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DEPOLARIZING ACTIONS OF γ-AMINOBUTYRIC ACID AND RELATED COMPOUNDS ON RAT SUPERIOR CERVICAL GANGLIA *IN VITRO*

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1 Potential changes in rat superior cervical ganglia were recorded *in vitro* with surface electrodes.

2 γ-aminobutyric acid (GABA) produced a transient, low-amplitude ganglion depolarization at rest, and a transient hyperpolarization in ganglia depolarized by carbachol. Depolarization was not prevented by preganglionic denervation. The log dose-response curve for depolarization was sigmoid with a mean ED_{50} of 12.5 μM.

3 The ganglion was depolarized in similar manner by the following compounds (mean molar potencies relative to GABA (=1) in brackets): 3-aminopropane sulphonic acid (3.4), γ-amino-β-hydroxybutyric acid (0.27), β-guanidino-propionic acid (0.12), guanidinoacetic acid (0.057), δ-aminovaleric acid (0.048), β-alanine (0.01), 2,4-diaminobutyric acid, γ-guanidino-butyric acid, taurine and *N*-methyl-GABA (all <0.01). The following compounds did not depolarize the ganglion at 10 mM concentrations: α- and β-amino-*n*-butyric acids, α-amino-*iso*-butyric acid, glycine and glutamic acid.

4 Depolarization declined in the continued presence of GABA. Ganglia thus 'desensitized' to GABA showed a diminished response to other amino acids but not to carbachol.

5 The effect of GABA was not antagonized by hyoscine and hexamethonium in combination, in concentrations sufficient to block responses to carbachol.

6 Responses to GABA were blocked more readily than those to carbachol by bicuculline (IC_{50}, 14 μM) and picrotoxin (IC_{50}, 37 μM). Strychnine (IC_{50}, 73 μM) was a relatively weak and less selective GABA-antagonist.

7 It is concluded that sympathetic ganglion cells possess receptors for GABA and related amino acids which are (a) different from the acetylcholine receptors and (b) similar to GABA receptors in the central nervous system.

Introduction

De Groat (1970) reported that γ-aminobutyric acid (GABA) can depolarize cat superior cervical ganglia *in vivo* when injected into the arterial blood supply, and can depress transmission of preganglionic nerve impulses through the ganglion. The depolarization appeared to differ from that observed with cholinomimetic agents, and instead resembled central inhibitory responses to GABA in terms of agonist and antagonist interaction.

No substantial evidence can as yet be presented for a transmitter function for GABA at this site, since neither GABA itself nor enzymatic mechanisms for its synthesis have so far been detected in ganglia (Nagata, Yokoi & Tsukada, 1966; McBride & Klingman, 1972). Nevertheless, the responses of ganglia to GABA seemed to warrant further study, particularly since a further parallel with the central nervous system appears to obtain with respect to transport processes for GABA (Bowery & Brown, 1972a).

In the present study we report some observations on the depolarizing action of GABA on rat isolated superior cervical ganglia. These experiments were concerned particularly with the chemical specificity of the response, which can be more easily assessed under *in vitro* conditions. For this we used an extracellular recording technique (Pascoe, 1956; Brown, 1966a). Other experiments on the mechanism of the depolarization, in which intracellular electrodes were used, have been

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reported briefly (Adams & Brown, 1973) and will be described in full elsewhere. A preliminary account of the present observations has been given (Bowery & Brown, 1972c).

Methods

Rats (males, about 250 g weight), were anaesthetized with urethane (1.5 g/kg intraperitoneally). The superior cervical ganglion was removed with suitable lengths of pre- and postganglionic trunks attached. The connective tissue sheath was stripped off and the ganglion immersed in 50 ml Krebs solution, previously bubbled with 95% oxygen : 5% carbon dioxide gas mixture and maintained at ambient temperature (between 22° and 28° C). The composition of the Krebs solution (and modifications thereof) used in the present study, were as previously described (Brown, Brownstein & Scholfield, 1972). Surface potentials were recorded from the ganglion by means of a moving-fluid electrode as described by Pascoe (1956) and Brown (1966a). The potential profiles were displayed on an X-Y plotter using the potentiometric mode at 1 mV/inch amplification. In some rats the preganglionic nerve was sectioned unilaterally between 12 and 17 days before experimentation under pentobarbitone anaesthesia with aseptic procedures, and the animals allowed to recover. Both ipsilateral 'denervated' and contralateral normal ganglia were then removed for experimentation as described above. The degree of denervation was checked by observing the ocular effect of preganglionic stimulation before removal of the ganglion.

