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INFLUENCE OF NEUROGLIAL TRANSPORT ON THE ACTION OF γ-AMINOBUTYRIC ACID ON MAMMALIAN GANGLION CELLS

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1 The effect of inhibiting the transport of γ-aminobutyric acid (GABA) by neuroglial cells on the depolarizing action of exogenous amino acids on isolated superior cervical ganglia of the rat was studied.

2 Transport (measured by uptake of [^{3}H]-GABA) was inhibited by (a) reducing external [Na^+] from 143 to 2 mM and (b) administering alternative carrier-substrates, 3-amino-*n*-butyric acid (β-amino-butyric acid, BABA) and (±)-nipecotic acid at a concentration of 1 mM.

3 All three procedures enhanced the depolarization produced by low concentrations of GABA ($\leqslant 10$ μM) but did not alter the maximum response, nor the response to 3-aminopropanesulphonic acid (a gabamimetic with low affinity for the neuroglial carrier).

4 It is concluded that the neuroglial uptake process can limit the action of exogenous GABA upon neurones, by reducing the interstitial GABA concentration.

Introduction

The central inhibitory neurotransmitter γ-amino-butyric acid (GABA) depolarizes neurones in sympathetic ganglia (De Groat, 1970; Bowery & Brown, 1974), by increasing Cl^- conductance (Adams & Brown, 1975). The neurones in this tissue are closely invested by neuroglial (satellite) cells, which possess a transport system capable of taking up exogenous radioactively-labelled GABA (Bowery & Brown, 1972; Young, Brown, Kelly & Schon, 1973). The question addressed in the present experiments is whether the effect of exogenous GABA on sympathetic neurones is modified by the activity of the adjacent glial carrier.

The results of our previous experiments are not clear on this point. Bowery & Brown (1974) and Adams & Brown (1975) observed no gross change in the depolarizing action of GABA on omitting Na^+ ions from the bathing medium, a procedure that inhibits carrier-mediated uptake of GABA by the glial cells (Bowery & Brown, 1972), but rather high concentrations of GABA were used in these experiments. On the other hand, Bowery, Brown, Collins, Galvan, Marsh & Yamini (1976: Figure 10) detected an increase in the depolarizing action of very low concentrations (< 10 μM) of GABA in Na^+-free solution.

In the present experiments the effect of reducing external [Na^+] on the depolarizing action of GABA has been assessed more carefully than hitherto. In addition, another method of reducing glial uptake of GABA, the application of false substrates (β-amino-butyric acid, BABA: Bowery *et al.*, 1976) and (±)-nipecotic acid (Krogsgaard-Larsen & Johnston, 1975) has been tested.

Methods

Superior cervical ganglia with their attached pre- and postganglionic nerve trunks were isolated from Wistar rats (230–270 g, either sex) anaesthetized with urethane (1.5 g/kg). Potentials were recorded from desheathed superfused preparations as described by Brown & Marsh (1975). The superfusion fluid was Krebs-Henseleit solution of composition described previously (Bowery *et al.*, 1976), gassed with 95% O_2 and 5% CO_2 and maintained at 25°C. In the present experiments ganglia were isolated the day before use and maintained overnight at 4°C: by allowing the initial demarcation potential to subside, this gives greater d.c. stability for high-gain recording, without altering the response of the ganglia to agonists or the

