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GABA RECEPTOR MULTIPLICITY

VISUALIZATION OF DIFFERENT RECEPTOR TYPES IN THE MAMMALIAN CNS

N. G. BOWERY, G. W. PRICE, A. L. HUDSON, D. R. HILL, G. P. WILKIN and M. J. TURNBULL
Department of Pharmacology, St Thomas's Hospital Medical School, London SE1, Department of Biochemistry, Imperial College, London SW7 and Bioscience II Department, ICI Pharmaceuticals Division, Macclesfield, Cheshire, England

Summary—Receptors for GABA in the mammalian brain are not homogeneous. A clear separation exists between receptors which recognize the antagonist bicuculline and a population which does not. These two classes have been designated $GABA_A$ and $GABA_B$ sites respectively. Within the $GABA_A$ category there may also be many subtypes which exhibit subtle pharmacological differences. Numerous centrally-active agents influence $GABA_A$ site function generally via an allosteric interaction. By comparison very few substances currently available interact with $GABA_B$ sites. The $GABA_A$ and $GABA_B$ sites show many contrasting characteristics not least of which is their distribution pattern within the rat brain. Autoradiographic analysis has indicated that although both receptors may be present within many regions, in some areas only one type is present. For example, $GABA_A$ sites only are present in the lamina molecularis of the olfactory bulb and granule cell layer of the cerebellum whereas $GABA_B$ sites are present in the interpeduncular nucleus without any evidence of $GABA_A$ sites. In the spinal cord $GABA_B$ sites are unevenly distributed with high densities in laminae I–IV. $GABA_A$ sites are more uniformly distributed throughout the dorsal and ventral horns. In conclusion, there is now good evidence for multiple GABA receptors and the way is open to determine the functional significance of the $GABA_B$ receptor in relation to the now classical $GABA_A$ site.

Key words: $GABA_A$, $GABA_B$, autoradiography, baclofen, [^{35}S]-*t*-butyl-bicyclophosphorothionate, GABA receptor localization.

There are undoubtedly many drugs of therapeutic importance which can directly influence GABA-mediated neurotransmission. These include the barbiturates, benzodiazepines, valproate and baclofen, all of which enhance GABA function. In addition, numerous convulsant compounds such as picrotoxin, bicuculline, pentylenetetrazole, penicillin and the bicyclophosphates are all capable of decreasing GABA-induced inhibition within the CNS. In most of these examples the site of interaction appears to be beyond the receptor or via an allosteric site. For example, benzodiazepines do not mimic the effect of GABA in its absence (Costa, Guidotti, Mao and Suria, 1975) and they do not compete with [^{3}H]-GABA for its receptor binding site on neuronal membranes (Olsen, Ticku, Van Ness and Greenlee, 1978b). Valproate is similarly inactive at GABA binding sites (Whittle and Turner, 1982) and is only weakly active in enhancing the neuronal response to muscimol or GABA (Harrison and Simmonds, 1982). In very few instances do drugs appear to act as ligands for the GABA recognition site. Nevertheless, the possibility that their therapeutic activity stems from an interference with GABA mechanisms has prompted numerous studies in this area.

To define the manner by which any drug influences a receptor mechanism it is essential to initially characterize the receptor. In particular, defining the pharmacological profile for activation is crucial. It is this more than any other characteristic which has lead to the emergence of multiple receptors for the majority of neurotransmitters. Even though GABA receptor subtypes have been described on the basis of many characteristics (e.g. Brennan and Cantrill, 1979; Karobath, Placheta, Lippitsch and Krogsgaard-Larsen, 1979; Nistri and Constanti, 1979; Allan, Evans and Johnston, 1980; Costa, 1981; Johnston, 1981; Alger and Nicoll, 1982), perhaps the clearest subdivision to emerge has derived from structure–activity studies performed in a variety of systems (see Bowery, 1982). The separate sites arising from this division have been designated $GABA_A$ and $GABA_B$ sites (Hill and Bowery, 1981). The $GABA_A$ sites represent all "classical" bicuculline-sensitive sites whereas $GABA_B$ sites are not affected by bicuculline and do not recognize many of the accepted GABA-mimetics such as isoguvacine, 3-aminopropanesulphonic acid and THIP (see Table 2). The most compelling reasons for believing this receptor separation to be clearcut are: firstly, the reproducibility with which $GABA_B$ sites occur in a variety of tissues of peripheral origin (e.g. Bowery, Doble, Hill, Hudson, Shaw, Turnbull and Warrington, 1981; Muhyaddin, Roberts and Woodruff, 1982a; Giotti, Luzzi, Spagnesi and Zilletti,

1983; Ong and Kerr, 1983). Secondly, the evidence derived from both neurochemical and electrophysiological studies which show the existence of $GABA_B$ sites (Bowery, Hill, Hudson, Doble, Middlemiss, Shaw and Turnbull, 1980; Ault and Nadler, 1982; Cain and Simmonds, 1982; Henry, 1982; Bowery, Hill and Hudson, 1983a). [In all other cases, the subtypes of GABA receptor so far described have only been observed in a single system, e.g. GABA autoreceptors, Brennan and Cantrill (1979) and $GABA_1$ and $GABA_2$ sites, Guidotti, Gale, Suria and Toffano (1979).] Finally, the separate locations of $GABA_A$ and $GABA_B$ sites within the CNS (see later). It seems unlikely that a single interconvertible receptor would show the same separation in distribution on repeated examination. A summary of the major distinctions between $GABA_A$ and $GABA_B$ sites is given in Table 1 although receptor distribution has been omitted. Undoubtedly, subtypes for $GABA_A$ sites exist which include autoreceptors, pre- and postsynaptic receptors, and synaptic and extrasynaptic sites. In each case, subtle differences separate them from other $GABA_A$ sites. By contrast, $GABA_B$ sites so far appear to be homogeneous.