The following compounds were used: γ-amino-*n*-butyric acid (4-amino-*n*-butyric acid, GABA; B.D.H.); β-alanine, (3-aminopropanoic acid; B.D.H.); glycine (aminoacetic acid, aminoethanoic acid; B.D.H.); δ-aminovaleric acid hydrochloride (5-aminopentanoic acid HCl; Sigma); ε-aminocaproic acid (6-amino-hexanoic acid; Sigma); taurine (2-aminoethane sulphonic acid; B.D.H.); 3-aminopropane sulphonic acid (K. & K. Laboratories); L-α-amino-*n*-butyric acid (L-2-amino-*n*-butanoic acid; Sigma); α-amino-*iso*-butyric acid (AIB, 2-amino*iso*butyric acid; Sigma); DL-β-amino-*n*-butyric acid (3-amino-*n*-butanoic acid; Sigma); L-2, 4-diamino-*n*-butyric acid dihydrochloride (L-2, 4-diamino-*n*-butanoic acid, DABA; Sigma); guanidinoacetic acid (guanido-acetic acid, glycocyamine; Sigma); β-guanidinopropionic acid (3-guanidopropionic acid, *N*-amidino-β-alanine; Sigma); γ-guanidinobutyric acid (4-guanidino-*n*-butyric acid; Sigma); L-glutamic acid, monosodium salt (2-amino-pentanedioic acid, α-aminoglutaric acid, Sigma); DL-α-amino-β-hydroxy-*n*-butyric acid (4-amino-3-hydroxy-*n*-butanoic acid, GABOB; Sigma); carbachol (carbaminoylcholine chloride, B.D.H.); hexamethonium bromide (Koch-Light); bicuculline (Pearce Chemicals); picrotoxin (Sigma); strychnine hydrochloride (Macfarlane Smith); and hyoscine hydrobromide (Martindale Samoore). *N*-methyl-GABA (4-methylamino-*n*-butyric acid) was synthesized according to the method of McElvain & Vozza (1949). γ-Aminobutyryl choline (4-amino-*n*-butyryl choline, GABA choline) was prepared as described previously (Bowery & Brown, 1972b). Agonist drugs were added to the bath rapidly, in a volume not exceeding 2 ml; antagonists were added at the final concentration from a reservoir. Doses refer to molar bath concentrations.

Results

GABA depolarized the isolated rat superior cervical ganglion when applied in a concentration of 1 μM or more (Figures 1-3). The observed responses showed two noticeable differences from those seen with an acetylcholine-receptor stimulant such as carbachol (cf. Brown, 1966a), concerning (respectively) their time-course and amplitude.

Firstly, whereas carbachol produced a sustained depolarization over several minutes application, the depolarization produced by GABA reached a peak within 15 to 30 s of application and thereafter diminished with GABA still present (Figure 1). On washing out the GABA, there was no after-hyperpolarization (associated with ionic recovery processes; Brown *et al.*, 1972), and full sensitivity to GABA was restored within 15 to 20 minutes.

Secondly, the maximum amplitude of the depolarization produced by GABA (average 1.9 mV at 100 μM and above, Fig. 3) was less than half of that which could be obtained with high concentrations of carbachol. Further, the two were not additive in effect, and addition of GABA while the ganglion was strongly depolarized by carbachol produced a hyperpolarization (Figure 2).

The small and rapidly-declining effect of GABA might result from secondary depolarization of the postganglionic nerve trunk, to which the reference electrode is attached. This can be circumvented by crushing the nerve trunk, to prevent electrical potential changes therein (see Brown *et al.*, 1972). This procedure did not alter the magnitude or time-course of the response to GABA, which must therefore reflect ganglionic responses *per se*.

The depolarization might also be abbreviated

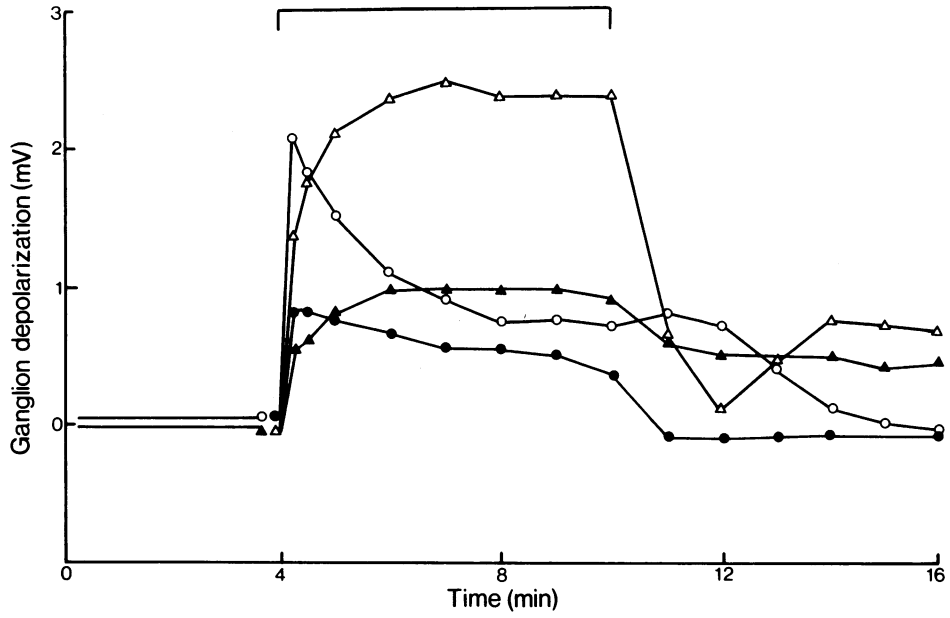


Fig. 1 Superimposed depolarization of a rat isolated superior cervical ganglion by γ-aminobutyric acid (●, 10 μM) and (○, 1 mM) and by carbachol (▲, 4.4 μM) and (△, 11 μM). Ganglion depolarization (mV) measured as a negative shift in the demarcation potential recorded between an electrode on the surface of the ganglion and a second electrode in contact with the postganglionic nerve trunk. (The resting potential difference between the electrodes was reset to zero prior to each drug application.) The drugs were added for 6 min, as indicated by the length of the horizontal bar.

