Biochemical Pharmacology, Vol 28, pp. 2697–2702
Pergamon Press Ltd. 1979. Printed in Great Britain.

COMMENTARY
GABA AGONISTS AND ANTAGONISTS

P. R. ANDREWS and G. A. R. JOHNSTON

Departments of Physical Biochemistry and Pharmacology,
Australian National University, Canberra, Australia

The role of γ-aminobutyric acid (GABA) as an inhibitory neurotransmitter in the mammalian central nervous system is well established and has been the subject of numerous comprehensive reviews. The aim of this commentary is to speculate on likely developments concerning GABA agonists and antagonists.

Many substances inhibit the firing of central neurones. In order to characterise an inhibitory action as likely to be the result of activation of GABA receptors, it is at least necessary to study the effects of certain antagonists. In general, the inhibitory action of GABA can be antagonised by the convulsant alkaloid bicuculline, but not by the convulsant alkaloid strychnine. On this basis, GABA agonists may be defined as bicuculline-sensitive, strychnine-insensitive neuronal inhibitors, and can thus be distinguished from glycine agonists, defined as strychnine-sensitive, bicuculline-insensitive neuronal inhibitors, and from other substances such as dopamine and noradrenaline, whose inhibitory actions are insensitive to both alkaloids [1]. This definition of GABA agonists almost certainly represents a convenient oversimplification. Evidence is accumulating in favour of a multiplicity of GABA receptors, and this implies the likely existence of a corresponding range of GABA agonist and antagonist classes. For example, four types of GABA receptors have been characterized in crayfish muscles on the basis of desensitization experiments and the antagonist effects of β-guanidinopropionic acid [2]. There are also a variety of indirectly acting GABA agonists and antagonists, as well as endogenous ligands which may modulate GABA-receptor interactions.

A substance may antagonise the action of GABA directly by competing with GABA for its receptors, or indirectly by allosterically modifying the receptors or by blocking GABA-activated ionophores. The two "classic" GABA antagonists, bicuculline and picrotoxinin, appear to act in different ways [3] consistent with electrophysiological observations that bicuculline can act as a competitive antagonist of GABA while picrotoxinin acts as a non-competitive antagonist. Ligand binding studies using bicuculline methiodide and dihydropicrotoxinin show that these substances bind to different sites on rat brain membranes and that GABA influences only the binding of bicuculline methiodide. These and other results indicate that bicuculline binds to a population of GABA receptors, while picrotoxinin binds to GABA ionophores, as indicated in Fig. 1.

A substance may activate GABA receptors directly as a true GABA-mimetic (e.g. muscimol) or indirectly

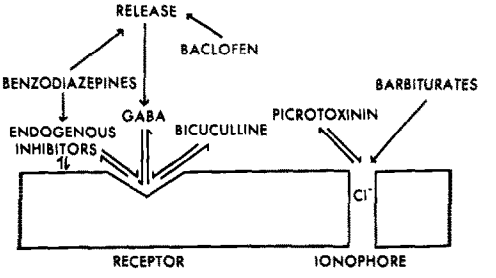

Fig. 1. Schematic diagram of drug interactions at a GABA receptor–ionophore complex.

in several ways. It might release GABA from intracellular stores (e.g. baclofen) or act as a pro-drug which yields the active GABA agonist at the site of action (e.g. SL 76.002). Potentiation of the action of tonically released GABA may result from displacement by a substance (e.g. diazepam) of an endogenous inhibitor of receptor binding, inhibition of GABA inactivation by uptake processes (e.g. nipecotic acid) or inhibition of GABA degradation by transamination (e.g. gabaculine). Aspects of GABA uptake and metabolism have been the subject of other Commentaries in this journal [4, 5]. A GABA-like action could also result from a substance bypassing GABA receptors and directly influencing GABA ionophores (e.g. pentobarbitone). The structures of these and related substances are shown in Fig. 2.