THE CHARACTERISTICS OF THE $GABA_A$ SITE

Numerous diagrams representing bicuculline-sensitive GABA receptors have already been presented by a variety of authors (e.g. Olsen, 1981; Braestrup and Nielsen, 1982). The representation shown in Fig. 1 has been designed so as to contrast with the $GABA_B$ site illustrated to the right of the $GABA_A$ site. Classical $GABA_A$ receptors are linked to chloride ion channels such that activation of the receptor increases the conductance of this anion across the neuronal membrane. The direction of the ion flow will depend on its electrochemical gradient. In the majority of cases an inward movement occurs (Dreifuss, Kelly and Krnjevic, 1969) but an outward movement is believed to occur, for example, in sympathetic and dorsal root ganglia (Adams and Brown, 1975; Deschenes, Feltz and Lamour, 1976) and at primary afferent terminals. The resulting depolarization in this latter case is associated with the phenomenon of presynaptic inhibition within the spinal cord (see Curtis, 1978 and also Ryall, 1978). This control of chloride channels has been represented in Fig. 1 by a revolving tap mechanism. GABA activation rotates the 'tap' into the open position. Similarly, GABA mimetics produce the same rotation whereas the receptor antagonist bicuculline, which competes for the GABA recognition site (Simmonds, 1980), would oppose this rotation. The effect of drugs which are known to affect this channel mechanism but which do not act at the GABA recognition site is represented by adjacent "wheels". These "wheels" may be considered as allosteric sites which, on activation or "rotation", alter the GABA/chloride channel complex. The cog-toothed wheel represents the picrotoxin binding site (see Olsen, 1981). When picrotoxin binds to this site,

Table 1. The contrasting properties of $GABA_A$ and $GABA_B$ receptor systems

	$GABA_A$	$GABA_B$	Reference
Ligands for recognition site			
Muscimol	potent agonist	weak agonist	1
Piperidine 4-sulphonic acid	agonist	IA	2
Isoguvacine	agonist	IA	1
(−)-Baclofen	IA	agonist	1, 2, 3
Bicuculline	antagonist	IA	1
δ-Aminovaleric acid	agonist	antagonist	4, 5, 6
Factors influencing binding to receptor			
Ca^{2+}/Mg^{2+}	independent	dependent	2, 3
GTP	no effect	decreases binding affinity	7, 8
Triton X-100	increases high affinity binding capacity	decreases binding capacity	2, 9
Benzodiazepines	enhance binding	no effect	5, 10, 11, 12
Barbiturates	enhance binding	no effect	13, 14, 15, 16
Post-receptor mechanism			
Cl^- channels	increase in conductance	no apparent link	1, 17
Ca^{2+} channels	no apparent link	decrease in conductance	18, 19, 20

IA = inactive.

1. Bowery, Doble, Hill, Hudson, Shaw, Turnbull and Warrington (1981). 2. Bowery, Hill and Hudson (1983a). 3. Hill and Bowery (1981). 4. Bowery and Brown (1974). 5. Muhyaddin, Roberts and Woodruff (1982a). 6. Muhyaddin, Roberts and Woodruff (1982b). 7. Bowery, Hill and Hudson (1982). 8. Hill, Bowery and Hudson (1983). 9. Enna and Snyder (1977). 10. Skerritt, Willow and Johnston (1982). 11. Doble and Turnbull (1981). 12. Bowery, Hill, Hudson, Price, Turnbull and Wilkin (1983b). 13. Willow and Johnston (1980). 14. Asano and Ogasawara (1982). 15. Whittle and Turner (1982). 16. Hill (1982). 17. Dreifuss, Kelly and Krnjevic (1969). 18. Dunlap (1981). 19. Desarmenien, Feltz, Loeffler, Occhipinti and Santangelo (1982). 20. McBurney (1983).

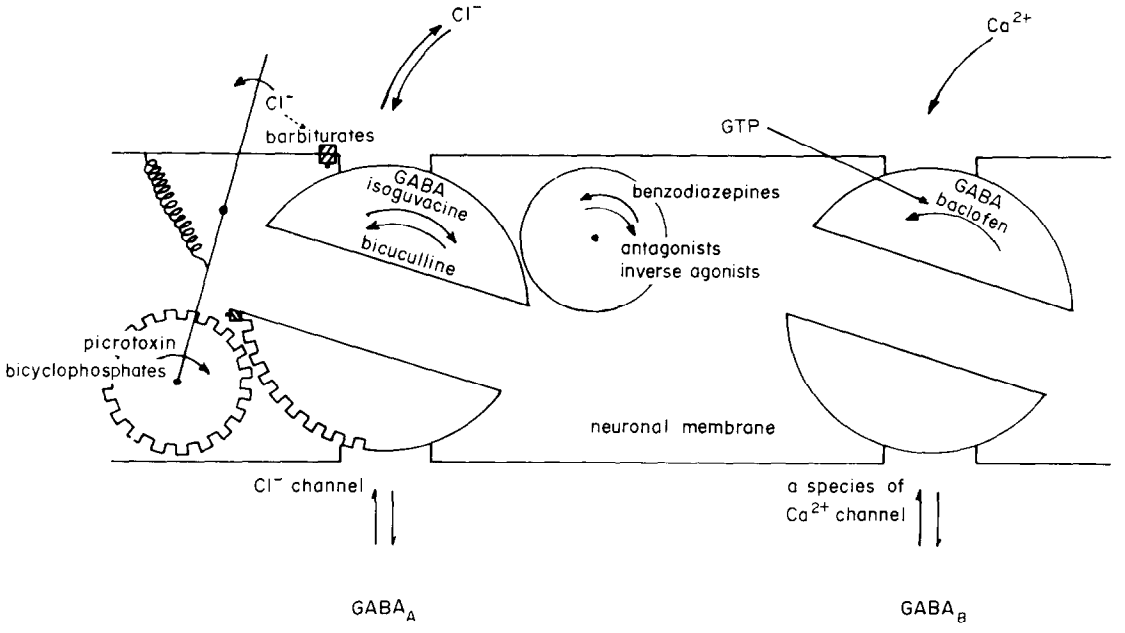

Fig. 1. Diagrammatic representation of $GABA_A$ and $GABA_B$ receptors sites and their associated mechanisms in a neuronal membrane. See text for explanation.

the rotation produced prevents the GABA-induced increase in Cl^- conductance. The cogs represent the obligatory and non-competitive nature of this effect. The influence of picrotoxin on GABA systems may be dependent on the external Cl^- concentration (see Supavilai, Mannonen, Collins and Karobath, 1982) and this action is represented in Fig. 1 by the pivoted arm. Movement of this mechanism engages the cogged teeth and Cl effects this engagement.