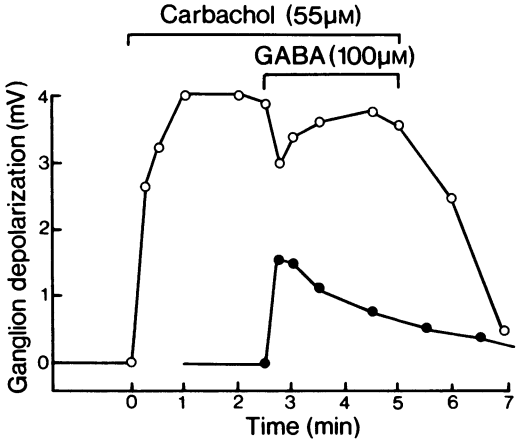


Fig. 2 Potential changes produced by γ-aminobutyric acid (GABA, 100 μM) applied to a ganglion at rest (●) and during a large depolarization induced by the prior addition of 55 μM carbachol (○). Note the reversal of the GABA-induced potential change. Scales as in Figure 1.

by metabolism or by removal of GABA from the extracellular fluid. Uptake of GABA in isolated ganglia can be inhibited by replacing extracellular Na^+ with Li^+ (Bowery & Brown, 1972a); metabolism in the ganglion can be prevented with amino-oxyacetic acid (Bowery, unpublished observations), as in other tissues (Baxter & Roberts, 1961; Neal & Starr, 1973). However, the time-course of the depolarization was not altered by substituting Li^+ for Na^+, nor by adding amino-oxyacetic acid (10 μM).

Dose-response curve and sensitivity

The relationship between the concentration of applied GABA and the peak amplitude of depolarization is shown in Figure 3. This relationship accorded with the conventional expression:

$$\frac{y}{y_{max}} = \frac{x^n}{x^n + K} \qquad (1)$$

where y is the observed response, y_{max} is the asymptotic response, x is the concentration of GABA, n is an integer (the Hill coefficient) and K

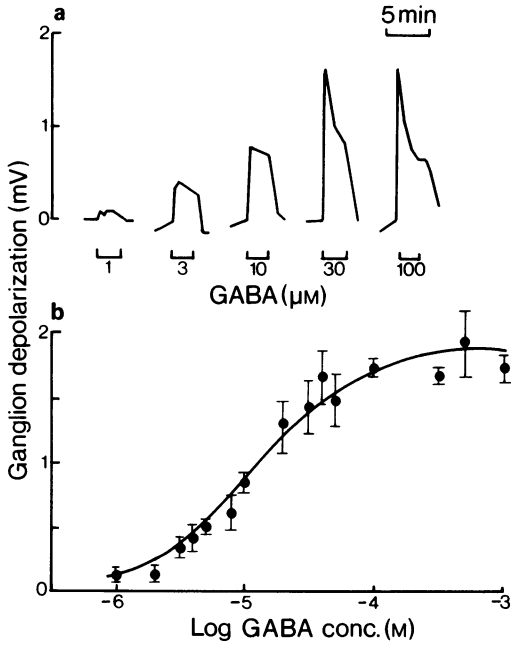


Fig. 3 Ganglion depolarization produced by increasing concentrations of γ-aminobutyric acid (GABA). (a) Records of surface potential changes in a single ganglion plotted as in Fig. 1 but on a contracted time scale. Each concentration of GABA (μM) was applied for 3 min at intervals of at least 15 min (indicated by breaks in the records.) (b) Mean log dose-response curve compiled from observations in 10 ganglia. Each point and vertical bar gives the mean and standard error of at least three measurements. The curve is drawn according to text-equation (1), with K = 12.5 μM (−4.9 log molar), n = 1, and V_{max} = 1.9 mV.

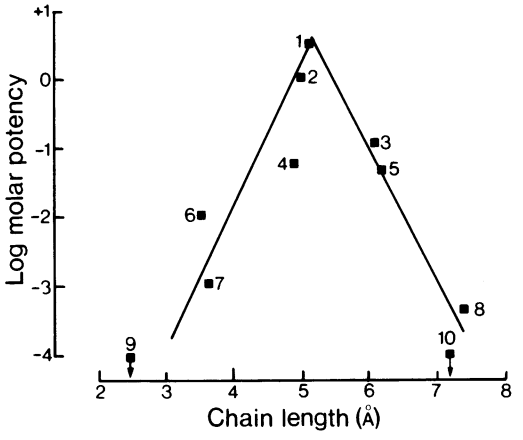


Fig. 4 Relation between ganglion depolarizing activity (log molar potency relative to γ-aminobutyric acid (GABA), see Table 1) and chain length derived from Dreiding models (Ångstroms) between the carboxyl carbon (or sulphonyl sulphur) and amino nitrogen atoms in the extended configuration. Numbers adjacent to points refer to compounds as follows: 1, 3-aminopropanesulphonic acid; 2, GABA; 3, β-guanidinopropionic acid; 4, guanidinoacetic acid; 5, δ-aminovaleric acid; 6, β-alanine; 7, taurine; 8, γ-guanidinobutyric acid; 9, glycine; 10, ε-aminocaproic acid. Arrows below 9 and 10 indicate potency as <0.0001 (no response being detected at 10 mM concentrations).

is a constant. The slope of the curve accords with a Hill coefficient of 1, in which case K is equal to the concentration of GABA producing a half-maximal response (the ED_{50}). The mean ED_{50} was 12.5 μM. The ED_{50} for carbachol was about 8 μM, in approximate agreement with that observed previously (28 μM; Brown, 1966a). Thus, notwithstanding the lower amplitude of depolarization the sensitivity of the ganglion to GABA is comparable with that to carbachol.

Preganglionic nerve degeneration did not obviously impair the response of the ganglion to GABA when compared with the contralateral normally-innervated ganglia (two experiments).