To establish whether or not a particular substance has any of the above actions, information is required from electrophysiological investigations of its effect on functional neurones, and from neurochemical studies of effects on ligand binding, enzymic activities, uptake and release phenomena. Electrophysiological studies can provide information with respect to relative potency, time course and ionic basis of action, desensitization, and susceptibility to antagonists. Ligand binding studies using ligands which show some selectivity for certain receptor (GABA, muscimol and bicuculline) and ionophore sites (dihydropicrotoxinin) can provide clues to likely sites of action. Uptake and release studies using tissue slices or synaptosomal preparations can show if a substance is able to release GABA, e.g. by heteroexchange, or to influence evoked release, e.g. by interaction with presynaptic receptors; relatively selective substrates are now available for labelling either presynaptic (*cis*-3-aminocyclohexanecarboxylic acid) [6] and glial (β-alanine) [4] transmitter pools.

BP 28/18—A

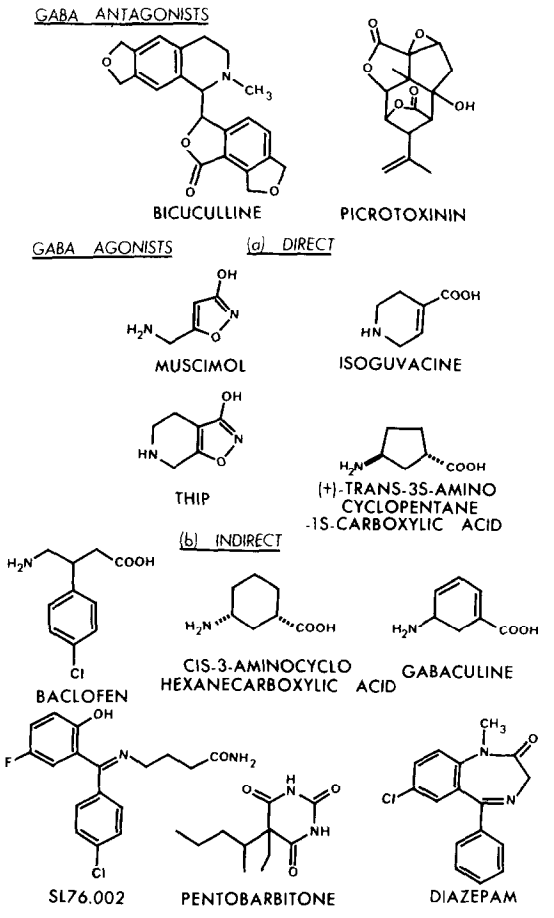

Fig. 2. Structures of some GABA antagonists and agonists.

MUSCIMOL AS A SELECTIVE GABA AGONIST

The action of muscimol, a psychotomimetic isolated from the mushroom *Amanita muscaria*, as a "GABA-like" amino acid was first reported in this journal some 10 years ago [7] and this isoxazole is now widely used as a selective GABA agonist. Muscimol shows a remarkable selectivity in its interaction with GABA receptors; the 3-isoxazolol moiety acts as a masked carboxyl group which is recognised efficiently by GABA receptors but not by other macromolecules that interact with GABA in the brain. Muscimol is not a substrate for GABA transaminase and is transported inefficiently by GABA uptake systems [8].

There are indications that the relative potency of GABA and muscimol as bicuculline-sensitive, strychnine-insensitive neuronal inhibitors *in vivo* differs in the cat spinal cord and cerebral cortex [9, 10]. This suggests the existence of GABA receptors of different agonist specificity in these tissues. Ligand binding studies using radioactive GABA and muscimol [11–13*] are consistent with muscimol being a selective GABA agonist binding to about half the total number of sites that bind GABA (Table 1). These binding studies indicate that muscimol binds to rat brain membranes with higher affinity than GABA and that both ligands share some common binding sites. Multiple binding sites are found for both ligands since they exhibit at least biphasic binding kinetics. Electrophysiological studies on crustacean GABA receptors suggest multiple binding sites [14]. Studies with some GABA analogues and inorganic salts show differences between GABA and muscimol binding: trans-4-aminocrotonic acid, (±)-*trans*-3-aminocyclopentanecarboxylic acid and ammonium thiocyanate are more potent inhibitors of GABA than of muscimol binding, while the reverse is true for 3-aminopropanesulphonic acid, bicuculline and sodium cyanide [11–13].