It is well recognized that barbiturates enhance GABA-mediated inhibition (e.g. Nicoll, Eccles, Oshima and Rubia, 1975) and this may be reflected in membrane preparations by an enhancement of GABA binding (Willow and Johnston, 1980). This binding phenomenon appears to be Cl^- dependent (Olsen and Snowman, 1982; see also Johnston, 1983). Barbiturates also compete with [^{3}H]dihydropicrotoxinin at the picrotoxin site (Ticku and Olsen, 1978). The picrotoxin "wheel" may therefore represent a barbiturate binding site. Electrophysiological studies on cultured neurones (Huang and Barker, 1980; Barker, Gratz, Owen and Study, 1983) indicate that the hypnotic barbiturates increase the lifetime of GABA activated Cl^- channels. This could be represented in the diagram as an anti-clockwise rotation of the picrotoxin "wheel" which prevents the reclosure of the channel. However, this does not account for any interaction of barbiturates with the GABA recognition site. Therefore, the influence of barbiturates has been represented by a "latch" mechanism which on activation keeps the channel in the open position.

Another group of compounds which can influence the binding of picrotoxin to synaptic membranes is the bicyclophosphates (Ticku and Olsen, 1979). These cage convulsants decrease GABA-mediated responses (Bowery, Collins and Hill, 1976a; Bowery, Collins, Hill and Pearson, 1976b, 1977) but, like picrotoxin, do not appear to have a significant effect at the GABA recognition site (Ticku and Olsen, 1979). Structure–activity studies within a series of these convulsants have demonstrated a positive correlation between: (i) their GABA antagonistic activity; (ii) their convulsant potency; and (iii) their ability to displace [^{3}H]dihydropicrotoxinin from its binding sites (Bowery *et al.*, 1976b, 1977; Ticku and Olsen, 1979). Recent studies within this area have been facilitated by the introduction of a radiolabelled bicyclophosphate, [^{35}S]-*t*-butyl-bicyclophosphorothionate ([^{35}S]-TBPS). Binding studies performed on synaptic membranes from brain which have been dialysed against EDTA and water, indicate that this ligand binds in a saturable manner with high affinity (Squires, Casida, Richardson and Saederup, 1983). Picrotoxin and picrotoxinin are potent inhibitors of [^{35}S]-TBPS binding whereas picrotin, the weakly active component of picrotoxin, is approx. 60 times less potent (Squires *et al.*, 1983). Squires *et al.* (1983) suggest, quite reasonably, that [^{35}S]-TBPS is a ligand for the picrotoxin site even though its binding properties differ in some respects from those exhibited by [^{3}H]dihydropicrotoxinin. In particular, GABA-mimetics such as muscimol, isoguvacine, 3-aminopropanesulphonic acid, THIP and even GABA itself can displace [^{35}S]-TBPS from its binding site (Squires *et al.*, 1983). This contrasts with the lack of effect of GABA and muscimol on the binding of [^{3}H]picrotoxinin (Olsen, Greenlee, Van Ness and Ticku, 1978a). Squires *et al.* (1983) propose that

"GABA(and presumably the analogues) exerts its inhibitory effect (on [^{35}S]-TBPS binding) by acting on a receptor different from the [^{35}S]-TBPS binding site". This would be a $GABA_A$ site allosterically influencing the binding of [^{35}S]-TBPS.

In an attempt to make further analysis of the binding characteristics of [^{35}S]-TBPS, its binding to brain sections has recently been studied. Rat cerebellar sections were chosen since the distribution and characteristics of [^{3}H]-GABA and [^{3}H]muscimol binding sites in this region have previously been studied (Palacios, Scott, Young and Kuhar, 1980; Wilkin, Hudson, Hill and Bowery, 1981). Cryostat sections (10 μm thick, 6 sections per microscope slide) were prepared from cerebella, which had previously been perfused-fixed *in vivo* with 200 ml 0.1% *para*-formaldehyde. The sections were stored at −20 °C for up to 2 weeks. For the binding experiments, the sections were thawed and immersed in Krebs–Henseleit solution for 60 min at 20 °C. After drying, the sections were covered with 100 μl Krebs–Henseleit solution containing [^{35}S]-TBPS (40 nM) for 20 min at 20 °C. Background (non-specific) labelling of the sections was routinely determined with unlabelled *t*-butylbicyclophosphate (100 μM) or isopropylbicyclophosphate (100 μM). Picrotoxin could also have been used since it displaced the radiolabelled ligand to the same extent as the bicyclophosphates. After incubation with [^{35}S]-TBPS the solution was aspirated off and the sections rinsed twice for 3 sec in fresh Krebs–Henseleit solution. Each dried slide was then placed in contact with LKB Ultrofilm or in close apposition to a dry coverslip previously coated with Ilford K5 emulsion (emulsion–water 1:1.5) (Palacios *et al.*, 1980; Wilkin *et al.*, 1981). Alternatively the slides were placed in scintillation vials together with 0.4 ml water before the addition of scintillation fluid (see below). The Ultrofilms were developed (Tecknol 1:10 in water) after 22–26 days contact with the sections whereas the coverslip emulsions were developed (Kodak D19) after 8–13 days. An example of the results obtained on Ultrofilm is shown in Fig. 2. Deposition of radioactivity is indicated by the blackness of the image. The granule cell layer shows the greatest concentration of binding sites with much less in the molecular layer. This correlates well with the distribution of $GABA_A$ sites within the cerebellum (Olsen and Mikoshiba, 1978; Palacios *et al.*, 1980; Wilkin *et al.*, 1981). It also supports the recent report by Gee, Wamsley and Yamamura (1983) who found a predominance of grains in the granule cell layer with greater amounts towards the white matter than in the areas close to the Purkinje cell layer. The present results showed the same uneven distribution and were identical irrespective of whether Ultrofilm or emulsion-coated coverslips were used.

