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INFLUENCE OF CELLULAR TRANSPORT ON THE INTERACTION OF AMINO ACIDS WITH γ-AMINOBUTYRIC ACID (GABA)-RECEPTORS IN THE ISOLATED OLFACTORY CORTEX OF THE GUINEA-PIG

D.A. BROWN, G.G.S. COLLINS[1] & M. GALVAN*[2]

Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX & *Department of Pharmacology, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ

1 Freshly cut guinea-pig olfactory cortex slices contained 2.2 mmol γ-aminobutyric acid (GABA)/kg tissue weight. This declined during *in vitro* incubation at 25°C in the absence of exogenous GABA, but increased to 6.95 mmol/kg after 1.5 h incubation in 1 mM GABA.

2 Uptake of [^{3}H]-GABA (1 μM) was inhibited by 1 mM (±)-nipecotic acid (−83%), β-amino-*n*-butyric acid (BABA) (−59%), L-2,4-diaminobutyric acid (DABA) (−63%), (±)*cis*-3-aminocyclohexane carboxylic acid (ACHC) (−53%), and 3-aminopropanesulphonic acid (3-APS) (−26%), but was *increased* by β-alanine (BALA) (+23%).

3 Autoradiographs showed steep concentration gradients of radioactivity across slices incubated for short periods in [^{3}H]-GABA.

4 Efflux of [^{3}H]-GABA from pre-loaded slices was accelerated strongly by nipecotic acid, BABA, DABA and ACHC but weakly or not all by BALA or 3-APS.

5 Nipecotic acid (1 mM) potentiated the surface-depolarization of the slice produced by GABA but not that produced by 3-APS.

6 The depolarizing actions of DABA, BABA, nipecotic acid and ACHC, but not that of 3-APS or BALA, were potentiated when the endogenous GABA content of slices was raised.

7 It is concluded that: (a) the depolarizing action of exogenous GABA is limited by cellular uptake; (b) surface-depolarizations produced by nipecotic acid, DABA, BABA and ACHC may be mediated by the release of GABA; and (c) neuronal, rather than glial, transport systems are responsible for these effects.

Introduction

In the central nervous system, γ-aminobutyric acid (GABA) transport is mediated by neurones and glial cells, whereas in the periphery only glial cells appear to possess the necessary carrier (Bowery & Brown, 1972; Young, Brown, Kelly & Schon, 1973; Schon & Kelly, 1974; Iversen & Kelly, 1975). It has long been thought that either or both of these systems are responsible for the inactivation of synaptically released GABA (see Martin, 1976 for a recent review), though direct evidence for this hypothesis is still lacking (see, for example, Curtis, Game & Lodge, 1976; Lodge, Johnston, Curtis & Brand, 1977). Nevertheless, there is some evidence to suggest that these transport systems are responsible for the control of interstitial GABA concentrations and can exert a profound influence on the action of exogenously-applied GABA-agonists. In particular, experiments using isolated sympathetic ganglia, which are depolarized by GABA (Bowery & Brown, 1974; Adams & Brown, 1975), have demonstrated two important points: first, the neuronal response to bath-applied GABA is augmented when uptake is inhibited (Brown & Galvan, 1977); and secondly, some analogues of GABA such as β-alanine and β-aminobutyric acid appear capable of indirectly activating the neuronal GABA-receptors by accelerating the release of GABA from endogenous stores (Bowery, Brown, Collins, Galvan, Marsh & Yamini, 1976a). Investigations using iontophoretic techniques have also shown an increased effectiveness

[1] Present address: Department of Pharmacology, The University, Sheffield, S10 2TN.

[2] Present address: Physiologisches Institut der Universität München, 8000 München 2, Pettenkoferstrasse 12, GFR.

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of GABA on cat central neurones *in vivo* when uptake was reduced (Curtis *et al.*, 1976; Lodge *et al.*, 1977; Lodge, Curtis & Johnston, 1978).

Activation of GABA-receptors leads to a depolarization of neural elements in olfactory cortex slices maintained *in vitro* (Brown & Galvan, 1979; Brown & Scholfield, 1979) and preliminary tests showed an avid GABA uptake system in this tissue (Galvan & Scholfield, 1978). In the present experiments, which were modelled on those previously performed on isolated sympathetic ganglia, we have attempted to assess the influence of cellular transport processes for GABA on the responses of the isolated olfactory cortex to bath-applied agonists. We have tested not only whether the two phenomena described above can be detected in brain tissue *in vitro*, but also which cell carrier is primarily responsible, neural or glial.

Methods

Guinea-pigs (approx. 350 g) were decapitated and slices of olfactory cortex prepared at a nominal fresh thickness of 0.5 mm in the manner described by Harvey, Scholfield & Brown (1974). Slices were maintained in Krebs solution (see Brown & Galvan, 1979) at 20° to 25°C, bubbled with 95% O_2 and 5% CO_2, and unless otherwise stated, contained 10 μM amino-oxyacetic acid to prevent metabolism of GABA (Wallach, 1961).

Endogenous GABA

Tissue concentrations in half-slices were measured by a microdansylation assay (Clark & Collins, 1976).