Table 1. Kinetic parameters for the binding of GABA and muscimol to rat membranes

Ligand	K_D (nM)	B (pmol/mg protein)	References
(a) 0·05%-Triton X100-treated rat brain membranes:			
GABA	16	0.6	[11]
	130	5	
Muscimol	1.8	2.6	[12]
(b) 0.5%-Triton X100-treated rat brain membranes:			
GABA	9.4	4.7	[13]
	37	6.5	
Muscimol	0.9	0.5	[13]
	5.8	2.2	
	32	3.1	

BICUCULLINE AS A SELECTIVE GABA ANTAGONIST

The convulsant alkaloid bicuculline is now generally accepted as a selective GABA antagonist [1]. Early doubts regarding the usefulness of bicuculline appear to have been related to its instability under certain conditions: at physiological pH and 37° it is hydrolysed to the relatively inactive GABA antagonist bicucine with a half life of only a few minutes [15]. Quaternary salts of bicuculline such as bicuculline methiodide ("*N*-methylbicuculline" and bicuculline methochloride are stable in the pH range 2–8 and much more water soluble than simple salts of bicuculline, and they are preferable to the simple salts for most experiments. Simple salts of bicuculline are essential, however, for studies of central effects following systemic administration since blood–brain barriers appear to exclude quaternary salts more efficiently than simple salts.

Bicuculline-sensitive synaptic inhibitions are found in all areas of the brain. This may reflect the widespread involvement of GABA as an inhibitory transmitter, but other bicuculline-sensitive inhibitors, including imidazole-4-acetic acid, δ-aminolevulinic acid, β-alanine and taurine, are found in the brain and thus not all bicuculline-sensitive synaptic inhibitions necessarily involve GABA. From neurochemical data, however, GABA does appear to be the transmitter involved in most bicuculline-sensitive inhibitions.

Some ligand binding studies have been carried out with radioactive bicuculline methiodide [16, 17]. Inorganic salts profoundly influence the binding which is routinely carried out in the presence of 50 mM sodium thiocyanate. Under these conditions only 30 per cent of the total bound radioactivity can be displaced by GABA or muscimol. Such "specific binding" can be increased to 50 per cent in the presence of 200 mM sodium perchlorate. The physiological relevance of

* And unpublished observations.

these salt effects is enigmatic. While GABA and muscimol appear to compete with bicuculline methiodide for some common binding sites, it seems likely that all agonist and antagonist sites are not identical. These results have been interpreted on the basis of two conformational states of the GABA receptor, one preferentially binding agonists and one preferentially binding antagonists [17], but the existence of a multiplicity of GABA receptors, not all of which interact with bicuculline and/or muscimol, may explain the observed differences between bicuculline, GABA and muscimol binding.

"ACTIVE CONFORMATIONS"

The structural requirements of a GABA agonist are not self-evident from the structure of GABA, which can exist in a large number of relatively stable conformations as a result of rotation around single bonds. The conformational mobility of GABA analogues can be restricted by the incorporation of unsaturation, ring structures or both into the basic GABA structure. Such conformational restriction often results in analogues having more selective actions and sometimes higher potency than that of GABA itself [13]. Muscimol is a GABA analogue of restricted conformation, the isoxazole ring structure effectively linking C1 and C3 of GABA and holding the carboxyl equivalent in a plane with C2, C3 and C4. Muscimol has a single degree of conformational freedom, rotation of the aminomethyl side chain with respect to the isoxazole ring. This limited freedom can be restricted still further by incorporating the side chain in a second ring, as in the relatively rigid derivative THIP, which still acts as a potent selective GABA agonist [18]. Extensive structure-activity studies on such derivatives led to the semi-rigid class of GABA agonists based on isoguvacine and to the proposal that GABA interacted with bicuculline-sensitive receptors in "a partially extended and almost planar conformation" [18].