In conjunction with these distribution studies, the radiolabelled deposition was also measured by liquid scintillation spectrometry. Radioactivity was eluted from the sections into scintillation fluid and the levels determined in a Rackbeta (LKB) counter with windows set for ^{35}S. This enabled the structure–activity profile for the binding site to be compared with that already described on synaptic membranes (Squires *et al.*, 1983). Saturation studies with [^{35}S]-TBPS or unlabelled TBPS were not performed since we had insufficient quantities of the labelled material and none of the unlabelled material. Nevertheless, displacement studies with unlabelled analogues were performed. The maximal displacement produced by the bicyclophosphates, *t*-butylbicyclophosphate and isopropylbicyclophosphate, and picrotoxin was identical in all experiments. This produced a specific/non-specific ratio of between 40 and 52% which despite being less than that observed in synaptic membranes (approx. 80%), was adequate for the displacement analysis and, moreover, was obtained in a physiological solution. By contrast, the studies performed on synaptic membranes utilized large concentrations of KBr to optimize the binding conditions (Squires *et al.*, 1983). The IC_{50} values for substances displacing [^{35}S]-TBPS from slices and membranes are given in Table 2. The membrane displacement values are derived from Squires *et al.* (1983). Results obtained in membrane binding studies with [^{3}H]dihydropicrotoxinin have also been included for comparison. The results from the two studies with [^{35}S]-TBPS are clearly dissimilar. All of the compounds were found to be active in the membrane assay whereas GABA, isoguvacine and 3-aminopropanesulphonic acid were inactive in slices. The isoxazoles, muscimol and THIP, however, were active displacers in slices as well as in the membrane assay. The reasons for these discrepancies remain to be elucidated but they may derive from the differences in incubation conditions or brain region. Even so, it is apparent that the characteristics of the [^{35}S]-TBPS

Table 2. Inhibition of [^{35}S]-*t*-butylbicyclophosphorothionate ([^{35}S]-TBPS) and [^{3}H]dihydropicrotoxinin ([^{3}H]-DHP) binding by GABA-related substances in rat cerebellar slices and brain synaptic membranes (IC_{50} values μM)

	[^{35}S]-TBPS		[^{3}H]-DHP
Displacer	Cerebellar slices (40 nM)	Forebrain membranes (2 nM)|	Cerebral cortex membranes (63 nM)
TBPO	0.05	0.073	2.0‡
IPPO	0.30	0.32	8.0‡
Picrotoxin	0.80	0.18	0.4†§
GABA	IA	0.34	IA§
Muscimol	0.13	0.059	IA§
3-Aminopropane sulphonic acid	IA	0.24	
THIP	5.1	5.0	
Isoguvacine	IA	0.93	
Bicuculline	IA*	IA	IA§
(±)-Pentobarbitone	IA	~80.0	50.0‡

TBPO = *t*-butylbicyclophosphate; IPPO = isopropylbicyclophosphate; THIP = 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol. IA = >100 or >1000 pentobarbitone.

* = Bicuculline methobromide. †Picrotoxinin. ‡ = Leeb-Lundberg and Olsen (1980). § = Olsen, Greenlee, Van Ness and Ticku (1978a). | = Squires *et al.* (1983).

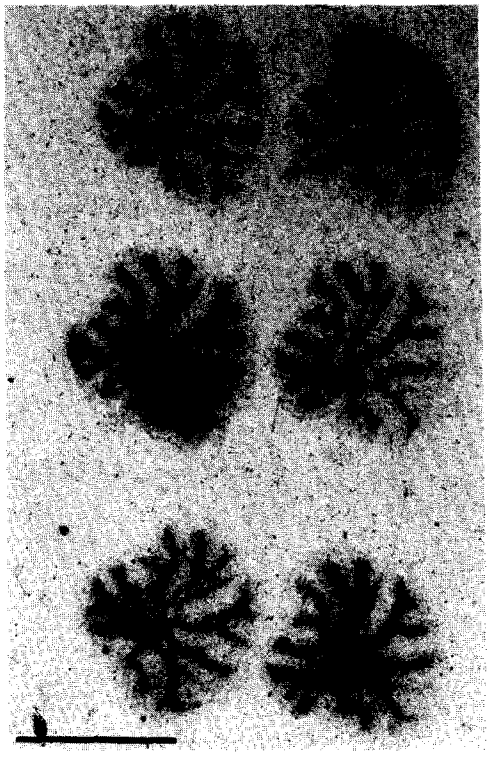

Fig. 2. Autoradiographic analysis of [^{35}S]-*t*-butylbicyclophosphorothionate ([^{35}S]-TBPS) in rat parasagittal cerebellar sections. Six sections of cerebellum (10 μm) from the same rat were prepared as described in the text. Each section was labelled with [^{35}S]-TBPS by incubating in Krebs–Henseleit solution (containing 40 nM [^{35}S]-TBPS) at 20°C for 20 min followed by 2 × 3 sec rinses in fresh solution. Background (non-specific) binding was determined in sections from the same cerebellum by incubation in [^{35}S]-TBPS solution containing excess (100 μM) unlabelled isopropylbicyclophosphate. Dried sections were placed in contact with LKB Ultrofilm for 23 days before developing in Tecknol (May & Baker) solution (diluted 1:10 in water) for 2 min at 21°C. The development was stopped with 3% acetic acid and the film fixed in super Amfix solution for 5 min. The final print was made on Kodak Transtar paper TPP5. Scale bar = 5 mm.

