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Uptake inhibitors potentiate γ-aminobutyric acid-induced contractile responses in the isolated ileum of the guinea-pig

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1 The γ-aminobutyric acid (GABA)-induced contractile responses in the guinea-pig isolated ileum, maintained in Krebs-bicarbonate solution (pH 7.4, 37°C), were significantly potentiated by inhibitors of GABA uptake, with a greater potentiation of the responses in the presence of (±)-*cis*-3-aminocyclohexane-carboxylic acid (ACHC) > L-2,4-diaminobutyric acid (DABA) > (±)-nipecotic acid > β-alanine, whilst simultaneous addition of DABA with β-alanine caused a greater potentiation of the GABA-induced responses than did nipecotic acid with β-alanine, or any of the uptake blockers applied alone.

2 The concentration-response curves for the GABA-induced ileal contraction were shifted to the left in the presence of the uptake inhibitors, this shift being more prominent over the lower concentration range of GABA (1–20 μM). By contrast, contractile responses to muscimol or 3-amino-1-propanesulphonic acid (3APS) were not potentiated by the uptake blockers, neither were their concentration-response curves altered.

3 Bicuculline methochloride shifted the GABA concentration-response curve to the right, whilst picrotoxinin both shifted the concentration-response curve for GABA to the right and depressed the maximum response. In the presence of the uptake inhibitors, the rightward shift of the concentration-response curves for GABA induced by bicuculline was less than that induced by bicuculline alone. The rightward shift with picrotoxinin was similarly reduced in the presence of the uptake inhibitors, without altering the depression of the maximum by picrotoxinin.

4 Bicuculline caused a rightward shift of the concentration-response curves for 3APS and muscimol, with the curve for 3APS most affected. Picrotoxinin similarly shifted the concentration-response curves for 3APS and muscimol but depressed the maximum, with the curve for 3APS again being most affected. None of the inhibitors of GABA uptake influenced the concentration-response curves for 3APS or muscimol in the presence of bicuculline or picrotoxinin.

5 In conclusion, a saturable GABA uptake system is present in the enteric nervous system of the guinea-pig intestine, where neuronal GABA uptake appears to predominate over glial uptake.

Introduction

High affinity [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) uptake has been demonstrated in the myenteric plexus of the guinea-pig intestine (Jessen *et al.*, 1979; 1983; Krantis & Kerr, 1981a; Krantis *et al.*, 1986). This uptake of [^{3}H]-GABA occurs into both neurones and glial cells of the enteric nervous system (ENS), with subsequent neuronal release being shown (Jessen *et al.*, 1983; Kerr & Krantis, 1983; Kerr & Ong, 1984). Furthermore, such uptake of [^{3}H]-GABA in the ENS can be prevented by compounds that inhibit GABA uptake into neurones or glia of the central nervous system (CNS) (Jessen *et al.*, 1979; Krantis & Kerr, 1981a; Kerr & Krantis, 1983; Krantis *et al.*, 1986). This suggests that a high affinity transport process exists for GABA in the ENS where it may be responsible for limiting and terminating the actions of endogenous GABA, as in the CNS (Martin, 1976). Such uptake of GABA into central neurones can be inhibited by L-2,4-diamino-n-butyric acid (DABA) (Iversen & Kelly, 1975), or by (±)-*cis*-3-aminocyclohexane car-

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boxylic acid (ACHC) (Beart *et al.*, 1972; Bowery *et al.*, 1976). In addition, (±)-nipecotic acid inhibits neuronal and, to a lesser degree, glial uptake of GABA (Krogsgaard-Larsen & Johnston, 1975; Johnston *et al.*, 1976), whilst β-alanine is a selective inhibitor of GABA uptake into glial cells (Schon & Kelly, 1975). By using such compounds, the inhibitory actions of synaptically released GABA is enhanced in the CNS, as is the duration of action and the effectiveness of exogenously applied GABA (Curtis *et al.*, 1976). The present study has examined the effects of a variety of inhibitors of GABA uptake on ileal contractile responses elicited by GABA, and by certain GABA analogues not subject to uptake, in order to determine if GABA uptake similarly limits GABA-mediated responses in the intestine.

Methods

Guinea-pig isolated ileal preparations

Guinea-pigs of either sex, weighing between 200–400 g, were stunned by a blow on the head and bled Segments of the distal ileum, 3–4 cm in length, taken 2–3 cm from the ileo-caecal valve, were quickly removed, cleared of their intra-luminal faecal contents and mounted vertically in a 10 ml organ bath containing modified Krebs-bicarbonate solution of the following composition (mM): Na^+ 151.0, K^+ 4.6, Mg^{2+} 0.6, Ca^{2+} 2.8, Cl^- 134.9, HCO_3^- 24.9, $H_2PO_4^-$ 1.3, SO_4^{2-} 0.6, glucose 7.7 (pH 7.4 at 37°C). The Krebs solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2. Isometric contractions of the longitudinal muscle of the tissue were recorded at a resting tension of 10 mN with a Grass Model FT03 force transducer coupled to a Grass polygraph recorder.