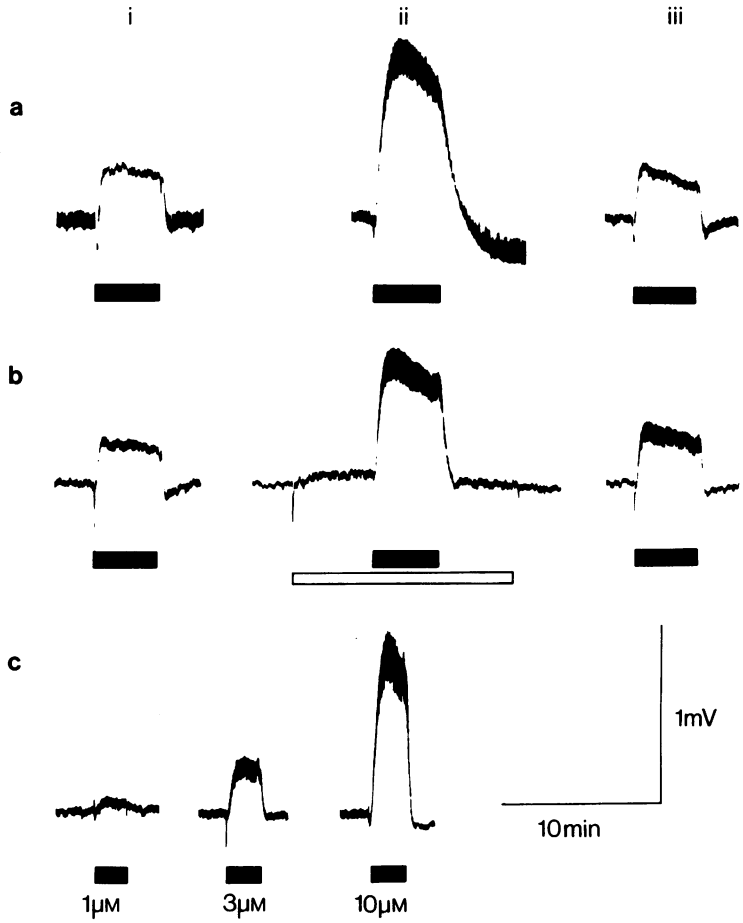

Figure 1 Depolarizations of an isolated ganglion of the rat produced by 4-min applications of 3 μM γ-aminobutyric acid (GABA, solid bars), (a) before (i), during (ii) and after (iii) exposure to low [Na^+] solution and (b) before, during and after exposure to 1 mM β-aminobutyric acid (open bar). (c) Shows responses to calibrating concentrations of GABA applied for 2-min periods at the end of the experiment. Scale: 1 mV, 10 minutes.

activity of the GABA carrier when compared with freshly-dissected ganglia.

The normal Na^+ concentration of the superfusing fluid was 143 mM (118 mM as NaCl, 25 mM as $NaHCO_3$). Low (2 mM) Na^+ solution was prepared by replacing all but 2 mM Na^+ with Li^+, as LiCl and Li_2CO_3.

Saturable influx of [^{3}H]-GABA into isolated ganglia at 25°C was measured as described by Bowery *et al.* (1976), with 10 μM amino-oxyacetic acid added to the incubation medium to preclude metabolism of label. Inhibitors of uptake were added 10 min before adding substrate.

The following compounds were used: γ-aminobutyric acid (4-amino-*n*-butyric acid, GABA: BDH); β-aminobutyric acid (3-amino-*n*-butyric acid, BABA: Sigma); and 3-aminopropanesulphonic acid (3-APS: K. & K.). (±)-Nipecotic acid was synthesized by C. Cooksey according to the method of Freifelder (1963). Agonists were superfused over the preparation for 4 min at 30-min intervals, unless otherwise stated. The superfusion rate was ≃2 ml/minute. Dead time was noted using an air-interface, and exposure times corrected accordingly. Concentrations refer to those in contact with the ganglion.