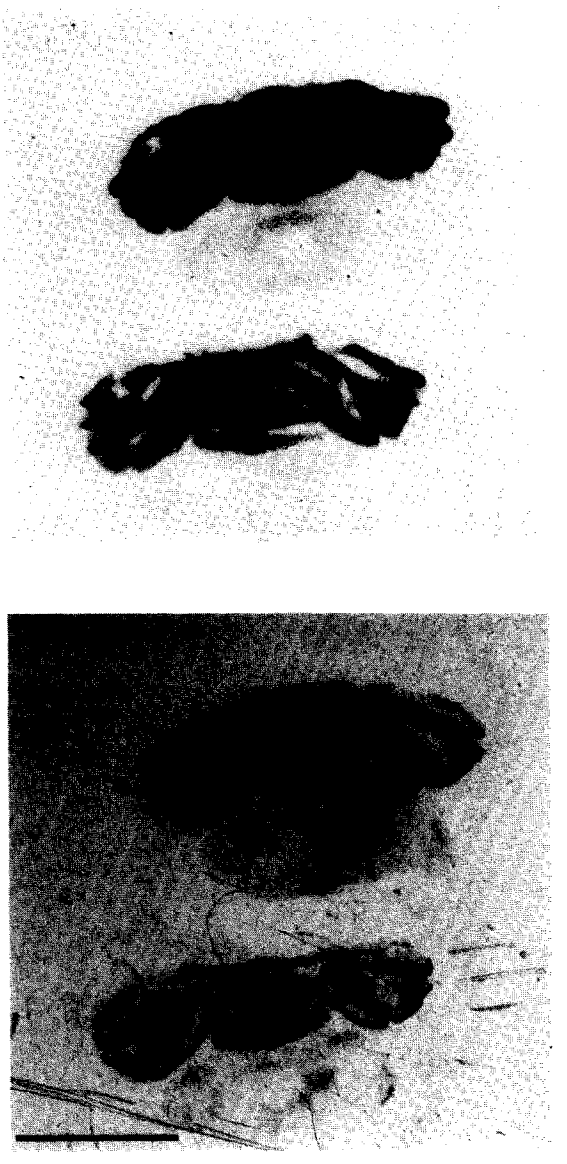

Fig. 4. Autoradiograms of [^{3}H]-GABA at $GABA_A$ and $GABA_B$ binding sites in transverse sections of a rat cerebellum and brain stem. Cryostat sections (10 μm) were prepared from tissue obtained from a rat, anaesthetized with pentobarbitone, followed by intracardiac perfusion with 0.1% *para*formaldehyde in phosphate-buffered saline (pH 7.4). $GABA_A$ sites (upper two pictures) were detected by incubation in Tris–HCl buffer solution (50 mM pH 7.4) containing 190 mM sucrose and 50 nM [^{3}H]-GABA. (±)-Baclofen (10 μM) was present to suppress any binding to $GABA_B$ sites. $GABA_B$ sites (lower two pictures) were detected by incubation in Tris–HCl–sucrose solution containing 2.5 mM $CaCl_2$ and isoguvacine (40 μM) to suppress all binding to $GABA_A$ sites. The concentration of [^{3}H]-GABA (50 nM) was the same as for $GABA_A$ sites. All sections were incubated for 15 min and then briefly rinsed twice. Dried sections were then placed in contact with LKB Ultrofilm as described in the legend to Fig. 2. Note (i) the heavy labelling over the granule cell layer in the upper pictures ($GABA_A$) and over the molecular layer in the lower pictures ($GABA_B$), (ii) the sparse labelling of the brain stem. Scale bar = 5 mm.

binding site do not readily conform with those described for the picrotoxin binding site.

Returning to the model (Fig. 1) for $GABA_A$ sites. Benzodiazepines are another group of sedatives capable of enhancing the binding of [^{3}H]-GABA to the $GABA_A$ recognition site (Skerritt, Willow and Johnston, 1982). The electrophysiological manifestation of this allosteric interaction appears to be an increase in the frequency with which the GABA-operated chloride channels open (Study and Barker, 1981). In the model this increase in frequency is represented by an increase in the rate at which the "channel wheel" rotates and this fast rotation is produced by the adjacent "wheel" on which specific benzodiazepine receptors exist. This receptor action can be prevented by the benzodiazepine antagonist R015-1788 (ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate) (Hunkeler, Mohler, Pieri, Polc, Bonetti, Cumin, Schaffner and Haefely, 1981). However, this blockade, unlike that produced at the picrotoxin site, does not prevent the GABA-induced increase in Cl^- conductance. The representative wheel has therefore been drawn with a smooth surface to indicate that the Cl^- channel can still open even though the benzodiazepine receptors are blocked. Inverse agonists of the benzodiazepine receptor such as the β-carboline esters (Polc, Bonetti, Schaffner and Haefely, 1982) exert the opposite effect to the normal benzodiazepine agonists on GABA mechanisms but their action can still be antagonized by R015-1788 (Haefely, 1983).

Undoubtedly, the diagram in Fig. 1 is an oversimplification of the $GABA_A$ receptor/ionophore complex and does not account for all observations which have been made at this site. For example, the ability of barbiturates and GABA-mimetics to enhance the binding of benzodiazepines (Tallman, Thomas and Gallager, 1978) and the antagonistic action of picrotoxin on this enhancement (Leeb-Lundberg, Snowman and Olsen, 1980). Nevertheless, it does provide a simplistic representation of the ways in which drugs may alter $GABA_A$ receptor function.

$GABA_B$ RECEPTORS

The $GABA_B$ sites have been defined (Hill and Bowery, 1981; Bowery, Hill and Hudson, 1983a) as bicuculline-insensitive GABA receptors which are:

1. Not activated by isoguvacine, 3-aminopropanesulphonic acid, piperidine-4-sulphonic acid or THIP as well as many other recognized GABA-mimetics.
2. Activated in a stereospecific manner by (−)-baclofen (β-*p*-chlorophenyl GABA).
3. Not influenced by barbiturates or benzodiazepines (see Bowery *et al.*, 1983b).
4. Sensitive to the presence of guanosine triphosphate (GTP) (Bowery, Hill and Hudson, 1982; Hill, Bowery and Hudson, 1983).
5. Not associated with chloride ionophores, and
6. Dependent on physiological concentrations of divalent cations Ca^{2+} or Mg^{2+}.