Structural similarities between GABA, muscimol and bicuculline were noted in the first paper describing the antagonist action of bicuculline, thus providing "an indication of the particular configurations of GABA and bicuculline recognized by the receptor" [19]. Binding studies have since shown that GABA, muscimol and bicuculline salts mutually inhibit each others binding to brain membranes, and thus it is reasonable to propose that these substances share some common binding sites. The "active conformations" of GABA at sites shared with bicuculline fall within a relatively narrow range defined by the known absolute stereochemistry (1*S*, 9*R*) of bicuculline and its available range of conformations as indicated by nuclear magnetic resonance and molecular orbital data [20]. Use of interactive computer graphics techniques to superimpose GABA on bicuculline at the midpoint of the latter's likely conformation range gives rise to the "bicuculline conformation" for GABA shown in Fig. 3. Muscimol cannot be fitted to this "bicuculline confor-

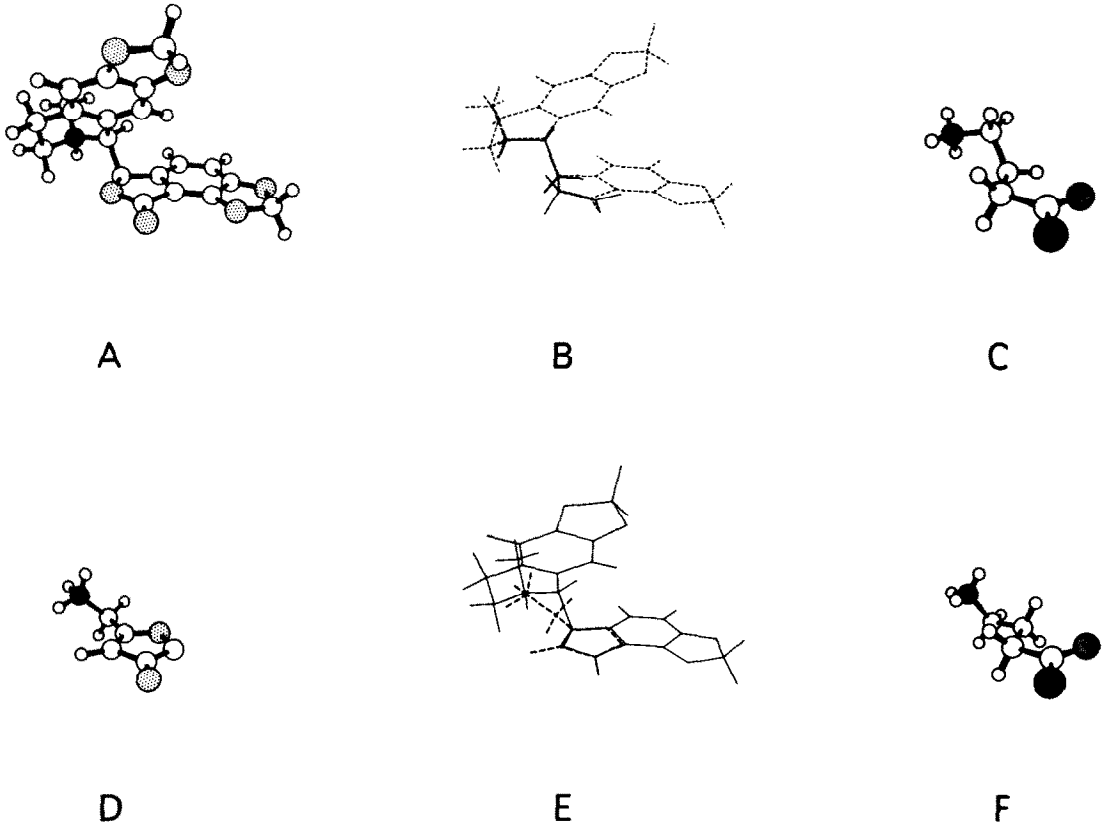

Fig. 3. Structural similarities between GABA, bicuculline and muscimol. (A) Bicuculline in a low energy form (H-C1–C9–H angle 90°). (B) GABA fitted to this form of bicuculline. (C) GABA in the "bicuculline conformation". (D) Muscimol conformation (N7–C6–C5–C4 torsion angle 39·) which affords maximum overlap with bicuculline. (E) Overlay of muscimol on bicuculline. (F) GABA in the "muscimol conformation" which differs only in the position of carbon atom 4 from the "bicuculline conformation".