Responses to other amino acids

Several other amino acids with some structural resemblances to GABA also depolarized the ganglion. From comparative dose-response curves, molar potencies of the amino acids relative to that of GABA were calculated as

$$\frac{\text{amino acid } ED_{50}\ (\mu M)}{\text{GABA } ED_{50}\ (\mu M)}$$

the ED_{50} values being determined with reference to the maximum response to GABA. Individual values and means are listed in Table 1. Some general points emerge from this Table with respect to the chemical configuration appropriate to depolarization:

(a) Within homologous series of straight-chain ω-amino acids, ω-sulphonic acids and ω-guanidino acids depolarizing activity depended critically upon the chain length in the extended configuration (cf. Kier & Truitt, 1970; Beart, Curtis & Johnston, 1971). Figure 4 shows the logarithm of the molar potency expressed as a function of the maximum distance between the terminal amino-N and the carbonyl-C or sulphonyl-S atoms, calculated from Dreiding models. Peak activity appears at a maximum $N^+ - C$ distance of 5.2 Å, corresponding to a maximum $N^+ - O^-$ distance of 6.3 Å. On increasing or reducing the chain length, potency diminished in a rather consistent manner,

Table 1 Relative potencies (γ-aminobutyric acid (GABA) = 1) of some amino acids and related compounds as depolarizating agents on the isolated rat superior cervical ganglion, assessed from dose-response curves (see text)

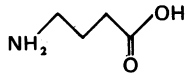
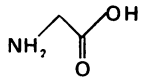
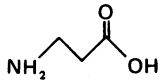
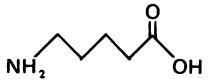
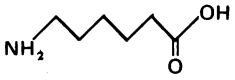
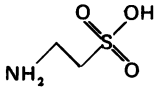
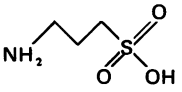
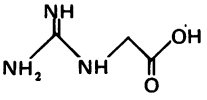
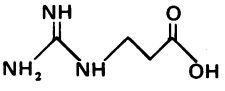
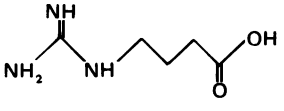
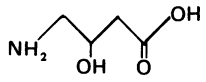
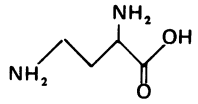
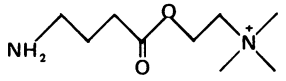
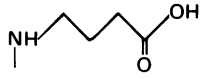
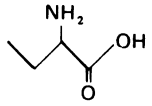
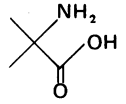
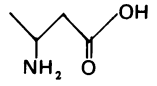
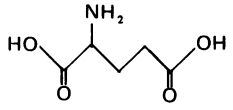
Compound	*Structure*	*Molar potency*	
		Individual values	*Mean*
γ-aminobutyric acid (GABA)			1.0
glycine			<0.0001**
β-alanine		0.01, 0.009, 0.015	0.01
δ-aminovaleric acid		0.087, 0.018, 0.04	0.048
ε-aminocaproic acid			<0.0001**
taurine		0.001, 0.0015, <0.001	0.001
3-aminopropane sulphonic acid		3.1, 2.7, 4.0, 4.0	3.4
guanidinoacetic acid		0.02, 0.1, 0.046, 0.062	0.057
β-guanidinopropionic acid		0.2, 0.06, 0.07, 0.07, 0.2	0.12
γ-guanidinobutyric acid		0.0004, 0.001	0.0007

Table 1—*continued*

Compound	*Structure*	*Molar potency* *Individual values*	*Mean*
γ-amino-β-hydroxy butyric acid		0.2, 0.33, 0.33, 0.2	0.27
2,4-diaminobutyric acid		0.001, 0.0013, 0.0013	0.0012
GABA-choline		0.47, 0.43, 0.50	0.47
GABA-choline with physostigmine*		0.013, 0.05, 0.013	0.025
N-methyl-GABA		0.005	0.005
α-amino-*n*-butyric acid			<0.0001**
α-amino-iso-butyric acid			<0.0001**
β-amino-*n*-butyric acid			<0.0001**
glutamic acid			<0.0001**

In each experiment one or more amino acids was compared directly with GABA: numbers give individual values and means of molar potencies.

* See Bowery & Brown (1972b).

** No depolarization observed at highest concentration applied (10 mM).

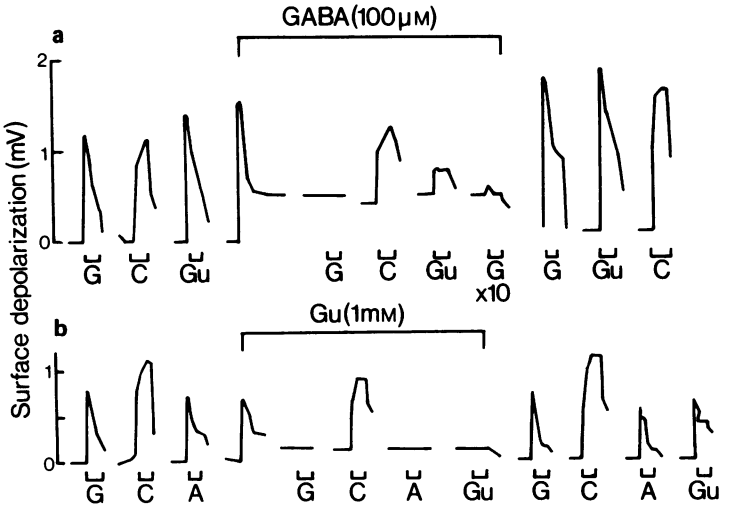


Fig. 5 Responses of two ganglia to γ-aminobutyric acid (GABA) (100 μM, G), carbachol (4.4 μM, C), β-guanidinopropionic acid (1 mM, Gu) and β-alanine (10 mM, A) applied for brief (4 min) periods at rest and in the presence of (a) 100 μM GABA and (b) 1 mM guanidinopropionic acid (present for the duration indicated by the horizontal bars above the records). Abscissae: time, expressed in a discontinuous manner, the bars below each response representing the period of addition (4 min) and the breaks representing rest periods of at least 15 minutes. Note that the responses to continuous applications of GABA and β-guanidinopropionic acid declined, and that during their presence responses to other amino acids but not to carbachol were occluded.

by about 2 log units per Å change from 5.2 Å. Inhibition of GABA transaminase with aminooxyacetic acid (10 μM) (see above) did not alter the relative potencies of GABA and 3-aminopropanesulphonic acid (cf. Beart & Johnston, 1973), suggesting the potency data to be uninfluenced by differential metabolism.