Uptake of radiolabelled GABA and analogues

Amounts of radiolabelled substrates accumulated by the slices were measured in essentially the same way as previously described for sympathetic ganglia (Bowery *et al.*, 1976a; Bowery, Brown, White & Yamini, 1979). Slices were pre-equilibrated for at least 2 h before adding labelled substrate. The subsequent incubation time was normally 30 min at about 20°C. A minimum volume of 2.5 ml fluid per 10 mg slice was used, to preclude depletion of radioactivity. Inhibitors were added 10 or 15 min before adding substrate to the incubation medium. In some experiments intact slices, average weight about 40 mg, were used and the incubation volume increased accordingly. Uptake was measured as the tissue/medium ratio of radioactivity (disintegrations per minute per mg tissue/disintegrations per min per μl incubation fluid i.e. d min^{-1} mg^{-1} tissue/d min^{-1} μl^{-1} incubation fluid). Average intracellular radioactivity and labelled substrate concentrations were calculated therefrom using the measurements of inulin and non-inulin space made previously (38 and 62% respectively of final wet wt.; Harvey *et al.*, 1974) as measures of extracellular and intracellular volumes. All incubation fluids contained 10 μM amino-oxyacetic acid.

Efflux experiments

Slices were 'loaded' with radiolabelled substrate by preincubation for 3 h in 1 mM [^{3}H]-GABA. Subsequent efflux of radioactivity into non-radioactive Krebs solution at 20°C was measured at 2 min intervals by continuous double-sided superfusion. Again, both loading and wash solutions contained 10 μM amino-oxyacetic acid.

Autoradiography

Light-microscope autoradiographs of glutaraldehyde-fixed slices were prepared as described by Young *et al.* (1973).

Table 1 Concentrations of GABA in guinea-pig olfactory cortex slices incubated at 25°C in Krebs solution *in vitro*

AOAA (*10 μM*)	*Solution content* *GABA* (*1 mM*)	*Incubation time* (*min*)	*GABA* (*mmol/kg tissue*) (*mean ± s.e.*) (*n = 4*)
–	–	0 (fresh)	2.23 ± 0.46
–	–	180	1.34 ± 0.19
+	–	90	1.17 ± 0.08
+	+	90*	6.95 ± 0.57
–	+	90*	4.67 ± 0.34

* Assayed after 30 min washing in GABA-free solution, with amino-oxyacetic acid (AOAA) if appropriate.