These criteria are all in direct contrast to the characteristics of $GABA_A$ receptor binding sites (Table 1) and therefore make the present diagram representing $GABA_B$ sites much simpler (Fig. 1). The $GABA_B$ site has been represented, like the $GABA_A$ site, as a rotating "tap" which acts as a channel gating mechanism. Evidence indicates that the $GABA_B$ site is unlikely to be associated with a Cl^- channel mechanism but possibly to a species of Ca^{2+} channel (Dunlap, 1981; Desarmenien, Feltz, Loeffler, Occhipinti and Santangelo, 1982; McBurney, 1983). Activation of the receptor appears to decrease the inward flux of Ca^{2+} and it is this decrease which may give rise to the reduction in evoked transmitter release frequently described as a $GABA_B$ effect on presynaptic terminals (Bowery and Hudson, 1979; Bowery *et al.*, 1980; Bowery *et al.*, 1981; Kaplita, Waters and Triggle, 1982; Henry, 1982; Hughes, Morgan and Stone, 1982; Muhyaddin *et al.*, 1982a,b; Fillenz and Fung, 1983; Giotti *et al.*, 1983; Kilpatrick, Muhyaddin, Roberts and Woodruff, 1983; Ong and Kerr, 1983). Whether Ca^{2+} channels are associated with $GABA_B$ sites which are on postsynaptic elements such as Purkinje cell dendrites in the cerebellum (Bowery, Price, Turnbull and Wilkin, 1983c) remains to be seen.

A pharmacological model which is often used as a determinant of the responsiveness of primary afferent terminals within the spinal cord is the dorsal root ganglion *in vivo* or *in vitro* (e.g. Feltz and Rasminsky, 1974). It is thought that the cell bodies may reflect the sensitivity of the axon terminals. The $GABA_B$ sites are present on the ganglion cell bodies of C and Aδ fibres and, when activated, decrease the duration of action potentials generated by the injection of current into the cells (Dunlap, 1981; Desarmenien *et al.*, 1982; McBurney, 1983). This effect is illustrated in Fig. 3. The record is taken from McBurney (1983) and shows the dual effect of GABA at $GABA_A$ and $GABA_B$ receptors on a cultured dorsal root ganglion neurone of the rat. Initially GABA (100 μM) depolarized the neurones by activation of $GABA_A$ sites. A prolongation of the action potential (at b) and a decrease in amplitude, due to membrane shunting with the increase in Cl^- conductance (at c), were associated with this. Subsequently, (at d) during the recovery phase of the depolarization as the GABA was washed away, the action potential amplitude was restored, but the duration of the action potential was diminished even though some depolarization was still evident. This depolarization would tend to increase rather than decrease the duration (as at b). Only this latter, slower onset, effect of GABA can be mimicked by (−)-baclofen and muscimol produces no changes in the duration (Dunlap, 1981). Thus, activation of $GABA_B$ sites can be attributed to this effect but, as McBurney (1983) points out, more information is

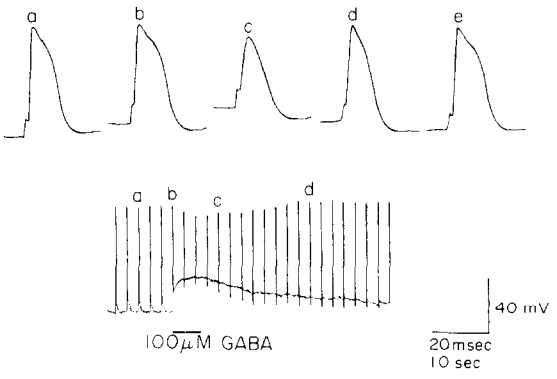

Fig. 3. Effects of GABA on a cultured dorsal root ganglion neurone of a rat. Intracellular recordings were made via one potassium acetate (150 mM) filled microelectrode whilst brief depolarizing current injections were made through a second electrode. The lower trace shows a GABA-induced depolarization (GABA 100 μM present in the bathing medium) upon which action potentials elicited by the current injections are superimposed. The upper trace shows individual action potentials captured at a faster sweep speed (20 msec calibration) at the points indicated by the letters a–e. Tetraethylammonium (10 mM) was present in the bathing medium throughout. At b the action potential is prolonged, at c the action potential amplitude is diminished and at d the action potential is shortened even though the neuron is depolarized to the same extent as at b. (Taken from McBurney 1983 with permission.)

needed about the membrane properites of the primary afferent terminals before the results obtained with GABA at the cell body can be extended to its terminals. Nevertheless, there is a high density of $GABA_B$ sites localized within the dorsal horn of the spinal cord, which coincides with the afferent terminal distribution (see later). In the diagram (Fig. 1), activation of $GABA_B$ sites, has therefore been represented as a channel closing event to contrast with activation of $GABA_A$ sites which results in channel opening.

The $GABA_B$ sites have been demonstrated on neuronal elements in the periphery as well as within the central nervous system. They are present on the inputs to heart, intestinal, vas deferens, anoccocygeus and vascular muscle (Bowery *et al.*, 1981; Starke and Weitzell, 1980; Anwar and Mason, 1982; Kaplita *et al.*, 1982; Muhyaddin *et al.*, 1982a,b; Giotti *et al.*, 1983; Kleinrok and Kilbinger, 1983; Ong and Kerr, 1983). In all cases activation of the receptor produces a diminution in release of neurotransmitter. Whilst this may be a purely pharmacological effect in the majority of tissues, the presence of GABA neurones within the enteric nervous system (see Ong and Kerr, 1983) could provide the source of a functional $GABA_B$ system in the intestine. Its most likely role would be to modulate peristaltic activity by attenuating the release of acetylcholine (Ong and Kerr, 1983; see also Kleinrok and Kilbinger, 1983) since the muscle-relaxant effect of activation of $GABA_B$ sites is absent in the presence of tetrodotoxin and hyoscine (Giotti *et al.*, 1983). The relaxant effect produced by stimulation of $GABA_B$ sites in the intestine is in striking contrast to the muscular contraction resulting from the activation of $GABA_A$ sites. These receptors, like $GABA_B$ sites, are present on the post-ganglionic cholinergic neurones since tetrodotoxin, atropine and hyoscine will prevent the contraction (Giotti *et al.*, 1983; Ong and Kerr, 1983). This clear separation in the intestine is one of the many differentials between $GABA_A$ and $GABA_B$ systems (Table 1).