(b) *N*-alkylated γ-amino- or α-amino and β-amino derivatives showed little activity, and 2- or 3-substituted γ-amino derivatives showed reduced activity. The potency of GABAcholine is spuriously high because of partial hydrolysis to free GABA (see Bowery & Brown, 1972b).

(c) The other postulated central inhibitory amino-acid transmitter, glycine, did not produce a detectable depolarization at 10 mM concentrations, and central excitatory dicarboxylic acids such as glutamate, aspartate and (±)-homocysteate failed to depolarize the ganglion at concentrations of 1 mM or more.

Further tests with some of the more active amino acids showed that their effect resembled that of GABA rather than of carbachol. They all produced a low amplitude depolarization of similar magnitude to that obtained with GABA, and the dose-response curves for GABA and amino-acid were approximately parallel. Also, the depolarization produced was transient and declined in the continued presence of the amino-acid, as with GABA. At such time, application of GABA failed to produce a depolarization, whereas carbachol remained effective (Figure 5). Conversely, a high concentration of GABA occluded the response to other amino acids. A partial exception to this mutual occlusion occurred with guanidinopropionic acid: although this compound occluded fully the response to GABA, it retained some slight depolarizing activity in the presence of GABA (100 μM).

Action of antagonists

Depolarization by GABA and carbachol could be distinguished quite clearly with acetylcholine-receptor blocking agents. In this tissue, carbachol depolarization is mediated through activation of both nicotinic and muscarinic receptors (Brown, 1966b). A muscarinic component is particularly noticeable with the low carbachol concentrations used to produce low-amplitude depolarizations matching those obtained with GABA, such that the response to carbachol is strongly depressed by hyoscine (Figure 6). Complete suppression of the carbachol responses requires a combination of both hyoscine and a nicotinic blocking agent such as hexamethonium. In contrast, neither hexamethonium nor hyoscine, nor a mixture of the two, antagonized the effect of GABA. The

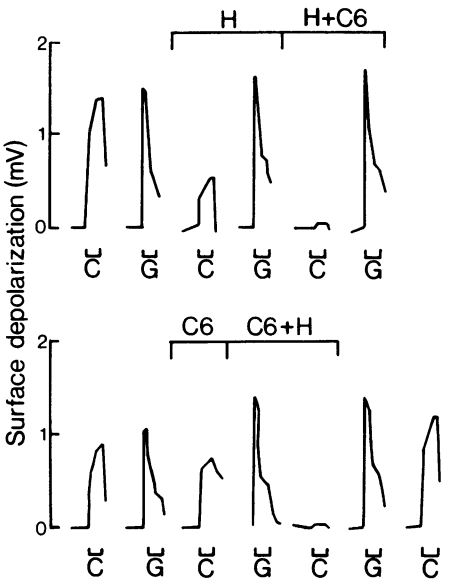


Fig. 6 Effects of hyoscine (H, 2.6 μM), hexamethonium (C6, 0.83 mM), and a mixture of the two on responses of two ganglia to carbachol (C, 4.4 μM) and γ-aminobutyric acid (GABA) (G, 100 μM). Scales as in Figure 5. GABA and carbachol were added for 4 min periods (bars below responses) at intervals of >15 min (breaks in records); hyoscine and/or hexamethonium were present for the duration indicated by the horizontal bars above the records.

discriminatory effect of hexamethonium (in the presence of hyoscine) is shown in Fig. 7d, where the responses to GABA and carbachol (tested in equally effective doses) are plotted against the concentration of added hexamethonium. The concentration required to depress the response to carbachol by 50% (IC_{50}) was about 50 μM whereas the IC_{50} versus GABA was in excess of 2 mM, giving a discrimination ratio of >40.

On the other hand, bicuculline was a more effective antagonist to GABA than to carbachol (Fig. 7a), the respective IC_{50} values being 14 and 230 μM. This accords with the qualitative observations of De Groat, Lalley & Block (1971) on cat ganglia *in vivo*. Bicuculline also antagonized other amino acids (β-alanine, 3-aminopropane sulphonic acid and β-guanidinopropionic acid) with equal facility (Figure 8). Antagonism by bicuculline was reversible on washing, and was fully surmountable on increasing the concentration of GABA, such that the GABA dose-response curve showed a parallel shift to the right without depression of the maximum response (Figure 9). However, the magnitude of the shift (0.63 log units for a ten-fold increase in bicuculline concentration) was rather less than that (0.95) expected for a simple competitive inhibition. No evidence for any depolarizing action of bicuculline itself, or for potentiation of GABA responses at sub-blocking concentrations, was obtained (cf. Straughan, Neal, Simmonds, Collins & Hill, 1971).

Picrotoxin also antagonized the response to GABA (as reported by De Groat, 1970), at somewhat higher concentrations than observed with bicuculline (IC_{50}, 37 μM). A concentration five times higher (180 μM) was required to depress the response to carbachol by 50%.

Strychnine was a weak GABA antagonist (IC_{50}, 73 μM), and not very selective in that appreciable depression of the response to carbachol was also observed (IC_{50} against carbachol, 174 μM).