Results

Figure 1 illustrates one type of experiment carried out. In the upper trace (Figure 1a) constant low-amplitude depolarizing responses to a just-suprathreshold con-

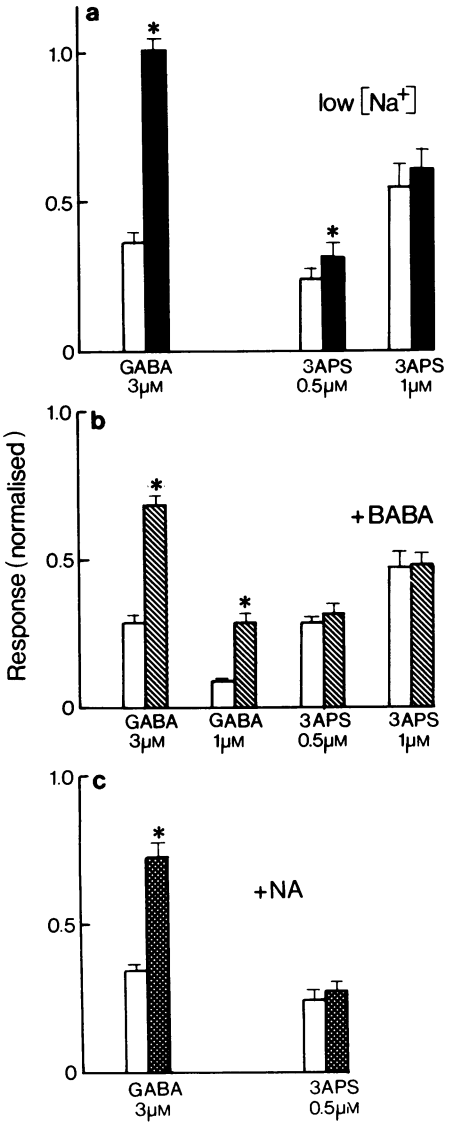

Figure 2 Effects of inhibiting glial transport of γ-aminobutyric acid (GABA) upon the depolarizing action of exogenous GABA and 3-aminopropanesulphonic acid (3-APS). Responses to low doses of GABA and 3-APS are shown in normal solution (open columns), (a) in low [Na^+] solution (closed columns), and during superfusion with either (b) 1 mM β-aminobutyric acid (BABA, hatched columns) or (c) 1 mM (±)-nipecotic acid (NA, cross hatched columns). Responses were normalized with respect to those produced by 10 μM GABA in normal solution (=1.0). Each column is the mean of 6 experiments except for experiments with (±)-nipecotic acid, where $n=4$. Vertical bars show s.e. means.

*Significant difference between means ($P<0.001$, Student's t test); unmarked columns, $P>0.05$.

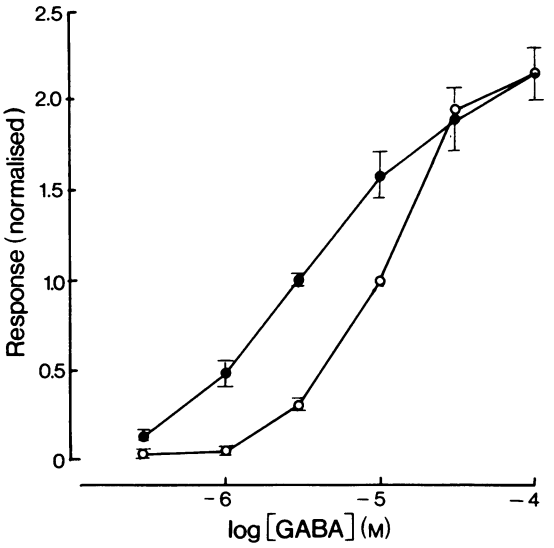

Figure 3 Dose-response curves to γ-aminobutyric acid (GABA) measured in normal solution (O) and low [Na^+] solution (●). Responses were normalized with respect to those produced by 10 μM GABA (=1.0) in normal solution to facilitate comparison between different ganglia. Each point is the mean of 6 determinations in different ganglia; bars=s.e. mean.

centration (3 μM) of GABA were obtained in normal Krebs solution containing 143 mM [Na^+] (Record i). This solution was then replaced with one containing 2 mM [Na^+] and 141 mM [Li^+] (Record ii). After the electrode junction-potential changes had stabilized, 3 μM GABA now gave a response equal to that produced by 10 μM GABA in 143 mM [Na^+] solution (Figure 1c). This potentiation was fully reversed on restoring Na^+ ions (Record iii).

