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γ-AMINOBUTYRIC ACID AGONISTS: AN *in vitro* COMPARISON BETWEEN DEPRESSION OF SPINAL SYNAPTIC ACTIVITY AND DEPOLARIZATION OF SPINAL ROOT FIBRES IN THE RAT

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1 Relative molar potencies, of a range of γ-aminobutyrate (GABA)-related agonists, for depolarization of isolated spinal roots have been compared with their potencies for depression of spontaneous synaptic activity, recorded in ventral roots of hemisected spinal cord preparations from 3 to 9-day-old rats. Both effects were sensitive to antagonism by bicuculline.

2 The depolarizing potencies of the series were not parallelled by their depressant potencies. This disparity was shown most strongly by 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) which was 20 times stronger than GABA in depolarizing root fibres and 20 times stronger than GABA in its depressant action and by (+)-*cis*-3-aminocyclopentane-carboxylic acid (17 times weaker than GABA on root fibres and 42 times stronger than GABA as a depressant).

3 The effect of uptake on these relative potencies is discussed and it is concluded that fibre depolarization and depression are probably mediated by different types of bicuculline-sensitive receptor.

4 The depolarizing potencies of the agonists showed a strong correlation with previously reported data for displacement of labelled GABA from *in vitro* rat brain membrane preparations (correlation coefficient 0.90, $P < 0.001$). However, the relative depressant potencies showed no such correlation with binding data (correlation coefficient 0.50, $P > 0.05$).

Introduction

γ-Aminobutyric acid (GABA) and related neutral amino acids produce two types of change in neuronal membrane potentials. Central neurones are hyperpolarized by GABA (Obata, Ito, Ochi & Sato, 1967; Curtis, Hösli, Johnston & Johnston, 1968), whereas primary afferent neurones (de Groat, Lalley & Saum, 1972; Feltz & Rasminsky, 1974; Gallagher, Higashi & Nishi, 1978), primary afferent terminals (Curtis, Lodge & Brand, 1977) and non-myelinated nerve fibres of various peripheral nerves (Brown & Marsh, 1978) are depolarized by GABA. In the present study the depolarization of dorsal or ventral root fibres, by a wide range of GABA-related compounds was compared with the depression of spontaneous synaptic activity in isolated spinal cord preparations from immature rats. The results suggest that fibre depolarization and neuronal depression may be mediated through two types of bicuculline-sensitive receptor.

Methods

The method for superfusing and recording the electrical polarity of dorsal or ventral roots of isolated spinal cord preparations, maintained at 25°C, from 3 to 9-day-old rats was similar to that described previously (Evans & Watkins, 1978). Upward deflection on the records indicates increased positivity of the recording electrode, corresponding to depolarization of motoneurones or afferent fibres. In the present study the following two types of recording were made.

Fibre recording. Dorsal or ventral roots were dissected from spinal cords and mounted with the central end in the stream of superfusing medium and the peripheral ganglionic end on the recording electrode. The length of root exposed to the superfusing medium was 1 to 2 mm. The remaining length of root between the superfusing medium and the recording electrode was immersed in a liquid paraffin/vaseline seal (Evans, 1978).

'Intraspinal' recording. Hemisected spinal cords were mounted essentially as described previously (Evans & Watkins, 1978) but care was taken to ensure that the liquid paraffin/vaseline seal extended over the complete length of the dorsal or ventral root and over the surface of the cord at the point of entry of the root. This precaution ensured that none of the extraspinal

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Table 1 Equimolar potencies of γ-aminobutyric acid (GABA) agonists (GABA = 1.0) for depolarization of dorsal or ventral root fibres, displacement of labelled GABA from rat brain membranes (IC_{50} GABA/IC_{50} test agonist, IC_{50} = concentration of agonist required for 50% inhibition of GABA binding).