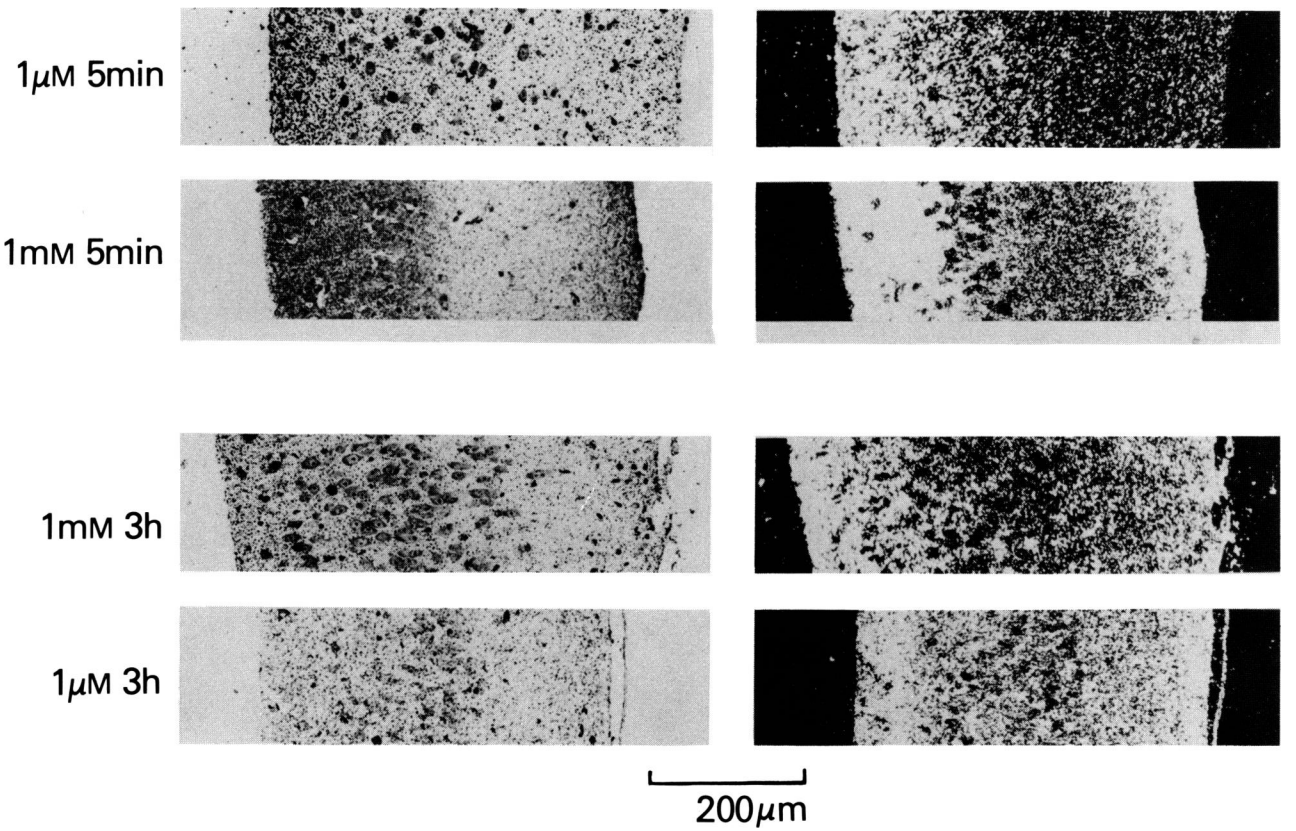

Figure 1 Light-microscopic autoradiographs of sections of guinea-pig olfactory cortex slices previously incubated in solutions containing [^{3}H]-GABA. Incubation conditions and exposure times (in parentheses) were as follows (above downwards): 1 μM GABA, 5 min (46 days); 1 mM GABA, 5 min (98 days); 1 μM GABA, 3 h (103 days); 1 mM GABA, 3 h (6 days). Solutions used for 3 h incubations also contained 10 μM amino oxyacetic acid. In each case the pial surface of the slice is on the right-hand side. Left side photographs were taken under transmitted light, right side photographs (of the same field) under incident light. Calibration bar: 200 μm.

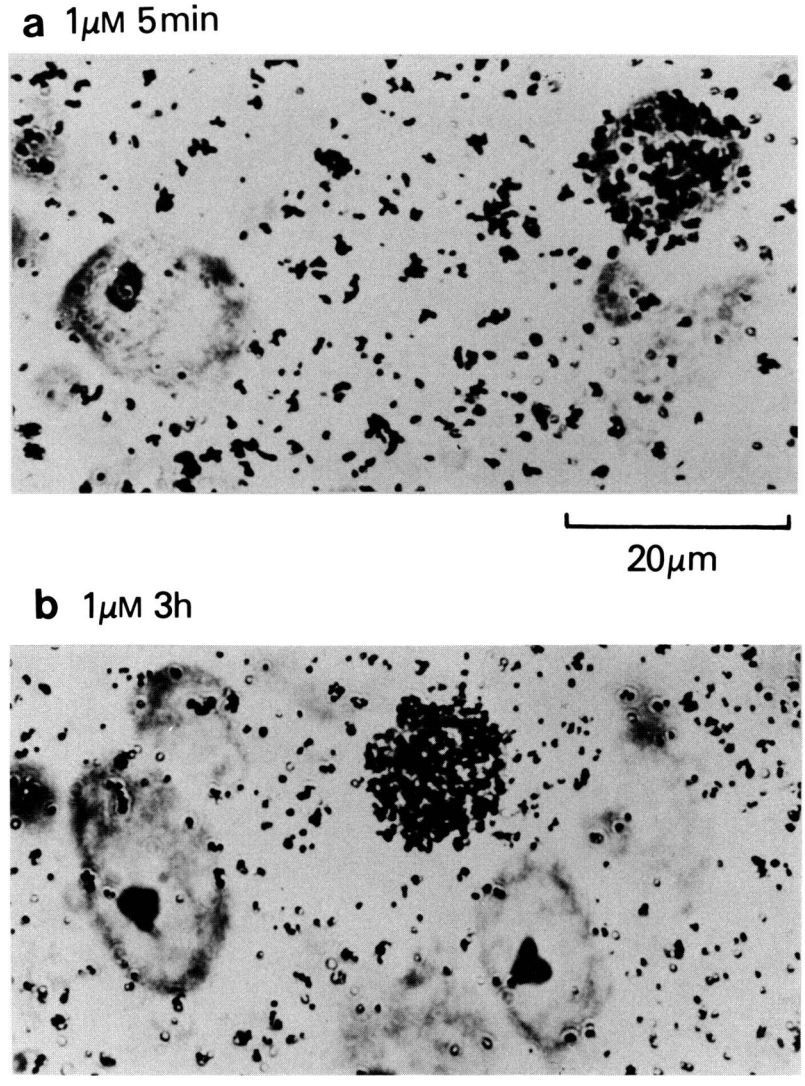

Figure 2 Light-microscopic autoradiographs showing the grain distribution after incubation of olfactory cortex slices in 1 μM [^{3}H]-GABA for (a) 5 min, and (b) 3 h. Note that only some cell bodies are heavily labelled. Photographs taken from the same sections shown in Figure 1. Calibration bar: 20 μm.

Extracellular electrical responses

Pial surface negativity ('surface depolarization') was measured with silver/silver chloride electrodes and d.c. amplification as described by Brown & Galvan (1979).

Results

Transport of GABA by olfactory slices

Endogenous GABA levels Immediately after excision, olfactory slices contained about 2.2 mmol GABA/kg tissue (Table 1). This declined by about 40% after 3 h incubation *in vitro* at 25°C; addition of amino-oxyacetic acid did not prevent this decline.

A progressive *increase* in the amount of GABA occurred on adding 1 mM exogenous GABA to the incubation fluid. Thus, after 1.5 h in 1 mM GABA in the presence of amino-oxyacetic acid, the tissue contained 6.95 mmol GABA/kg, representing a net uptake of 5.8 mmol/kg (Table 1). This uptake occurred against a concentration gradient, since the final intracellular/extracellular concentration ratio was about 10.6:1. Nipecotic acid (1 mM), an inhibitor of GABA-transport (Krogsgaard-Larsen & Johnston, 1975), reduced net uptake by half.

Uptake of radiolabelled GABA After 30 min incubation in 1 μM [^{3}H]-GABA the tissue/medium tritium ratio was about 22:1 (Table 2), corresponding to an average intracellular/extracellular concentration gradient of 35:1; at 300 μM external [^{3}H]-GABA, the intracellular:extracellular concentration ratio was about 10:1.