An area of study which is currently proving to be significant in our understanding of the differences between $GABA_A$ and $GABA_B$ sites is the determination of their regional distributions within the brain. This is being conducted by an autoradiographic analysis similar to that described earlier for the [^{35}S]-TBPS location studies except that [^{3}H]-GABA is used as the ligand (see Wilkin *et al.*, 1981).

In many regions of the brain, $GABA_A$ and $GABA_B$ sites can both be detected. For example, in the cerebral cortex there is a high density of $GABA_A$ sites with always a lower concentration of $GABA_B$ sites. However, in many regions a clear separation occurs. Four regional examples of this are shown in Fig. 4–7. In each pair of photographs, adjacent sections from the same brain have been labelled for $GABA_A$ or $GABA_B$ sites and the radioactive deposits located on LKB Ultrofilm. The figures illustrate some of the major contrasts so far observed. These are in (i) the cerebellum (Figs 4 and 6); (ii) the lamina molecularis of the olfactory bulb (Figs 5 and 6); (iii) the thalamic nuclei (Fig. 6); and (iv) the interpeduncular nucleus (Fig. 7). In the cerebellum $GABA_A$ sites predominate in the granule cell layer (Olsen and Mikoshiba, 1978; Palacios *et al.*, 1980; Wilkin *et al.*, 1981) whereas $GABA_B$ sites are confined to the moleclular layer (Wilkin *et al.*, 1981). In the olfactory bulb, where a large concentration of bicuculline-sensitive [^{3}H]muscimol and [^{3}H]-GABA ($GABA_A$) binding sites is present (Palacios, Wamsley and Kuhar, 1981; Quinn and Cagan, 1981), $GABA_B$ sites are few in number. In particular, the precise band of $GABA_A$ sites evident in the lamina molecularis is devoid of grains in the $GABA_B$ labelled sections (Fig. 5). The thalamic nuclei exhibit a high density of $GABA_B$ sites (Fig. 6) although their specific loci have yet to be determined. [^{3}H]muscimol ($GABA_A$) sites have already been described within these nuclei and their distribution is uneven (Palacios *et al.*, 1981). The greatest concentration of [^{3}H]muscimol sites appear to be in the ventral nucleus, lateral nucleus pars posterior and dorsal lateral geniculate nucleus. The lowest level was in the medial nucleus pars medialis (Palacios *et al.*, 1981). Present observations so far indicate that this is probably the region of the thalamus where the concentration of $GABA_B$ sites is greatest.

Perhaps the region showing the most striking distinction so far is the interpeduncular nucleus. In this area, there is a very large concentration of $GABA_B$

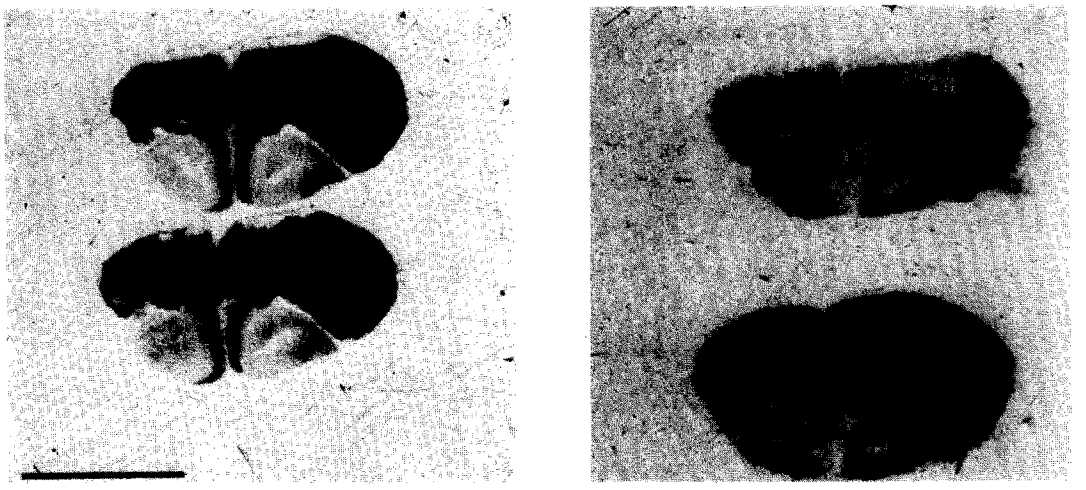

Fig. 5. Autoradiograms of [^{3}H]-GABA at $GABA_A$ and $GABA_B$ binding sites in transverse sections of olfactory bulb and frontal cortex of a rat brain. Procedure exactly as described in Fig. 4. $GABA_A$ sites are shown in the two pictures on the left and $GABA_B$ sites in the two pictures on the right. Scale bar = 5 mm.