	Agonist	*Fibre depolarizing potency* *Dorsal root*	*Ventral root*	*GABA displacement potency*	*Depressant potency* *Mean*	*Lowest*
A	Muscimol	4.4 ± 0.25 (4)	4.8 ± 0.49 (5)	14.2	833 ± 99 (4)	692
B	Dihydromuscimol	3.7 ± 0.6 (4)	3.6 ± 0.40 (5)	6.1	1127 ± 179 (4)	912
C	3-APS	2.4 ± 0.7 (3)	1.30 ± 0.12 (4)	2.9	108 ± 28 (4)	44
D	(+)-*trans*-3-Aminocyclopentanecarboxylic acid	1.2 ± 0.24 (3)	1.47 ± 0.20 (4)	3.4	1068 ± 165 (4)	617
E	(±)-Aminocyclopent-1-enecarboxylic acid	1.0 ± 0.16 (3)	1.10 ± 0.05 (4)	0.44	1039 ± 195 (4)	501
F	*trans*-4-Aminocrotonic acid	0.88 ± 0.14 (4)	0.90 ± 0.04 (4)	2.43	0.85 ± 0.19 (4)	0.51
G	Piperidine-4-sulphonic acid	0.30 (2)	0.28 ± 0.03 (4)	1.0	464 ± 47 (4)	400
H	Isoguvacine	0.27 ± 0.05 (4)	0.40 ± 0.04 (5)	0.24	353 ± 145 (4)	100
I	Imidazole-4-acetic acid	0.15 (2)	0.082 ± 0.014 (4)	1.42	16.9 ± 3.3 (5)	7.2
J	4-Amino-3-hydroxybutyric acid	0.11 (2)	0.13 ± 0.02 (4)	0.28	4.0 ± 0.8 (4)	3.1
K	(+)-*cis*-3-Aminocyclopentanecarboxylic acid	0.66 ± 0.009 (3)	0.097 ± 0.012 (4)	0.23	69 ± 12 (4)	42
L	β-Guanidinopropionic acid	0.057 ± 0.021 (3)	0.13 ± 0.02 (4)	0.11	0.90 ± 0.009 (4)	0.62
M	THIP	0.054 ± 0.006 (4)	0.083 ± 0.009 (5)	0.13	62 ± 22 (11)	20

IC_{50} values obtained from the following sources: A, B, H and M: Krogsgaard-Larsen *et al.* (1978); C, I, J and L: Greenlee, Van Ness & Olsen (1978); E and F: Johnston, Allan, Kennedy & Twitchin (1978a); D and K: Johnston, Allan, Andrews, Kennedy & Twitchin (1978b) and G: Krogsgaard-Larsen, Falch, Schousboe, Curtis & Lodge (1980).

part of the root was exposed to the superfusing medium.

Potency ratios were measured from the horizontal distance between log dose-response curves for GABA and responses produced by test agonists at approximately 2 to 3 times the threshold concentration required to produce an effect. Since spinal root fibres are sensitive to amino acids (see below) the positioning of the grease seal, used in the recording system, has a strong influence on changes in root polarity induced by amino acids. Thus previously observed variations between preparations in potency of antagonists (Evans, 1978) and agonists (Evans, 1979) are more likely to have been caused by differences in length of the proximal unsealed part of the root, rather than the age of the animal as suggested by Evans (1979).

3-Aminopropane sulphonic acid (3-APS), (+)-*trans*-3-aminocyclopentanecarboxylic acid, (+)-*cis*-3-aminocyclopentanecarboxylic acid, (±)-4-aminocyclopent-1-enecarboxylic acid, *trans*-4-aminocrotonic acid, (±)-*cis*-3-aminocyclohexanecarboxylic acid, and nipecotic acid were prepared by R.D.A.

Muscimol, dihydromuscimol, isoguvacine, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) and piperidine-4-sulphonic acid were gifts from Dr P. Krogsgaard-Larsen. Other chemicals were obtained from commercial sources.

Results

The relative molar potencies for the depolarization of isolated dorsal or ventral root fibres, by 13 compounds structurally related to GABA are shown in Table 1.

The relative potencies of the agonists on dorsal root fibres were not significantly different from their relative potencies on ventral root fibres, suggesting a close similarity between receptors on dorsal and ventral root fibres. In most preparations the threshold concentration of GABA required to depolarize dorsal root fibres was 1 μM and the corresponding threshold concentration for ventral root fibres was 10 μM. Two preparations superfused with bicuculline hydrochloride (20 μM) still responded to glycine (ventral root fibres), or kainate (dorsal root fibres), but responses to the agonists listed in Table 1 were decreased by 80% or more. The following compounds produced either no, or just detectable, depolarization at the highest concentration tested (2 mM); γ-guanidinobutyric acid, *cis*-3-aminocyclohexanecarboxylic acid, nipecotic acid, valproic acid and baclofen. It is apparent that THIP was the least potent compound, in Table 1, which depolarized root fibres, by a bicuculline-sensitive mechanism, being 20 times less potent than