Discussion

The observations on rat ganglia *in vitro* described in this paper confirm and extend those of De Groat (1970) on cat ganglia *in vivo*. They show that GABA depolarizes rat ganglia, apparently through a direct action on the ganglion cells since its effect persisted in preganglionically-denervated ganglia. Depolarization was not mediated through acetylcholine receptors because it was not blocked by concentrations of hexamethonium or hyoscine (separately or in combination) sufficient to antagonize the effect of carbachol. Instead, a separate and single amino acid receptor appears to be involved. Thus, the active amino acids were antagonized to the same extent by bicuculline (Fig. 8) and showed mutual occlusion (Fig. 5), probably reflecting receptor desensitization (see below). Since optimal potency was observed for GABA itself and its sulphonic acid derivative (Fig. 4), it seems reasonable to term the receptor a 'GABA receptor'.

Several questions are raised by these experiments, of which the following appear worth consideration at this stage: (a) how does the ganglionic GABA-receptor compare with those at other sites in terms of chemical specificity? (b) what is the ionic mechanism of the depolarization, and why does GABA depolarize the ganglion rather than hyperpolarize it? (c) how does GABA-depolarization affect transmission? (d) what significance might attach to the presence of GABA receptors in ganglia?

(a) Comparison with other GABA-receptors

The structure-activity relations for ganglionic depolarization in Table 1 accord broadly with the

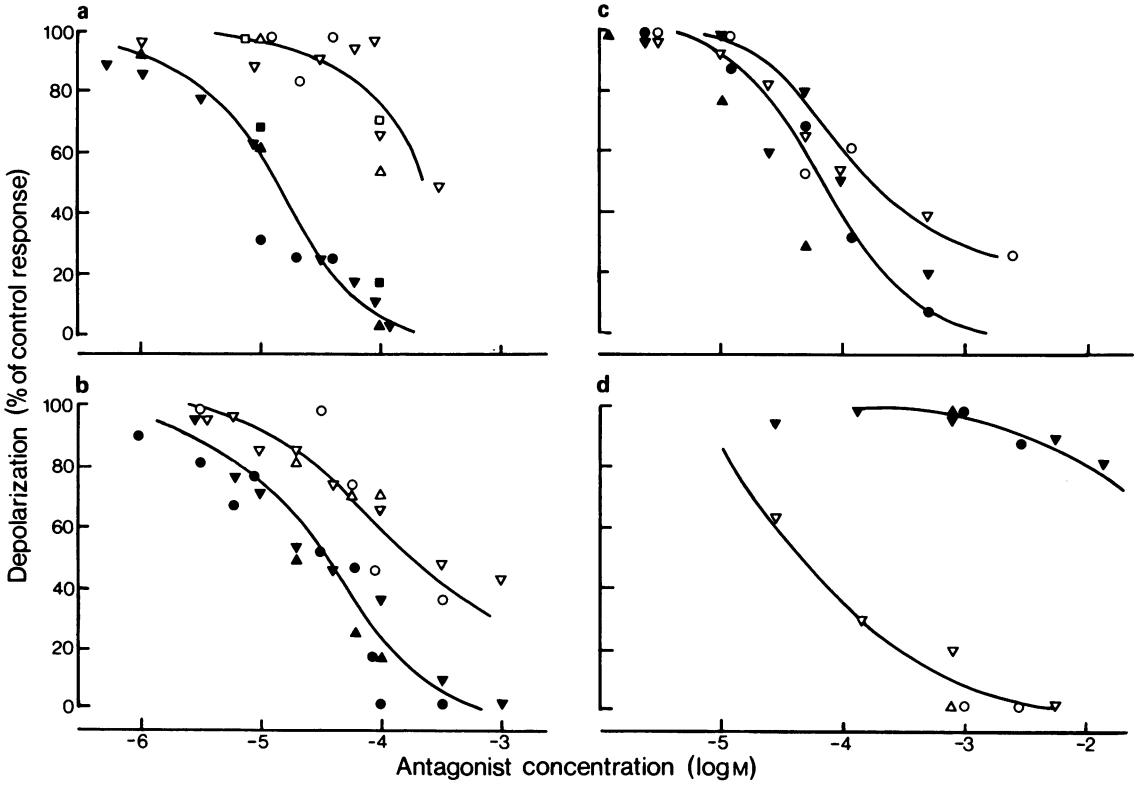


Fig. 7 Effects of increasing concentrations of (a) bicuculline, (b) picrotoxin, (c) strychnine and (d) hexamethonium (in the presence of 2.6 μM hyoscine, see Fig. 6) on ganglion depolarization produced by equi-effective concentrations of γ-aminobutyric acid (GABA) (filled symbols) and carbachol (open symbols). Depolarization is expressed as % of that attained in the absence of antagonist by each of the agonists. In each experiment the two agonists were applied alternately for 4 min periods at 15 min intervals in the absence (= 100% response) and presence of cumulative concentrations of one of the antagonists. Each symbol pair (○ ●, △ ▲, ▼ ▽, □ ■) represents a separate experiment. Lines are drawn by eye.