mation" of GABA, but favours the closely related "muscimol conformation", which differs only in the position of C4. These two conformations of GABA may define the limits of the range of "active conformations" at bicuculline-sensitive receptors. Within this range, the agonists muscimol, THIP, *trans*-4-aminocrotonic acid and (+)-*cis*-3*R*-aminocyclopentane-1*S*-carboxylic acid may adopt conformations close to the "muscimol conformation", isoguvacine an intermediate conformation, and (+)-(*trans*-3*S*-aminocyclopentane-1*S*-carboxylic acid and 4,5-dihydromuscimol may adopt the "bicuculline conformation", while GABA may take up any conformation within the range. Although stereoisomers of the simple molecule GABA do not exist, these "active conformations" have a defined stereochemistry such that analogues in which elements of these conformations are preserved should exhibit stereospecificity [21]. Binding data on the stereoisomers of *trans* 4-aminopent-2-enoic acid and 5-(1-aminoethyl)-3-isoxazolol indicate that in these compounds, which are likely to adopt a "muscimol conformation", a methyl substituent at the pro-*R* position of the carbon next to the amino group inhibits interaction with the receptor. 4*R*-Aminopentanoic acid, however, is equipotent with its 4*S*-isomer: this compound can adopt the "bicuculline conformation" which moves the pro-*R* substituent to a region where methyl substitution does not influence interaction with the receptor.

Certain of the conformationally restricted GABA agonists may show selectivity for different classes of bicuculline-sensitive receptors. In this context, it is interesting that muscimol does not appear to bind to all GABA binding sites [13] and that (±)-*trans*-3-aminocyclopentanecarboxylic acid is a more potent inhibitor of GABA than of muscimol binding [11, 12]. Furthermore, isoguvacine is as potent as muscimol in inhibiting the firing of cat spinal neurones [18], but is some 20 times less potent than muscimol in depolarizing rat sympathetic ganglionic neurones [22]. It seems very likely that relatively selective agonists (and thus antagonists) for different classes of bicuculline-sensitive receptors may be developed on the basis of speculations regarding the likely "active conformations" of GABA at these receptors.

In view of the structural resemblance between the "active conformations" of GABA, muscimol and bicuculline, what structural features differentiate between agonist and antagonist activity? Steward and colleagues [23] have noted that the polarity of the lactone ring in bicuculline is considerably less than that of the GABA carboxyl group with which it is isosteric. They suggest that the smaller net charges in bicuculline may result in weaker receptor binding and thence antagonist action. However, the net atomic charges in the isoxazol ring of muscimol are also substantially less than those in the GABA carboxyl group although muscimol binds more tightly than GABA as well as being a more potent agonist. In the molecular regions isosteric with GABA, both conformational and electronic factors may be ruled out as explanations for antagonist action which appears to be due to additional interactions involving the two substituted aromatic rings. Currently available structure–activity data [24] do not define the prerequisite structural features of an antagonist molecule, and further systematic studies of a range of bicuculline derivatives are needed.

BICUCULLINE-INSENSITIVE GABA AGONISTS?

GABA receptors that are relatively insensitive to antagonism by bicuculline have been demonstrated in crustacea. The existence of some bicuculline-insensitive GABA receptors in the mammalian CNS could explain the action of certain analogues of GABA in folded conformations which inhibit neuronal firing in a bicuculline-insensitive, strychnine-insensitive manner. These analogues include *cis*-4-aminocrotonic acid, *trans*-2-aminocyclohexanecarboxylic acid and *trans*-3-aminocyclohexanecarboxylic acid. These conformationally restricted analogues of GABA, among which structural similarities have been noted [24], may constitute another class of GABA agonists. GABA might interact with bicuculline-sensitive receptors in extended conformations and with bicuculline-insensitive receptors in folded conformations.