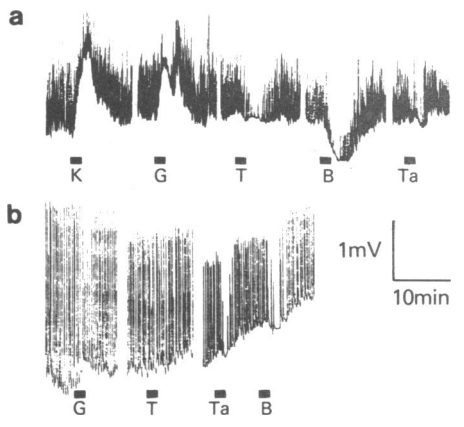

Figure 1 Ventral root recording of spontaneous synaptic activity recorded from the ventral root of a hemisected spinal cord. KCl 5 mM (K), γ-aminobutyric acid (GABA) 4 mM (G), THIP 0.1 mM (T), baclofen 2 μM (B) and taurine 1 mM (Ta) were superfused during the periods indicated below the records. (a) Before addition of bicuculline, (b) 20 min after introduction of bicuculline (100 μM) to the superfusing medium.

GABA. In some experiments spinal roots from mature animals and sciatic nerve branches from mature and immature animals were tested for sensitivity to GABA and THIP. Dorsal roots from mature animals (Brown & Marsh, 1978) and sciatic nerve branches from immature animals were depolarized by GABA and THIP with relative potencies corresponding to those given in Table 1 for spinal roots. Ventral roots and sciatic nerve branches from mature animals did not respond to GABA (5 mM) or THIP (1 mM).

Isolated hemisected spinal cords of immature rats, bathed in Mg^{2+}-free medium show marked spontaneous synaptic activity recorded in ventral roots (Evans, 1978). Such spontaneous activity can be diminished by depressant compounds, invariably resulting in a hyperpolarization recorded in ventral roots. Figure 1 shows records from one preparation with depressant effects produced by THIP, GABA, baclofen and taurine.

In most preparations the threshold concentration of GABA for such a depressant effect was 2 mM, contrasting strongly with the much lower concentrations required to depolarize fibres. However, the threshold concentration of THIP required to depress synaptic activity was similar to the concentration required to depolarize fibres. Thus the depressant potency of THIP was at least 20 times greater than that of GABA. The action of GABA was biphasic (Figure 1a) consisting of depolarization combined with the depressant effect. The depressant actions of THIP and GABA were diminished by bicuculline hydrochloride (100 μM) whereas the depressant actions of baclofen

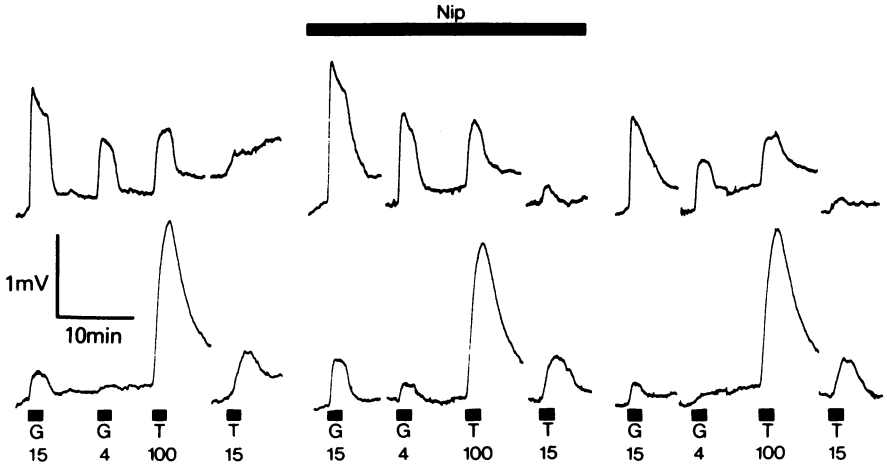

Figure 2 Effect of (±)-nipecotic acid (Nip) 200 ϱM on responses induced by γ-aminobutyric acid (GABA, G) and THIP (T). Recovery is shown 35 min following reintroduction of control bathing medium. The upper trace shows responses of fibres, in an isolated dorsal root, synchronous with the responses in the lower trace from an intact dorsal root arranged for intraspinal recording as described under Methods. Compounds were applied for the periods indicated by the bars above and below the records. Concentrations shown in ϱM. The medium contained 1 mM procaine hydrochloride throughout.