Uptake at 1 μM [^{3}H]-GABA was inhibited, in descending order of effectiveness, by 1 mM concentrations of the following compounds: (±)-nipecotic acid > L-2,4-diamino-butyric acid (DABA) > β-amino-*n*-butyric acid (BABA) > (±)*cis*-3-aminocyclohexanecarboxylic acid (ACHC) > 3-aminopropane sulphonic acid (3-APS). β-Alanine (BALA) *increased* uptake.

Autoradiographic distribution of accumulated [^{3}H]-GABA Samples of autoradiographs are illustrated in Figures 1 and 2. The following observations were made: (a) Except after long exposures to high concentrations (1 mM) of [^{3}H]-GABA, there was a steep gradient of accumulated radioactivity from the undercut surface inwards, and a lesser gradient from the pial surface inwards (Figure 1). This suggests that penetration may be initially from the cut surface rather than through the pia. (b) Most of the radioactivity was located in the neuropil between the neurone somata, but a few scattered neurones (less than 10% of the total) showed intense labelling (Figure 2).

Release of accumulated [^{3}H]-GABA During continuous perfusion the rate coefficient for [^{3}H]-GABA release from preloaded slices fell gradually to a minimum level of $5.0 \pm 1.7 \times 10^{-4}$ min^{-1} (mean ± s.d., $n = 13$). This is similar to that previously measured in isolated sympathetic ganglia (see Bowery *et al.*, 1976a). Addition of unlabelled GABA to the perfusion fluid (≥100 μM) increased the efflux rate coefficient in a concentration-dependent manner, without attaining a clear ceiling (Figure 3). At concentrations ≤1 mM, the peak efflux rate was attained within 4 min of application, but longer periods were necessary at higher concentrations. Efflux acceleration produced by

Table 2 Uptake of [^{3}H]-GABA by isolated guinea-pig olfactory cortex slices

GABA concentration (μM)	*Inhibitor*	*Tissue/medium ^{3}H-ratio* (*mean ± s.e.*) (*n*)	*Change of uptake* (%)	*Difference from control* (*P*)
1	None (controls)	22.20 ± 1.24 (7)	0	—
	(±)-Nipecotic acid (1 mM)	3.84 ± 0.25 (4)	−83	<0.001
	L-2,4-Diaminobutyric acid (1 mM)	8.21 ± 0.30 (4)	−63	<0.001
	β-Amino-*n*-butyric acid (1 mM)	9.05 ± 0.66 (4)	−59	<0.001
	1,3-*cis* Aminocyclohexanecarboxylic acid (1 mM)	10.51 ± 0.73 (4)	−53	<0.001
	3-Aminopropanesulphonic acid (1 mM)	16.47 ± 1.21 (4)	−26	<0.02
	β-Alanine (1 mM)	27.36 ± 1.31 (7)	+23	<0.02
300	None (controls)	6.72 ± 0.36 (4)	0	
	(±)-Nipecotic acid (0.5 mM)	2.75 ± 0.20 (3)	−59	<0.001
	(±)-Nipecotic acid (1 mM)	2.60 ± 0.09 (4)	−61	<0.001

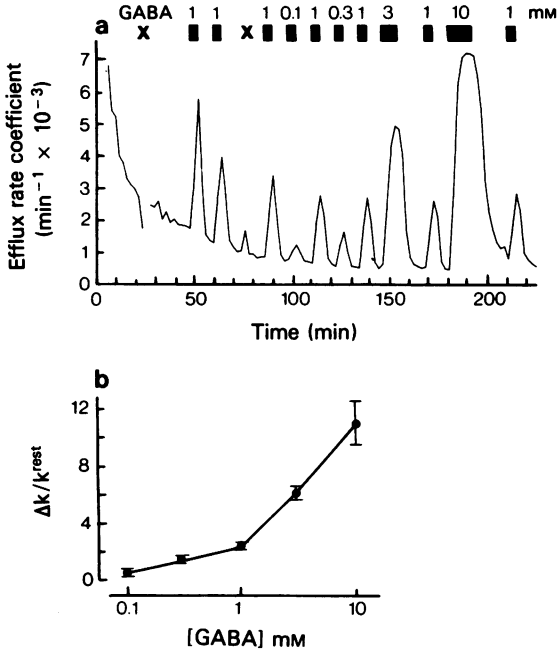

Figure 3 Effect of unlabelled GABA on the rate coefficient for efflux of [^{3}H]-GABA ($min^{-1} \times 10^{-3}$) from isolated olfactory cortex slices pre-incubated for 3 h in 1 mM [^{3}H]-GABA. Both incubation and wash solutions contained 10 μM amino-oxyacetic acid. The plot in (a) shows the result of a single experiment, with time (abscissa scale) running from the end of the loading period. The graph in (b) shows the mean results from 4 experiments, with acceleration (ordinates) plotted as the increase of rate coefficient/resting rate coefficient ($\Delta k/k^{rest}$). (The break and × in the upper record represent flow artefacts.)

GABA concentrations ⩽1 mM was similar to that previously seen in isolated ganglia (Bowery *et al.*, 1976a); however, peak rate coefficients achieved with GABA concentrations >1 mM were much greater.