criteria for inhibitory amino acid structure given by Curtis & Watkins (1965). A comparison of the relative potencies of some of the more active amino acids at the ganglion and at presumptive GABA-transmitting junctions is given in Table 2. There is clearly a close parallel between the ganglion and crustacean junctions, for example with respect to the effect of chain length upon activity. A similarity to GABA-receptors in the vertebrate central nervous system is apparent on other grounds (see below), but is rather obscured in Table 2 by the presence on central neurones of additional receptors for short-chain amino acids such as glycine, distinguished from GABA-receptors by their sensitivity to strychnine (Curtis, Hösli & Johnston, 1968; Davidoff, Aprison & Werman, 1969; Obata, Takeda & Shinozaki, 1970; Curtis, Duggan & Johnston, 1971). An interaction with glycine-receptors probably explains the pronounced effect of β-alanine and taurine on central neurones (Curtis *et al.*, 1968; Hösli, Tebecis & Filias, 1969; Obata *et al.*, 1970). The ganglion does not appear to possess glycine-receptors: glycine itself had negligible activity (<1/10,000th of that of GABA) and the potency-chain length spectrum (Fig. 4) showed no inclination toward a bimodal distribution. The optimum configuration for activation of ganglionic GABA-receptors corresponds with that previously deduced for central receptors in terms of the maximum distance between the $N^+ - C\text{-}O^-$ charge centres (see Kier & Truitt, 1970; Beart *et al.*, 1971). The latter were derived from consideration of the activity of rigid GABA-analogues: this

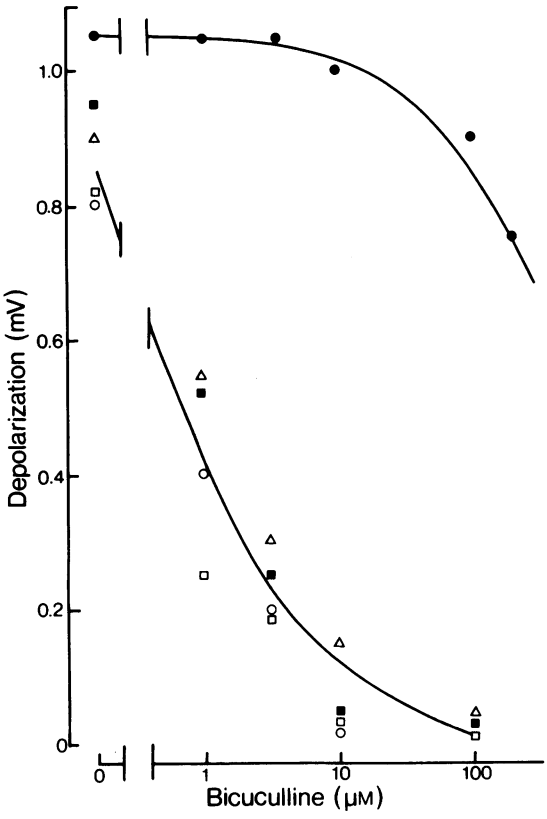


Fig. 8 Effect of cumulative increasing concentrations of bicuculline on the depolarization of a single ganglion produced by carbachol (●, 4.4 μM), γ-aminobutyric acid (■, 20 μM) 3-aminopropanesulphonic acid (○, 7 μM), β-alanine (□, 2.2 mM) and β-guanidinopropionic acid (△, 150 μM). The agonists were applied in rotation for 4 min periods at 15 min intervals.

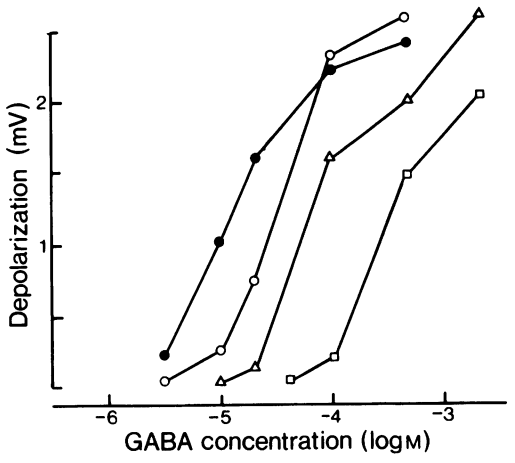


Fig. 9 Log concentration-depolarization relationships for γ-aminobutyric acid (GABA) in the absence (●) and presence of 1 μM (○), 10 μM (△) and 100 μM (□) bicuculline. Observations on a single ganglion with cumulative addition of bicuculline. GABA doses were applied for 4 min periods at 15 min intervals; bicuculline was added 30 min before testing, to allow equilibration.

implied that GABA normally interacts with the receptors in a fully-extended configuration, in spite of an apparent tendency toward partial folding in crystalline form (Steward, Player, Quilliam, Brown & Pringle, 1971). The symmetrical potency-chain length graph in Fig. 4 lends support to the extended configuration hypothesis.

The antagonistic effects of bicuculline and picrotoxin on the ganglionic responses to GABA accord with their reported effects on central neurones (Curtis, Duggan, Felix & Johnston, 1970; Hill, Simmonds & Straughan, 1972). Their selectivity *vis-à-vis* GABA and carbachol support the view of a specific block of the GABA-receptor. On the other hand, some differences emerge between ganglionic and crustacean GABA-receptors concerning the relative activities of bicuculline and picrotoxin and their surmountability by excess GABA (cf. Takeuchi & Takeuchi, 1969; Takeuchi & Onodera, 1972). A further difference obtains with β-guanidinopropionic acid, which behaves as a GABA-analogue on the ganglion but a GABA-antagonist on crustacean muscle (Dudel, 1965; Feltz, 1971). Thus, viewed from an antagonist viewpoint, the ganglionic GABA-receptors resemble those in the vertebrate central nervous system rather more closely than those in invertebrates.

(b) Mechanism of depolarization

Extracellular recording techniques are not suited to an examination of the ionic basis for the depolarization. However, parallel experiments with intracellular electrodes (Adams & Brown, 1973) have shown a comparable permeability change (to chloride ions) to that in central neurones (Krnjević & Schwartz, 1967). Depolarization might then result if the intracellular chloride concentration were sufficiently high to result in a net efflux of chloride during the action of GABA; reversal of this electrochemical gradient during carbachol depolarization would explain the ganglion hyperpolarization produced by GABA in the presence of carbachol (Figure 2). The waning depolarization in

Table 2 Comparison of the activities of γ-aminobutyric acid (GABA)-like amino acids on the rat superior cervical ganglion (column 1, taken from the present paper) with those previously published for several other test preparations.

