, 70] THUS THROWING FURTHER DOUBT ON THEIR NEUROTRANSMITTER ROLE. HOWEVER, AN IN VITRO MECHANISM FOR THE FORMATION OF 1-4 BENZODIAZEPINES HAS BEEN DESCRIBED [8].

CONCLUSION

BENZODIAZEPINES PRODUCE THEIR SEDATIVE, HYPNOTIC, ANXIOLYTIC, ANTICONVULSANT AND ANTIINOCICEPTIVE EFFECTS BY INTERACTIONS WITH GABA. THE BENZODIAZEPINES MODULATE THE EFFECTS OF GABA AT THE SUBSET OF GABA_A RECEPTORS. THESE ARE LIGAND-GATED CHLORIDE ION CHANNELS, PROBABLY CONSISTING OF FIVE SUBUNITS. THE SUBUNIT COMPOSITION VARIES IN DIFFERENT PARTS OF THE BRAIN, ALTHOUGH THE VARIATIONS IN SUBUNIT STRUCTURE HAVE NOT BEEN LINKED WITH DIFFERENT FUNCTIONS. THE TYPE OF SUBUNIT DETERMINES THE BENZODIAZEPINE PHARMACOLOGY—ANXIOLYTIC OR SEDATIVE. EACH SUBUNIT CONSISTS OF A CHAIN OF AMINO ACIDS WITH FOUR HYDROPHOBIC SEGMENTS THAT SPAN THE LIPID CELL MEMBRANE. THE INTRACELLULAR CHAINS JOINING THESE MEMBRANE-SPANNING SEGMENTS MAY BE PHOSPHORYLATED BY INTRACELLULAR PHOSPHOLIPASES. THIS MAY LEAD TO MODULATION OF THE EFFECT OF BOTH BENZODIAZEPINES AND GABA BINDING ON THE CELL SURFACE PART OF THE RECEPTOR. MANY INTERACTIONS OF THE BENZODIAZEPINE GABA COMPLEX WITH OTHER DRUGS AND NEUROTRANSMITTERS ALTERING INTRACELLULAR CYCLIC AMP CONCENTRATIONS ARE POSSIBLE. AMONG THESE ARE THE OPIOIDS, THAT ARE WELL KNOWN BY ANAESTHETISTS TO POTENTIATE THE SEDATIVE EFFECTS OF THE BENZODIAZEPINES. THERE ARE MANY POSSIBILITIES FOR DIFFERENT SUBUNIT STRUCTURES OF THE GABA_A RECEPTORS WHICH RESPOND TO BENZODIAZEPINES. THE EXISTENCE AND FUNCTION OF ENDOGENOUS BENZODIAZEPINES IS CONTROVERSIAL BUT IT
may be that some specialization of GABA- 
benzodiazepine receptors provides more specific targets for these endogenous compounds.

REFERENCES


56. Price GW, Kelly JS, Bowery NG. The location of GABAb receptor binding sites in mammalian spinal cord. Synapse 1987; 1: 530-538.


70. Wildmann J, Mohler H, Vetter W, Ranalder U, Schmidt K, Maurer R. Diazepam and N-desmethyldiazepam are found in the rat brain and adrenal and may be of plant origin. Journal of Neural Transmission 1987; 70: 383-398.