[^{3}H]-GABA efflux was also increased by unlabelled BABA and DABA (Figure 4a and b) and by nipecotic acid and ACHC (see Figure 10), but not to any appreciable extent by BALA or 3-APS (Figure 4c and d). The relative effectiveness of these compounds as releasers diminished in the approximate order: nipecotic acid > BABA > DABA > ACHC > BALA ≈ 3-APS; this is in accordance with their relative effectiveness as uptake inhibitors (cf. Table 2).

Depolarization of the olfactory cortex

Effect of uptake inhibition on the action of GABA (±)-Nipecotic acid was the most effective inhibitor of GABA-uptake: 1 mM reduced the uptake of 1 μM [^{3}H]-GABA by 83% (Table 2). Figure 5 shows the effect of 1 mM nipecotic acid, applied for 3 min, on the submaximal depolarization of an olfactory slice produced by GABA and 3-APS. The response to 300 μM GABA was increased such that it approximately matched that previously produced by 1 mM GABA, whereas the response to 3-APS was unchanged. This selective enhancement of GABA responses was confirmed in 3 more experiments (Figure 6).

Figure 7 shows the change in the dose-response curve for GABA-induced depolarization produced by nipecotic acid. The curve was shifted to the left in a non-parallel manner; the effect of a low concentration was increased more than that of a high concentration and the threshold was reduced from 100 μM to below 30 μM.

Depolarizing actions of GABA-releasers. It is difficult to deplete nervous tissue of its endogenous GABA and so, as in previous experiments on isolated sympathetic ganglia (Bowery *et al.*, 1976a), evidence that depolarization might be due to GABA release was sought by artifically *raising* the endogenous GABA concentration and then testing whether the neuronal depolarization produced by the suspected releasing agent was potentiated. The agent was tested before and after applying 1 mM GABA (+10 μM amino-oxyacetic acid) for 1.5 h, a procedure that increased the slice GABA content about 6 times, from 1.2 to 6.9 mmol/kg tissue (Table 1).

Figure 8 illustrates the changes produced in one such experiment. The 'loading' procedure increased the depolarization produced by 3 mM BABA, a strong releasing agent (cf. Figure 4), but not that produced by 3-APS or (in this case) by GABA itself. Figure 9a shows the mean depolarizations produced by BABA, DABA, 3-APS, and BALA, before and after 'loading', normalized with respect to the effect of GABA itself. There was a significant increase in the effects of BABA and DABA, but not of BALA or 3-APS, which accords with the different releasing effects of these agents (Figure 9b).

In a few tests the actions of nipecotic acid (3 mM; 2 expts) and ACHC (3 mM; 1 expt) also showed an increase after loading (Figure 10a); both of these agents can release [^{3}H]-GABA (Figure 10b).

The loading procedure usually potentiated the effect of GABA itself, the depolarization produced by 10 mM GABA (a near maximal dose) being increased by 31 ± 17% (mean ± s.d., n = 7). Although pre-incubation of slices in unlabelled GABA reduced the subsequent accumulation of [^{3}H]-GABA (0.1 μM, 30 min incubation) by 45 ± 10% (mean ± s.d., n = 4), Figure 7 shows that uptake inhibition by nipecotic acid did not alter the effect of high doses of GABA. Whatever the explanation for the enhancement, it does not affect

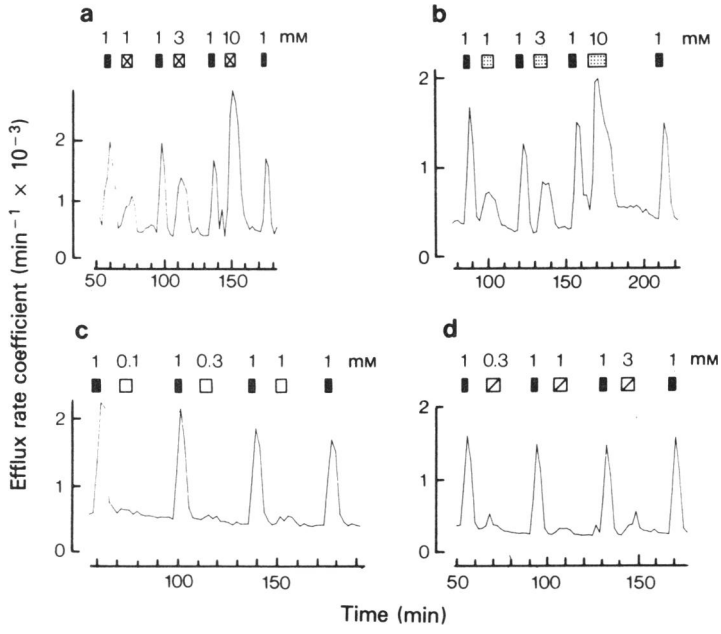

Figure 4 Effects of (a) β-aminobutyric acid (BABA), (b) L-2,4-diaminobutyric acid (DABA), (c) 3-aminopropanesulphonic acid (3-APS) and (d) β-alanine (BALA) on the rate coefficient for efflux of [^{3}H]-GABA from isolated olfactory slices (4 experiments: see Figure 3 for loading conditions). In each experiment 1 mM GABA (solid bars) was added for comparison with the effect of the test compound (see also Figure 9b).