GABA IONOPHORES

Olsen and colleagues [25] have suggested that the GABA antagonist picrotoxinin, and other drugs which affect GABA synaptic activity without displacing GABA from its receptor sites, may exert their effects through an interaction with GABA-activated chloride ionophores. Convulsant and depressant drugs may antagonise or potentiate GABA responses by binding to the ionophores and favouring inactive or active states respectively. Using a biologically active labelled analogue of picrotoxinin, [^{3}H]-α-dihydropicrotoxinin, as a probe for the ionophores, they found that a number of convulsant drugs, including barbiturates, bicyclophosphates, and bicyclocarboxylate ortho esters displace dihydropicrotoxinin, but not GABA, from binding sites on rat brain membranes. Of these, the so-called cage structures are known to inhibit GABA postsynaptic responses; this may be the result of ionophore antagonism.

Anaesthetic and anticonvulsant barbiturates mimic and potentiate GABA-mediated postsynaptic inhibition. Since these barbiturates also displace dihydropicrotoxinin, albeit weakly, they may be acting in part as ionophore agonists [26]. Pentobarbitone-induced changes in membrane conductance of neurones in tissue culture can be antagonised by picrotoxin [27].

BACLOFEN

Introduced as a lipophilic derivative of GABA better able to penetrate blood–brain barriers, baclofen, β-(*p*-chlorophenyl)-GABA, is used in the treatment of spasticity originating from various neurological disorders [28]. Much indirect evidence suggests that baclofen has a GABA-mimetic action, but it is not a direct GABA agonist at most bicuculline-sensitive receptors and is relatively ineffective in displacing GABA or bicuculline bound to brain membranes [1]. Its GABA-mimetic properties are perhaps related to GABA release since it has been shown to enhance the release of GABA from slices of rat globus pallidus [29] and from rat brain synaptosomes [30]. This stimulation of GABA release is stereoselective, being confined to the (+)-isomer [29], while the (−)-isomer influences dopamine metabolism [31]. The latter effect is not antagonised by picrotoxin and is thus independent of the major GABA

pathways. Experiments on brain slices have shown that baclofen inhibits the evoked release of glutamate and aspartate at lower doses than are needed to stimulate release of GABA [32], and its depressant action on primary afferent transmission in the spinal cord seen at low doses is more likely to be a presynaptic action depressing transmitter release [33]. Thus, the antispastic effects of baclofen are best explained by inhibition of release of excitatory transmitters rather than any GABA-mimetic action.

ENDOGENOUS INHIBITORS

The kinetics of GABA and muscimol 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 (see Table 1). This is due to the removal of endogenous inhibitors of GABA binding [34]. There are at least three such inhibitors which can be separated by partitioning between chloroform–methanol and water, and subsequent cation exchange chromatography of the aqueous phase. The chloroform–methanol soluble inhibitory activity appears to be associated with phosphatidylethanolamine [34*]. Inhibitory activity not retained by cation exchange chromatography is associated with an acidic protein [35], while that eluted by dilute base may be due to occluded GABA [36]. These inhibitors are naturally incorporated in brain membranes and may modulate the affinity of GABA for its receptor sites and the total number of available receptor sites.

Kinetic studies show that phosphatidylethanolamine acts as a non-competitive inhibitor of the high affinity binding of GABA to rat membranes, thus masking the availability of high affinity binding sites [34*]. Treatment of brain membranes with phospholipase C increases the availability of high affinity binding sites [37], as does base exchange with ethanolamine [34*]. There is evidence that a deficiency of phospholipid inhibitor may be responsible for the increased number of GABA high affinity binding sites found in some brain areas of patients with Huntington's disease [37]. Increased brain levels of glycerophosphoethanolamine are found in Huntington's disease and it is likely that abnormal phospholipid metabolism is linked to the neuropathogenesis of this disease.