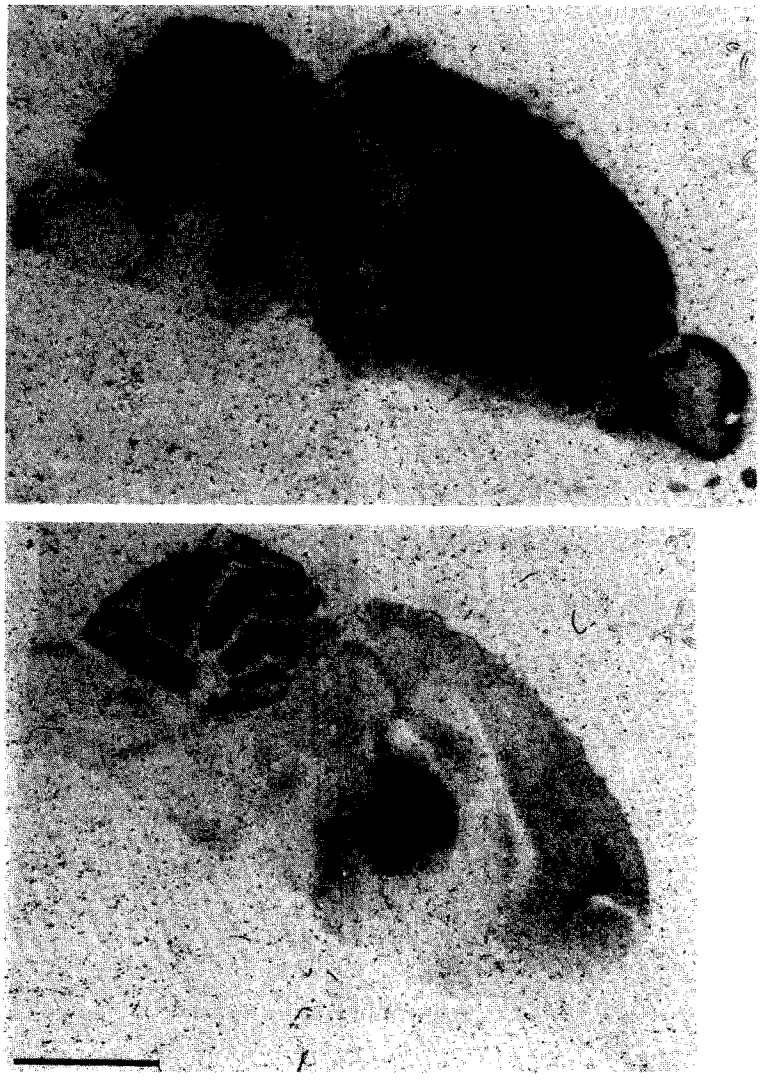

Fig. 6. Autoradiograms of [^{3}H]-GABA at $GABA_A$ and $GABA_B$ binding sites in adjacent parasagittal sections of rat brain. Sections cut at 700 μm from the midline. Binding site labelling performed exactly as described in legend to Fig. 4. Upper picture $GABA_A$ sites, lower picture $GABA_B$ sites. Note the density labelling over the olfactory bulb in the upper picture and the thalamic region in the lower picture. Scale bar = 5 mm.

Fig. 7. Autoradiograms of [^{3}H]-GABA at $GABA_A$ and $GABA_B$ binding sites in transverse sections of a rat brain. Sections correspond to A1950 μm in König and Klippel (1967). Procedure exactly as described in legend to Fig. 4. Upper picture $GABA_A$ sites, lower picture $GABA_B$ sites. Note the very dense labelling of $GABA_B$ sites in the region of the interpeduncular nucleus at the base of the brain (lower picture) with much less labelling of this region in the upper picture ($GABA_A$ sites). Scale bar = 5 mm.

binding sites with very few $GABA_A$ sites (Fig. 7). The significance of this is currently under investigation, but even this far it seems reasonable to conclude that $GABA_B$ sites are likely to be associated with neuronal systems which are not necessarily linked with $GABA_A$ sites. However, in regions where both receptor types are present there may not be an anatomical separation. For example, in the dorsal horn of the spinal cord where bicuculline-sensitive GABA receptors occur on afferent nerve terminals (Curtis, Duggan, Felix and Johnston, 1971; Levy, 1974) there is now evidence for $GABA_B$ sites which are also present on the sensory afferent terminals (Bowery, Price, Turnbull and Wilkin, 1983d; Price, Wilkin, Turnbull and Bowery, 1984). The $GABA_B$ sites are concentrated in laminae **I–IV** of the spinal cord whereas $GABA_A$ sites are more uniformly distributed throughout the dorsal and ventral laminae (**I–X**). After neonatal administration of capsaicin the concentration of $GABA_B$ sites in the dorsal horn of the adult rat is reduced by 40–50%. Since this neurotoxin causes a selective degeneration of primary sensory afferents (Jancso, Kiraly and Jancso-Gabor, 1977) it seems reasonable to conclude that the decrease in $GABA_B$ sites is due to the loss of these afferent fibres. It has been reported that treatment with capsaicin also decreases the number of [^{3}H]muscimol binding sites by 20–30% (Singer and Placheta, 1980). This would correspond to a diminution in $GABA_A$ sites which is consistent with the view that these sites are present presynaptically. However, the greater concentration of $GABA_B$ sites and the larger decrease after capsaicin could place a greater emphasis on these receptors in the modulation of afferent transmitter release. It is possible that the receptors may even function in concert.

In conclusion, it is now apparent that GABA receptors can no longer be considered as homogeneous. Subtypes of bicuculline-sensitive receptors ($GABA_A$ sites) which exhibit subtle distinguishing features have been described (see Bowery, 1984) but there is also clear evidence for a population of GABA receptors ($GABA_B$ sites) which differ in many respects from the classical GABA receptor associated with chloride ion channels. This receptor may provide a target for substances, the therapeutic actions of which are difficult to predict. Undoubtedly analgesia is one likely effect of an agonist (Bartolini, Bartolini, Biscini, Giotti and Malmberg, 1981; Sawynok and La Bella, 1982) but the effect(s) of an antagonist are an open question. In contrast to a $GABA_A$ site antagonist, convulsive activity is unlikely to result from its administration. The prospect of examining the effects of a selective antagonist are exciting not only to discover what happens *in vivo* but also as a means of providing evidence for a functional role for the $GABA_B$ receptor system. Whilst the endogenous ligand (GABA) is present in the brain there is still insufficient evidence to assign a role to this receptor system.

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Note added in proof—Since submitting this article, Crossman A. R., Latham A., Patel S. and Slater P. have reported (*Br. J. Pharmac.* **80:** 532P) that a high concentration of $GABA_B$ sites occurs in the rat interpeduncular nucleus.