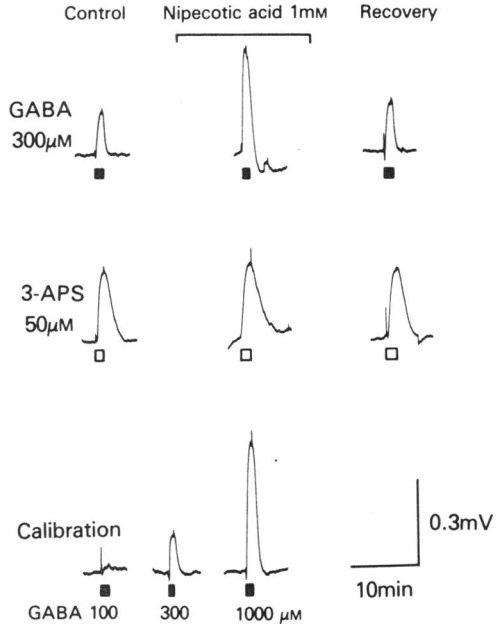

Figure 5 Effect of 1 mM (±)-nipecotic acid on the surface depolarization of a brain slice produced by (a) 300 μM GABA and (b) 50 μM 3-aminopropanesulphonic acid (3-APS). Agonists were applied until the peak response was obtained; nipecotic acid was added for 3 min before second agonist application. The lowest line shows responses to calibrating concentrations of GABA obtained at the end of the experiments. Scale 0.3 mV, 10 min.

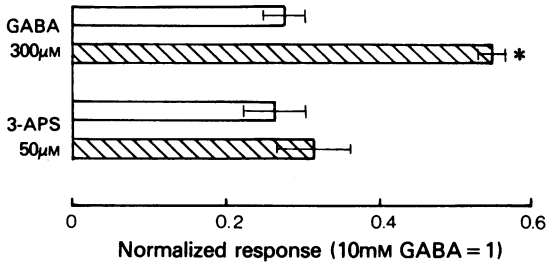

Figure 6 Mean depolarizing effects of GABA (300 μM) and 3-aminopropanesulphonic acid (3-APS) (50 μM) before (open columns) and during (hatched columns) respectively application of 1 mM (±)-nipecotic acid. Responses were measured as shown in Figure 5 and normalized as a fraction of that produced by 10 mM GABA before adding nipecotic acid. Each column is the mean of 4 experiments; horizontal lines show s.e. mean. Significant change $*P < 0.001$ (Students t test).

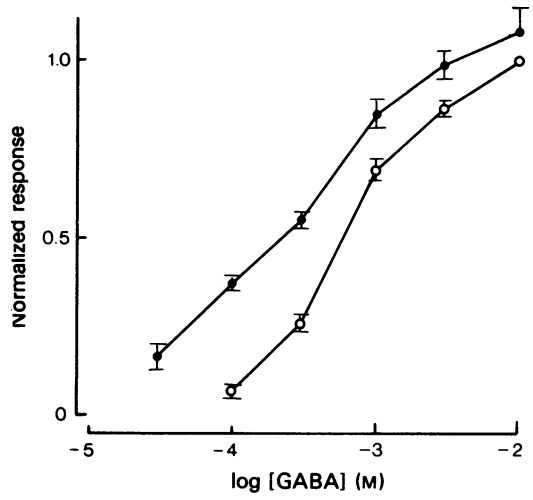

Figure 7 Dose-response curves to GABA determined in control solution (○) and in the presence of 1 mM (±)-nipecotic acid (●). Responses were normalised with respect to those produced by 10 mM GABA in control solution (= 1.0). Each point in the mean of 4 determinations in different slices; bars = s.e. mean.

the interpretation of our results since this increase is allowed for in the normalization procedure used in Figure 9a.

Discussion

Two main questions were addressed in the present experiments: (a) to what extent is the previously-described (Brown & Galvan, 1979) depolarizing action of GABA on the isolated olfactory cortex affected by cellular uptake of GABA? (b) can sufficient endogenous GABA be released by a GABA-analogue to depolarize olfactory neurones, i.e. can releasers act indirectly on GABA-receptors?

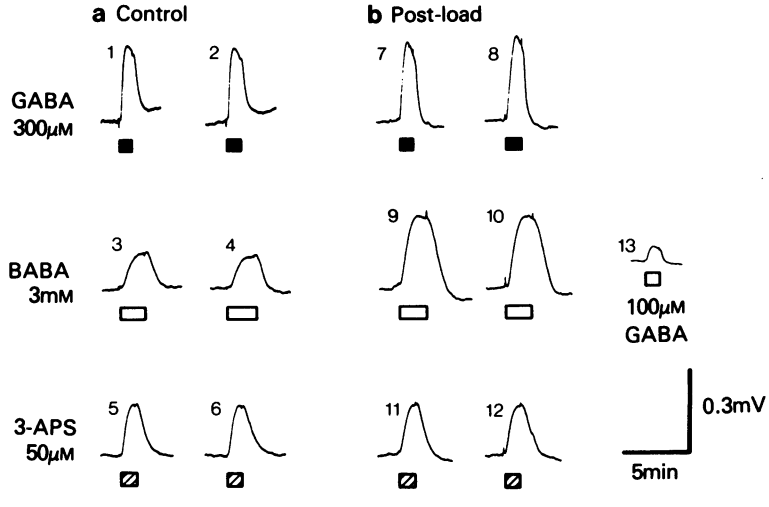

Figure 8 Surface depolarizations of a brain slice produced by 300 μM GABA, 3 mM β-amino-*n*-butyric acid (BABA), and 50 μM 3-aminopropanesulphonic acid (3-APS) recorded (a) before and (b) after loading for 90 min in 1 mM GABA, followed by 60 min washing. All solutions contained 10 μM amino-oxyacetic acid. Agonists were applied in the order shown until the peak surface-potential change was reached; response 13 is a calibration dose. Scale: 0.3 mV, 5 min.