	Relative activity (GABA = 1)								
Amino acid	*Rat ganglion (1)*	*Dog B.P. (2)*	*Crayfish stretch receptor (3)*	*(4)*	*Crayfish neuromuscular junction (5)*	*(6)*	*Toad spinal cord (7)*	*Cat moto-neurones (8)*	*Cat cortical inhibition (9)*
GABA	1	1	1	1	1	1	1	–––	++++
glycine	<0.0001	0	0.0008	0	0.00014	0	0.67	––	++
β-alanine	0.01	0.007	0.05	0.11	0.033	0.02	1.4	–––	++++
δ-aminovaleric acid	0.048	0.01	0.07	0.04	0.02	0.05	0.22	––	++
ε-aminocaproic acid	<0.0001	0	0.01	0.02	0.0005	<0.001		–	0
taurine	0.001	0.001	0.005	0.004		<0.02	1	––	0
3-aminopropane sulphonic acid	3.4						3.3		
guanidinoacetic acid	0.057		0.57	0.57	0.33		1	0	++++
β-guanidinopropionic acid	0.12		0.71	1.23	0.5		0.17		++++
γ-guanidinobutyric acid	0.0007		0.12	0.03	0.01		0	0	++
α-amino-*n*-butyric acid	<0.0001	0				0		0	0
β-amino-*n*-butyric acid	<0.0001				0.0003	<0.001	0	0	0
α-amino-*iso*-butyric acid	<0.0001	0	0.002	0.002				–	
γ-amino-β-hydroxy butyric acid	0.27			0.14	0.5			––	++++
2,4-diaminobutyric acid	0.0012		0.005			<0.01	0.14	–	+
N-methyl-GABA	0.005								

Numbers in columns (1) to (7) give molar potencies as defined in the text: GABA = 1. In columns (8) and (9) relative activities are given in the arbitrary units employed by the original investigators (see references). In (8) the number of – signs is inversely related to the amount of current required to discharge an effective amount of drug iontophoretically; in (9) the number of + signs is proportioned to the depression of evoked responses in the cerebellar cortex on topical application of the compounds in 0.1-1% solution.

(1) Present paper; (2) Stanton & Woodhouse (1960); (3) Edwards & Kuffler (1959); (4) McGeer, McGeer & McLennan (1961); (5) Dudel (1965); (6) Robbins (1959); (7) Curtis, Phillis & Watkins (1961); (8) Curtis & Watkins (1960); (9) Purpura, Girado, Smith, Callan & Grundfest (1959).

the continued presence of GABA results principally from receptor-inactivation rather than from exhaustion of the ionic battery, since the membrane conductance change also declines under these conditions (Adams & Brown, 1973 and unpublished observations; see also Dreifuss, Kelly & Krnjević, 1969). (Inactivation of GABA as a contributory factor is excluded by the experiments with lithium and amino-oxyacetic acid.) The amino acid occlusion phenomenon then implies interaction with a common receptor, rather than simply with the same ionic channels. Rapid desensitization may explain the low Hill coefficient ($n = 1$) for the dose-depolarization curve (Fig. 3): in crayfish muscle, where desensitization is less pronounced, Hill coefficients of 2 (Takeuchi & Takeuchi, 1967) and 3 (Feltz, 1971) have been reported.

(c) Transmission

De Groat (1970) reported that GABA depressed the transmitted action potential in cat ganglia *in vivo*. We did not routinely monitor transmission in the present experiments but have noted a reduced postganglionic response on other occasions (Bowery, unpublished observations). A reduced ganglion cell spike amplitude or failure of spike generation has also been seen with intracellular electrodes (Adams & Brown, 1973). De Groat's (1970) experiments showed the chemical requirements for ganglion depolarization and depression of transmission to be quite similar. Also, the structural requirements for GABA-like hypotensive activity in the dog, which probably results from impaired ganglionic transmission, accord fairly well with those for rat ganglion depolarization (see Stanton & Woodhouse, 1960, and Table 2). The latter authors report that cats are more resistant to the hypotensive effect of GABA; there appears to be no direct information on ganglion-blocking and hypotensive actions of GABA and its derivatives in rats.

(d) Significance

The presence of receptors for GABA in sympathetic ganglia need not imply a functional role for this compound within the ganglion. Such a dissociation is not without precedent, for example, the presence of acetylcholine receptors within non-innervated tissues such as amnion (Cuthbert, 1962) and foetal cardiac and skeletal muscle (Burn, 1954; Diamond & Miledi, 1962; Carmel, Gimeno & Leyden, 1966). GABA receptors on ganglion cells might relate to their embryonic origin from neural crest tissue: the absence of any response to glycine and glutamate may then appear the more surprising. In this context it is worth noting that 'autonomic' sensory neurones in the nodose ganglion are also depolarized by GABA (De Groat, 1972; and Bowery, unpublished observations).

Notwithstanding such teleological considerations, the present observations support the view of De Groat (1970) that the ganglion can provide a helpful model of mammalian GABA-sensitive neurones for certain experimental purposes. Studies on mammalian central neurones may be limited by (a) difficulties in quantitation of responses inherent in iontophoretic application techniques (cf. Curtis *et al.*, 1971); (b) the presence of separate glycine receptors mediating a qualitatively similar response; and (c) the possibility that GABA-like substances may release endogenous GABA in addition to (or instead of) acting directly on the GABA-receptors. These difficulties may be obviated by the use of isolated sympathetic ganglia.

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