In the experiment illustrated in Figure 1b, a similar procedure was used except that 1 mM β-aminobutyric acid (BABA) was added to inhibit GABA uptake. The response to 3 μM GABA now became equivalent to that of 6 μM GABA in the absence of BABA.

A number of experiments of this type were performed, some using as an additional agonist 3-aminopropanesulphonic acid (3-APS). The results are summarized in Figure 2. There were consistent and substantial increases in the responses to 3 μM GABA in low-Na^+, BABA and (±)-nipecotic acid solutions, but no corresponding increase in the equivalent-amplitude responses to 3-APS.

The effect of low-Na^+ solution on the dose-response curve to GABA is shown in Figure 3. The control curve, in 143 mM [Na^+] (ED_{50} 11 μM) is comparable with that previously described by Bowery & Brown (1974) (ED_{50} 12.5 μM). Low-Na^+ solution increased the responses to low (≤10 μM) GABA concentrations,

without altering the maximal response, thereby producing a non-parallel shift to the left and a new ED_{50} of 3.5 μM.

Uptake inhibition

The percentage inhibition of saturable [^{3}H]-GABA uptake at the substrate and inhibitor concentrations used in the present experiments are given in Tables 1 and 2.

At 3 μM [^{3}H]-GABA, saturable uptake was inhibited by 94% in 2 mM [Na^+], 70% in 1 mM (±)-nipecotic acid and 35% in 1 mM BABA. Inhibition in 2 mM Na^+ solution was constant at substrate concentrations from 0.3 to 100 μM, i.e., over the range in Figure 3.

Discussion

In these experiments, procedures that reduced the uptake of [^{3}H]-GABA into ganglionic neuroglial cells (reducing external [Na^+] to 2 mM, or adding 1 mM β-aminobutyric acid (BABA) or (±)-nipecotic acid) strongly potentiated the depolarization of the ganglionic neurones produced by GABA. Available evidence suggests these two effects to be causally related for the following reasons:

(1) The same procedures did not appreciably affect the action of 3-APS. The concentrations of 3-APS used (≤1 μM) are so far below its K_T for the GABA carrier (0.8 mM: Bowery, 1974) that it could be transported only at a very low rate. (A slight, though significant, augmentation at the lowest concentrations of 3-APS could be detected (see Figure 2): this is to be expected through the small additive effect of endogenous GABA released during uptake inhibition, Bowery *et al.*, 1976).

(2) The degree of potentiation showed some relationship to the amount of uptake inhibition produced by the three procedures. Potentiation by BABA is, perhaps, disproportionately great: this could be due to its weak direct agonist activity, which is just around threshold at the concentration used (1 mM: cf. Bowery *et al.*, 1976): (±)-nipecotic acid appears to

Table 1 Uptake of [^{3}H]-γ-aminobutyric acid measured after 30 min incubation in 3 μM substrate solution at 25°C in the presence of 10 μM amino oxyacetic acid, expressed as the tissue/medium ratio (concentration of label in ganglion/concentration in incubation medium), in the absence and presence of transport inhibition.

	Tissue/medium ratio mean ± s.e. (n)		*Mean % inhibition*
Control	7.99 ± 0.63	(10)	0
2 mM [Na^+]	0.50 ± 0.05	(8)	94
1 mM (±)-nipecotic acid	2.41 ± 0.11	(4)	70
1 mM β-aminobutyric acid	5.19 ± 0.27	(8)	35

Uptake values are corrected for the minor non-saturable infiltration (Bowery *et al.*, 1976).