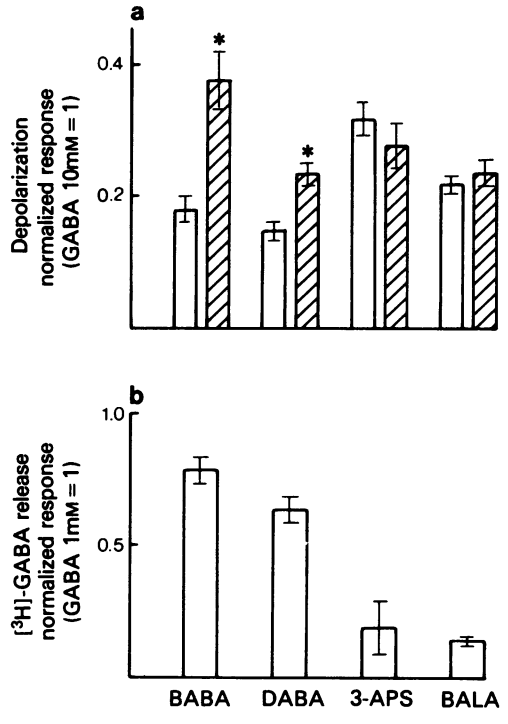

Figure 9 (a) Effects of 'loading' olfactory slices with GABA on depolarizing responses to β-amino-*n*-butyric acid (BABA, 3 mM), L-2,4-diaminobutyric acid (DABA, 3 mM), 3-aminopropanesulphonic acid (3-APS, 50 μM) and β-alanine (BALA, 3 mM), measured as indicated in Figure 8. The histograms show the mean depolarization (normalized with respect to that produced by 10 mM GABA) measured before (open columns) and after (hatched columns) loading (mean of 4, vertical lines show s.e. mean). *Significant change: $P < 0.01$. (b) Relative increase in efflux of [^{3}H]-GABA produced by the same agonists from preloaded slices, measured as shown in Figure 4, normalized with respect to that produced by 1 mM GABA.

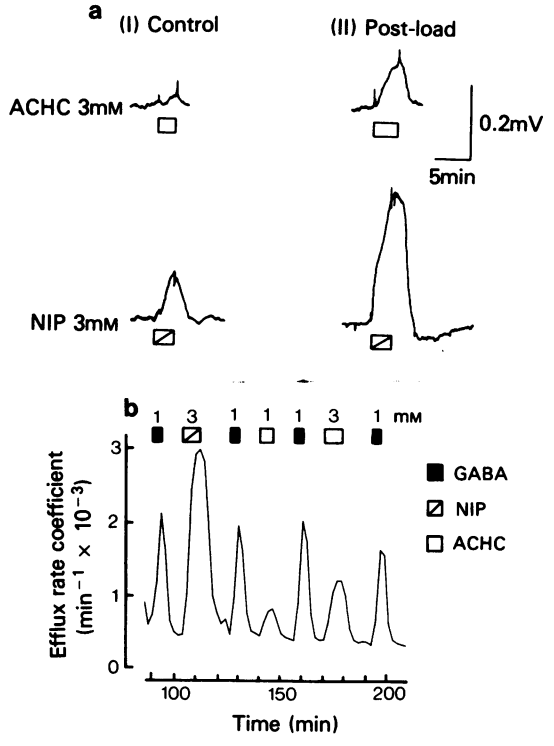

Figure 10 (a) Effects of 'loading' on the depolarizing actions of (±)-*cis*-3-aminocyclohexane carboxylic acid (ACHC) and (±)-nipecotic acid (NIP), observed as described in Figure 8. (b) Acceleration of [^{3}H]-GABA efflux by these two compounds, measured as in Figures 3 and 4.

Uptake

Application of nipecotic acid, in a concentration producing substantial inhibition of GABA-uptake, clearly potentiated the action of exogenous GABA. Since there was no increase in the action of 3-APS, which is, at most, a weak substrate for GABA-carriers (see Table 2; also Bowery & Brown, 1972; Beart & Johnston, 1973; Olsen, Bayless & Ban, 1975), the effect of nipecotic acid cannot be readily attributed to receptor-sensitization without the additional postulate that the manner of receptor activation by 3-APS and GABA differs. The simplest explanation is that the effect of exogenous GABA is attenuated by uptake.

The shift in the GABA dose-depolarization curve produced by nipecotic acid (Figure 7) suggests that, at an external concentration of 100 to 300 μM, uptake normally reduces the effective concentration at the receptors responsible for depolarization by about 80%. This almost certainly underestimates the true clearance since the concentration of nipecotic acid used did not produce complete inhibition of uptake at 1 μM external GABA, and would be even less effective at higher substrate concentrations (see Table 2). Quite probably, if uptake could be completely inhibited, the threshold concentration for depolarization of the olfactory slices by GABA might be reduced to that (about 1 μM) observed on peripheral tissues (Bowery *et al.*, 1976a; Brown & Marsh, 1978), where the GABA influx velocity is about 1000 times slower than that in the brain (see Martin, 1976).