and taurine were still evident despite the considerable increase in synaptic excitation induced by this concentration of bicuculline (Figure 1). The other agonists listed in Table 1 also showed depressant actions similar to THIP but combined with a variable depolarizing component as produced by GABA. The depolarizing action of the agonists was least evident for those compounds having the lowest potency on root fibres (Table 1). Depressant activity was measured by comparison of the amplitude of spontaneous synaptic depolarizations before and during the application of the agonist. The relative potencies determined for THIP on 11 preparations indicated a skewed distribution towards the top end of the range. Hence the lowest potency ratio measured for each agonist is listed also in Table 1. The depolarization produced by some agonists complicated these measurements. However, the reduction in amplitude of synaptic activity which occurred during potassium-evoked depolarizations was always less than the corresponding reduction in amplitude which occurred during similar depolarizations evoked by GABA (Figure 1a). Relative depressant activities of the agonists are listed in Table 1. 4-Amino-3-hydroxybutyric acid (GABOB) and 3-APS (Curtis, Hösli & Johnston, 1968), muscimol, dihydromuscimol, isoguvacine and THIP (Krogsgaard-Larsen, Hjeds, Curtis, Lodge & Johnston, 1979) and *trans*-4-aminocrotonic acid (Johnston, Curtis, Beart, Game, McCulloch & Twitchin, 1975) have the following order of potency as determined by a semiquantitative comparison of depression following electrophoretic application onto cat spinal neurones *in vivo:* muscimol = dihydromuscimol = 3-APS > isoguvacine > THIP > GABA = *trans*-4-aminocrotonic acid > GABOB. The depressant values in Table 1, with the exception of GABOB, are in reasonable agreement with this order of depressant potency.

Role of uptake

The marked difference between the potency of THIP to GABA for depolarization of fibres and for production of synaptic depression (Table 1) may indicate that different receptors are involved in mediation of these two actions. However, this difference in relative potency could just as well be produced through the operation of a removal system within spinal tissue which is more effective for GABA than for THIP (Krogsgaard-Larsen & Johnston, 1978). In order to depress synaptic transmission within the cord, agonists would have to penetrate more deeply into the tissue than would be required for the depolarization of dorsal or ventral root fibres. Hence the relative potency of THIP compared to GABA might be expected to be greater for depression of transmission than for depolarization of fibres.

Figure 2 shows a comparison of the depolarizing action of GABA on an isolated dorsal root and on intraspinal parts of a dorsal root connected to a hemicord preparation; regenerative activity was blocked with procaine 1 mM (Evans, 1978). In such preparations the threshold concentration of GABA required to produce a significant depolarization of fibres was 2 to 3 times higher for intraspinal responses

than for responses of isolated root fibres. Nipecotic acid is an inhibitor of GABA uptake (Krogsgaard-Larsen & Johnston, 1975) and has been shown previously to potentiate GABA-induced responses in isolated spinal cord preparations (Evans, 1979). In the present experiments nipecotic acid (0.05 to 1 mM) produced, at the most, a three-fold potentiation of depolarizations induced by GABA, in intraspinal dorsal root fibres, whereas depolarizations induced by THIP were not significantly affected (Figure 2). GABA-induced depolarizations of extraspinal dorsal root fibres (Figure 2, upper trace) were also potentiated by nipecotic acid. Autoradiographic studies have shown that GABA uptake by rat dorsal roots is principally into glial cells (Schon & Kelly, 1974) and nipecotic acid has been shown to compete with GABA for uptake into glial cells (Minchin, 1979).

The difference between the threshold concentrations of THIP required to depolarize intraspinal and extraspinal dorsal root fibres (compare upper and lower traces Figure 2) is unlikely to be caused through uptake of THIP (Krogsgaard-Larsen & Johnston, 1978). This difference in sensitivity to THIP between intraspinal and extraspinal fibres possibly reflects a difference in receptor type between these two regions.

The effect of nipecotic acid on depolarizing responses induced by GABA or THIP suggests that the 400 fold difference between the relative potencies of these agonists for fibre depolarization and depression of synaptic activity (cf. Table 1) is unlikely to be explained through differential uptake between intraspinal tissue and roots.

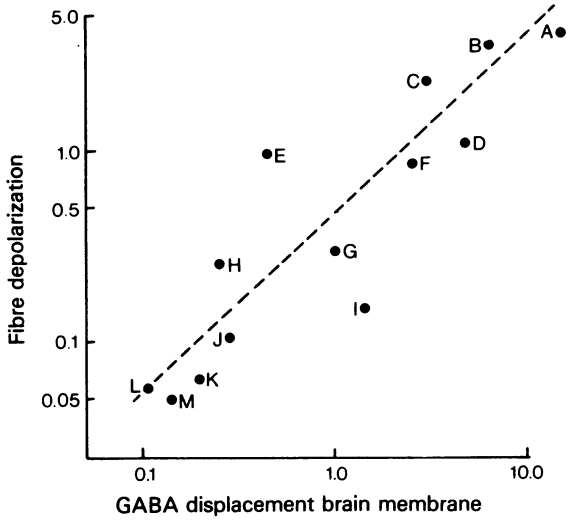

Figure 3 Correlation of dorsal root depolarizing potencies of agonists listed in Table 1 (ordinate scale) with previously reported potencies (IC_{50} GABA/IC_{50} test compound) for displacement of labelled GABA from rat brain membrane preparations. Letters indicate the compounds as listed in Table 1. The linear regression of the parameters (by method of least squares) is indicated by the broken line. For further details see Table 1.