The acidic protein inhibitor has a molecular weight of some 15,000 and influences both the number of high affinity binding sites and their affinity [35, 38]. This protein also inhibits benzodiazepine binding and it can be displaced from brain membranes by incubation with diazepam [38]. It thus forms a link to explain the GABA potentiating actions of benzodiazepines which may act by displacing this endogenous inhibitor of GABA receptors. Chronic administration of diazepam or phenobarbital to rats leads to a decrease in the number of GABA receptor sites in the striatum; this supports the notion of regulatory components of GABA receptors being involved in the action of benzodiazepines and barbiturates [39]. Of course these drugs have other actions on the GABA system, barbiturates on ionophores as discussed above and benzodiazepines stimulating the evoked release of GABA [40].

Occluded GABA in membrane preparations is in a non-exchangeable, osmotically insensitive pool which may be associated with cryptic GABA receptors buried in the membrane. This appears to influence both the availability and affinity of GABA receptors [34*]. This could be the basis of the apparent desensitization of GABA responses seen *in vivo* with spinal motoneurones [33], GABA combining with receptors and both being buried in the membrane.

Removal of endogenous inhibitors of GABA receptors could underlie phenomena such as denervation supersensitivity. Evidence suggesting denervation supersensitivity of GABA receptors has been obtained following unilateral kainic acid-induced lesions in rat striatum. This enhances behavioural responses to muscimol injected into the substantia nigra and increases the apparent density of GABA binding sites in membranes prepared from the substantia nigra [41].

Endogenous inhibitors of GABA receptors might be involved in many aspects of the function of this major inhibitory transmitter. They are obvious sites for drug action. They could serve to tune GABA dose response curves to suit specific situations as in the desensitization and supersensitivity examples above, and may play an important role in synaptic development and in synaptic changes involved in learning and memory. Endogenous inhibitors thus far appear to be unique to GABA receptors amongst the known transmitter systems, and this may reflect the relative importance of the major inhibitory transmitter system as an organising influence on brain function.

CONCLUSION

There are now clear indications for a multiplicity of GABA receptors in the CNS and different classes of GABA agonists and antagonists are emerging. Furthermore, endogenous inhibitors may modulate the number and activity of GABA receptors. These observations should serve as a stimulus for the development of substances which have very selective actions on GABA function and lead to a more rational basis for GABA pharmacology.

REFERENCES

1. G. A. R. Johnston, *A. Rev. Pharmac. Toxic.* **18**, 269 (1978).
2. J, Dudel and H. Hatt, *Pflügers Arch. ges. Physiol.* **364**, 217 (1976).
3. R. W. Olsen, D. Greenlee, P. Van Ness and M. K. Ticku, in *Amino Acids as Chemical Transmitters* (Ed. F. Fonnum), p. 467. Plenum Press, New York (1978).
4. L. L. Iversen and J. S. Kelly, *Biochem. Pharmac.* **24**, 933 (1975).
5. B. W. Metcalf. *Biochem. Pharmac.* **28**, 1705 (1979).
6. M. J. Neal and N. G. Bowery, *Brain Res.* **138**, 169 (1977).
7. G. A. R. Johnston, D. R. Curtis, W. C. de Groat and A. W. Duggan, *Biochem. Pharmac.* **17**, 2488 (1968).
8. G. A. R. Johnston, S. M. E. Kennedy and D. Lodge, *J. Neurochem.* **31**, 1519 (1978).
9. D. R. Curtis, A. W. Duggan, D. Felix and G. A. R. Johnston, *Brain Res.* **32**, 69 (1971).
10. D. R. Curtis, A. W. Duggan, D. Felix, G. A. R. Johnston and H. McLennan, *Brain Res.* **33**, 57 (1971).
11. S. J. Enna and S. H. Snyder, *Molec. Pharmac.* **13**, 442 (1977).

* And unpublished observations.