The concentration gradients revealed by the autoradiographs are also indicative of severe access limitations to exogenous GABA. Although these show the amounts taken up by cells, rather than the extracellu-

lar concentration, it seems reasonable to suppose that the two are closely related. The restricted penetration after a 5 min exposure (even at 1 mM external GABA) to within 150 μm of the cut surface and about 50 μm of the pial surface may be particularly relevant to electrical measurements of cell properties made over short time-periods. Although the origin within the slice of the surface-recorded responses is unclear, intracellular recording has revealed neuronal depolarization to GABA at all depths within the slice but with diminishing sensitivity on penetrating more than 150 μm from the cut surface (C.N. Scholfield, personal communication). Curtis *et al.* (1976) and Lodge *et al.* (1978) have presented evidence that uptake may limit the effect of iontophoretically-applied GABA in the cerebral cortex and spinal cord *in vivo*. The present experiments suggest an even more severe limitation on the effect of bath-applied GABA, such that the external concentration may bear little relation to that active at the receptors. Under these conditions, pharmacological definition of the receptors using the endogenous agonist is unwise; a non-transported agonist should be used, even for measurement of antagonist affinity.

The observation that only a few neurone somata in the olfactory cortex accumulate [^{3}H]-GABA is in agreement with a previous *in vivo* study of the cerebral cortex (Hökfelt & Ljungdahl, 1972), in which the labelled cells were identified as inhibitory interneurones (stellate cells). Although we have not yet attempted morphological identification of marked neurones (Figure 2), it is possible that these are also inhibitory interneurones postulated to exist in this cortical region (Biedenbach & Stevens, 1969; Halliwell, 1976).

Release

The relative effects of BABA, DABA and BALA as GABA releasing agents agrees with those previously reported by Crnic, Hammerstad & Cutler (1973) in isolated cerebral cortex. The enhanced depolarization produced by BABA, DABA, nipecotic acid and ACHC after endogenous GABA stores have been elevated may be most readily attributed to their ability to release GABA. This theory is supported by the finding that BALA and 3-APS, which were very weak releasers of [^{3}H]-GABA, showed no such enhancement. This type of experiment shows that releasing agents are *capable* of inducing indirect effects, but does not establish how far they might do so under normal circumstances. The obvious test procedure of depleting the tissue GABA stores is not very practicable. However, it should be noted that the concentration of GABA in the isolated cortex slices was initially *less* than that in freshly-dissected slices, so that the loading procedure increased the content to, at most, about three times the fresh tissue level. Further, DABA and ACHC are very weak agonists on GABA-receptors in peripheral tissues (Bowery & Brown, 1974; Bowery, Jones & Neal, 1976b), from which they are unable to release GABA. Hence, it seems more than plausible that a substantial component of any GABA-mimetic effect which these agents produce in the brain is indirect in origin.

The term 'releasing agent' is not intended to suggest any formal mechanism of release. In the sympathetic ganglion, the maximum substrate-stimulated increase in efflux rate was relatively small (3 to 4 times the resting rate) and approximately equal to that produced simply by removing external Na^+ ions (Bowery *et al.*, 1976a). Therefore it appeared that the increased efflux rate and consequent elevation of interstitial GABA concentrations produced by addition of BABA or BALA might result from inhibition of the reuptake of GABA (which is constantly leaking out of the cells), rather than accelerated efflux *per se*. In the olfactory slice, substrate-stimulated efflux acceleration was much greater (up to 10 times the resting rate, see Figure 3) and far exceeded that obtained by removing external Na^+ ions (M. Galvan, unpublished observations). This accords with previous suggestions (Raiteri, Frederico, Coletti & Levi, 1975) that a component of accelerated exchange diffusion may be involved in the release process. In this context, it is odd, though in agreement with the observations of Simon & Martin (1973), that an elevated intracellular content of carrier-substrate appeared to *inhibit* uptake in our experiments rather than accelerate it.

Neuronal and glial carriers

From available information regarding the substrate specificity of neuronal and glial GABA transport mechanisms (see Iversen & Kelly, 1975), the neuronal carrier appears to be predominantly responsible both for inactivating exogenous GABA in the olfactory slice and for mediating the effects of the releasing agents. In particular, BALA, a relatively specific glial carrier substrate (Schon & Kelly, 1975), had little effect on either uptake or release of GABA, and showed no evidence of indirect activity, whereas DABA and (±)ACHC, substrates for the neuronal carrier (Neal & Bowery, 1977; Weitsch-Dick, Jessell & Kelly, 1978), both accelerated [^{3}H]-GABA release and showed increased depolarizing activity after GABA-loading (q.v. Figures 9 and 10). In a mixed neuronal and glial population, a predominant short-term influence of neuronal transport is to be expected, given the much higher velocity of the neuronal transport system (see, for example, Lasher, 1975). The fact that in the periphery, where the neuronal system is absent, the short-term effects of glial transport (Bowery *et al.*, 1976a; Brown & Galvan, 1977) are essen-

tially similar to those demonstrated here for neuronal transport, presents an interesting duplication of function. Whilst it has been suggested that glial transport may be more concerned with the overall long-term regulation of interstitial GABA levels (Bowery *et al.*, 1979), the function of central glial transport remains uncertain.

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