Discussion

The results show that the relative molar potencies of the GABA agonists in depolarizing rat spinal root fibres were not parallelled by their relative molar potencies in depressing spontaneous synaptic activity. As explained above, differences in uptake are probably insufficient to explain these differences in relative potency. Iversen & Johnston (1971) obtained a value of 0.015 mmol kg^{-1} min^{-1} for the V_{max} of GABA uptake by adult rat spinal cord at 25°C. The threshold concentration of GABA required to depress synaptic activity (1 to 2 mM) would saturate this process. Thus it is unlikely that the change in potency of THIP, from 20 times weaker than GABA on fibres to over 20 times stronger than GABA on synaptic depression (Table 1), can be accounted for by differential uptake of the two agonists. Furthermore if differences in the uptake characteristics of agonists were sufficient to explain the differences between relative potencies for depolarization of fibres and relative potencies for synaptic depression, then it should be possible to predict, from a comparison of the potencies given in Table 1, the efficacy of agonists as uptake substrates. The properties of just two compounds, *trans*-4-aminocrotonic acid and (±)-4-aminocyclopent-1-enecarboxylic acid, serve to illustrate the inconsistency of this argument. Both of these compounds are equipotent with GABA in competition for the uptake carrier (Johnston, Allan, Kennedy & Twitchin, 1978a) and the former compound is equipotent with GABA for both depressant and fibre depolarizing activity (Table 1). The latter compound is also equipotent with GABA for fibre depolarization yet is at least 500 times more potent than GABA as a depressant (Table 1). It must be allowed that the above argument does not take into consideration the possible existence of uptake mechanisms which are independent of the GABA-carrier.

The existence of two types of bicuculline-sensitive receptor, which may be termed 'fibre' receptors and 'synaptic' receptors, is a more likely explanation for the non-parallelism of the fibre depolarizing and depressant potencies shown in Table 1. Thus THIP or (+)-*cis*-3-aminocyclopentanecarboxylic acid would be the agonists showing greatest preference for the synaptic receptors and GABA the agonist with greatest preference for fibre receptors with other agonists being intermediate. A similar difference in GABA receptor type has been proposed previously in order

to account for differences between dose-ratios for antagonism of GABA-induced responses of motoneurones and afferent fibres (Evans, 1978). Different classes of bicuculline-sensitive receptors for GABA have been proposed also on the basis of the chemical structure of certain GABA agonists and antagonists (Johnston, Allan, Andrews, Kennedy & Twitchin, 1978b; Andrews & Johnston, 1979).

The fibre receptors present on spinal roots (Evans, 1980) which are characterized by Table 1 are probably identical to the receptors which occur on unmyelinated peripheral nerve fibres (Brown & Marsh, 1978) because the relative potencies of GABA, 3-APS, GABOB and β-guanidinopropionic acid (Bowery & Brown, 1974), isoguvacine, muscimol (Bowery, Collins, Hudson & Neal, 1978) for depolarization of sympathetic ganglia agree closely with the values of Table 1.

A comparison is given in Figure 3, between relative values for displacement of GABA binding to rat brain membranes (Johnston *et al.*, 1978b) and the present relative potencies of the compounds listed in Table 1. A positive correlation (correlation coefficient 0.90, $P < 0.001$), was observed between displacement of GABA binding and fibre depolarizing potencies (Table 1). However, there was no correlation (correlation coefficient 0.50, $P > 0.05$) between displacement of GABA binding and depressant potency. The results of this comparison suggest that the binding of GABA to rat brain membranes is dominated by fibre receptors and that fibre depolarization and GABA displacement are of limited value in predicting the depressant potency of GABA analogues.

GABA has biphasic actions on hippocampal neurones (Langmoen, Andersen, Gjerstad, Mosfeldt-Laursen & Ganes, 1978), and Alger Nicoll (1979) have shown that GABA-mediated depolarizing or hyperpolarizing synaptic responses can be produced at different sites on these neurones. It is possible that the synaptic effects at each site could be mediated exclusively by each of the receptor types discussed above. A comparison between the actions of GABA and (+)-*cis*-3-aminocyclopentanecarboxylic acid or THIP at each of these sites might prove instructive.

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