Table 2 Effect of reducing [Na^+] from 143 mM to 2 mM on uptake of [^{3}H]-γ-aminobutyric acid (GABA) at different GABA concentrations

	Uptake velocity (μmol per l cell fluid/min)		
μM GABA	*143 mM [Na^+]*	*2 mM [Na^+]*	*% Inhibition*
0.3	0.189	0.017	91.0
1.0	0.804	0.051	93.7
3.0	1.80	0.146	91.9
10.0	5.44	0.346	93.6
30.0	8.99	0.706	92.2
100.0	9.98	1.02	89.8

Uptake is expressed as mean saturable influx velocity over a 30-min incubation period after subtraction of non-saturable uptake; comparisons at each substrate concentration are made between paired contralateral ganglia from the same rat.

have no direct agonist activity (N.G. Bowery, personal communication).

(3) The concentration-sensitivity of low-Na^+ effects (Figure 3) follows from the saturable nature of carrier-mediated transport, such that proportionately less GABA is cleared from the interstitial spaces as the apparent Michaelis constant ($K_T \sim 7\,\mu M$; Bowery & Brown, 1972) is approached or exceeded (the fractional inhibition of uptake in low Na^+ being independent of substrate concentration).

Further consideration of the lateral shift in the dose-response curve in Figure 3 suggests that, at a nominal external GABA concentration of 10 μM in normal (143 mM [Na^+]) solution the effective interstitial concentration is only around 3 μM, i.e., the steady-state concentration in the tissue is reduced some 70% by the glial transport process. At a nominal external concentration of 3 μM, a larger reduction to 0.6 μM interstitial concentration appears to result. One consequence of this is that the previously-obtained potency measurements, made in normal Na^+ solution with a functional carrier (Bowery & Brown, 1974) need revision. In particular, if corrected for the influence of the carrier, GABA becomes equipotent with 3-APS as an agonist, instead of one-third as potent as previously deduced (the potency measurements having been made from the apparent ED_{50} values).

Thus, the present observations imply that neuroglial cells in sympathetic ganglia are capable of net inward GABA transport at a rate sufficient to reduce the interstitial concentration of GABA. This may have some functional significance, since the concentration of GABA in the plasma has been estimated at 2–4 μM in cats (Crowshaw, Jessup & Ramwell, 1967). Inspection of Figure 3 shows that, in the absence of carrier-transport, such a concentration may produce an appreciable depolarization of the ganglion cells, but that carrier-mediated transport can reduce this effect to negligible levels. This would accord with inferences to be drawn from measurements of labelled GABA influx, which appears to drive towards a steady-state intracellular/extracellular concentration gradient of around 10^3. At a resting intracellular concentration of some 0.5–1 mM (cf. Bowery *et al.*, 1976; Bertilsson, Suria & Costa, 1976), the glial cells would tend to 'buffer' interstitial GABA to about 1 μM, sufficient to preclude untoward effects on neuronal excitability (cf. Adams & Brown, 1975). The same considerations might well apply to other peripheral neurones sensitive to GABA, such as autonomic and somatic sensory neurones (De Groat, 1972; De Groat, Lalley & Saum, 1972), which are also invested by GABA-transporting neuroglial cells (Bowery & Brown, 1972; Young *et al.*, 1973; Schon & Keily, 1974).

Recently, Curtis, Game & Lodge (1976) have found that inhibitors of GABA transport in the brain (nipecotic acid, L-2,4-diaminobutyric acid and 2,2-dimethyl-β-alanine) applied by iontophoresis to a variety of central neurones potentiated the inhibitory effect of iontophoretically-applied GABA, thus indicating a role for membrane transport in reducing extracellular GABA levels in the central nervous system. At this site clearance may be affected through transport into either neurones or glial cells or both. As the authors point out, their experiments did not clearly indicate the relative importance of neural and glial transport processes, but the effect of the β-alanine derivative suggests at least a partial role of glial transport (cf. Johnston & Stephanson, 1976). The present experiments, on a tissue without a neural carrier, support this inference.

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