12. K. Beaumont, W. S. Chilton, H. I. Yamamura and S. J. Enna, *Brain Res.* **148**, 153 (1978).
13. G. A. R. Johnston, R. D. Allan, S. M. E. Kennedy and B. Twitchin, in *GABA-Neurotransmitters* (Eds H. Kofod, P. Krogsgaard-Larsen and J. Scheel-Krüger), Munksgaard, Copenhagen in press.
14. A. Constanti, *Neuropharmacology* **16**, 357 (1977).
15. R. W. Olsen, M. Ban, T. Miller and G. A. R. Johnston, *Brain Res.* **98**, 383 (1975).
16. H. Möhler and T. Okada, *Nature, Lond.* **267**, 65 (1977).
17. H. Möhler and T. Okada, *Molec. Pharmac.* **14**, 256 (1978).
18. P. Krogsgaard-Larsen, G. A. R. Johnston, D. Lodge and D. R. Curtis, *Nature, Lond.* **268**, 53 (1977).
19. D. R. Curtis, A. W. Duggan, D. Felix and G. A. R. Johnston, *Nature, Lond.* **226**, 1222 (1970).
20. P. R. Andrews and G. A. R. Johnston, *Nature, New Biol.* **243**, 29 (1973).
21. G. A. R. Johnston, R. D. Allan, P. R. Andrews, S. M. E. Kennedy and B. Twitchin, in *Proceedings of the 7th International Congress of Pharmacology*, Vol. 2, *Neurotransmitters* (ed. P. Simon) in press. Pergamon Press, Oxford (1979).
22. N. G. Bowery, J. F. Collins, A. L. Hudson and M. J. Neal, *Experientia* **34**, 1193 (1978).
23. E. G. Steward, P. W. Borthwick, G. R. Clarke and D. Warner, *Nature, Lond.* **256**, 600 (1975).
24. G. A. R. Johnston, in *GABA in Nervous System Function* (Eds E. Roberts, T. N. Chase and D. B. Tower) p. 395. Raven Press, New York (1976).
25. M. K. Ticku, P. C. Van Ness, J. W. Haycock, W. B. Levy and R. W. Olsen, *Brain Res.* **150**, 642 (1978).
26. R. W. Olsen, M. K. Ticku, D. Greenlee and P. Van Ness, in *GABA-Neurotransmitters* (Eds M. Kofod, P. Krogsgaard-Larsen and J. Scheel-Krüger). Munksgaard, Copenhagen in press.
27. J. L. Barker and B. R. Ransom, *J. Physiol., Lond.* **280**, 355 (1978).
28. J. W. Faigle and H. Keberle, in *Spasticity—A Topical Survey* (Ed. W. Birkmayer) p. 94. Hans Huber Publications, Vienna (1972).
29. R. Kerwin and C. Pycock, *J. Pharm. Pharmac.* **30**, 622 (1978).
30. P. J. Roberts, H. K. Gupta and N. S. Shargill, *Brain Res.* **155**, 209 (1978).
31. P. C. Waldmeier and L. Maitre, *Eur. J. Pharmac.* **47**, 191 (1978).
32. S. J. Potashner, *Can. J. Physiol. Pharmac.* **56**, 150 (1978).
33. S. Fox, K. Krnjević, M. E. Morris, E. Puil and R. Werman, *Neuroscience* **3**, 495 (1978).
34. G. A. R. Johnston and S. M. E. Kennedy, in *Amino Acids as Chemical Transmitters* (Ed. F. Fonnum) p. 507. Plenum Press, New York (1978).
35. G. Toffano, A. Guidotti and E. Costa, *Proc. natn. Acad. Sci. U.S.A.* **75**, 4024 (1978).
36. D. V. Greenlee, P. C. Van Ness and R. W. Olsen, *Life Sci.* **22**, 1653 (1978).
37. K. G. Lloyd and L. Davidson, NINCDS Symposium on Huntington's Disease, San Diego, California (1978).
38. A. Guidotti, G. Toffano and E. Costa, *Nature, Lond.* **275**, 553 (1978).
39. H. Möhler, T. Okada and S. J. Enna, *Brain Res.* **156**, 391 (1978).
40. P. R. Mitchell and I. L. Martin, *Neuropharmacology* **17**, 317 (1978).
41. J. L. Waddington and A. J. Cross, *Nature, Lond.* **276**, 618 (1978).