The isolated tissues were allowed to equilibrate in the organ bath for 60 min before any effects of drug treatments were examined on the resting tissues. The time intervals between each drug application into the bath were 15–20 min and antagonists were added at least 5–10 min before agonists were tested, depending on the experiment. In experiments where inhibitors of specific GABA uptake for neurones and glial cells were used respectively or in combination to test their effects on responses induced by exogenously applied agonists, these uptake blockers were allowed to incubate in the bath for 5–10 min before further drug application. Control responses to GABA were always obtained before any drug application, and were re-established after washing out the drug. The volumes of drugs used were never more than 1% of the bath volume in each experiment. Student's *t* test for paired and unpaired samples was used to assess the significance ($P < 0.05$) of differences between mean values of the dose-response effects. All experiments were performed in duplicate, and were repeated at least 6 times on tissues from at least 4 different animals. Concentration-ratios for dextral shifts in concentration-response curves due to bicuculline methochloride were calculated from EC_{50} values using pooled control curves for GABA; this appears valid sinced there was little variation in the control EC_{50} values between preparations, and the pooled control curve is very close to that in Ong & Kerr (1984) constructed under identical conditions.

In order to avoid an excessive number of figures, only a representative concentration-response curve for GABA in the presence of one inhibitor (DABA) is shown in the results. Instead, antagonist-induced dextral shifts of the concentration-response curves for GABA and GABA analogues, as altered by uptake inhibitors, are expressed as calculated ratios, the significance of the alterations being assessed by Student's *t* test. With bicuculline, for each uptake inhibitor, three such ratios were determined from EC_{50} values: (a) for agonist + uptake inhibitor in the presence of bicuculline versus agonist alone, (b) for agonist + uptake inhibitor in the presence of bicuculline versus agonist + uptake inhibitor, and (c) agonist (no uptake inhibitor) with bicuculline versus agonist alone. With picrotoxinin as the antagonist, a similar method was used taking EC_{25} values from log-probit plots, the latter being used since the maximum depression of the concentration-response curve with picrotoxinin generally exceeded 50%, only ratios of agonists in the presence of uptake inhibitor and picrotoxinin versus agonsist alone were calculated.

Sources of drugs and chemicals

Acetylcholine chloride (ACh), β-alanine, γ-aminobutyric acid (GABA), 3-amino-1-propanesulphonic acid (3APS), atropine sulphate, L-2,4-diaminobutyric acid (DABA), histamine dihydrochloride, 5-hydroxytryptamine hydrochloride, muscimol, (±)-nipecotic acid, picrotoxinin (dissolved in 1:9 absolute alcohol and distilled water), tetrodotoxin (TTX, Sigma); bicuculline methochloride (Pierce); (±)-*cis*-3-aminocyclohexane carboxylic acid (ACHC) (Dr D.I.B. Kerr, University of Adelaide, South Australia).

Results

Potentiation of GABA-induced ileal contractions by inhibitors of GABA uptake

In the isolated ileum, exogenously applied GABA induced a transient contraction, sensitive to atropine (0.7 μM), bicuculline methochloride (10 μM), and TTX (0.7 μM). This contraction was followed by a delayed,

prolonged, bicuculline-insensitive 'after-relaxation' (cf. Ong & Kerr, 1983a,b), but the latter has not been included in the present study. The contractile responses to GABA were potentiated by 15 min preincubation with DABA (0.1 mM), a neuronal uptake inhibitor for GABA (Figure 1a), and further potentiated by preincubation with a combination of DABA (0.1 mM) and β-alanine (0.1 mM) to inhibit both neural and glial GABA uptake (Figure 1b); repeated washing with Krebs solution restored the control response. Such potentiation was seen following preincubation for 15 min with each of the uptake inhibitors used, β-alanine (0.1 mM), ACHC (10 μM), DABA (0.1 mM) or (±)-nipecotic acid (0.1 mM), and further potentiation occurred if β-alanine (0.1 mM) was preincubated in combination with DABA (0.1 mM) or (±)-nipecotic acid (0.1 mM). At these concentrations, the uptake inhibitors themselves did not alter the baseline of the ileal preparations, neither did they affect responses to exogenously applied ACh, histamine or 5-hydroxytryptamine. However, at higher concentrations (ACHC > 1 mM, β-alanine > 0.5 mM, DABA > 1 mM, (±)-nipecotic acid > 5 mM), slight ileal contractions were seen which were sensitive to bicuculline (10 μM) and picrotoxinin (10 μM). Such high concentrations were therefore avoided, particularly since they generally had a depressant action on the GABA-induced contractile responses when left in the bath for 15 min or longer.

Concentration-response curves for GABA-induced ileal contractions were constructed using GABA alone, and in the presence of the uptake inhibitors. As seen in Figure 2, DABA (0.1 mM, 15 min preincubation) produced a leftward shift of the GABA concentration-response curve, and the threshold was reduced some 10 fold. This leftward shift was more prominent over the lower dose range of GABA (< 10 μM), and even more so following 15 min preincubation with β-alanine (0.1 μM) and DABA (0.1 mM) in combination. Similar shifts in the concentration-response curves for GABA-induced ileal contractions were also seen in the presence of ACHC (10 μM), and of (±)-nipecotic acid (0.1 mM) alone or in combination with β-alanine (0.1 mM), again with 15 min preincubation. Taking log-probit plots, at the EC_{16} (Table 1), ACHC (10 μM) and DABA (0.1 mM) were equi-active in potentiating GABA-induced contractions but more active than (±)-nipecotic acid, whilst (±)-nipecotic acid (0.1 mM) was more active than β-alanine (0.1 mM); DABA (0.1 mM) combined with β-alanine (0.1 mM) gave the most marked potentiation ($P < 0.05$ for each combination). During construction of the concentration-res-

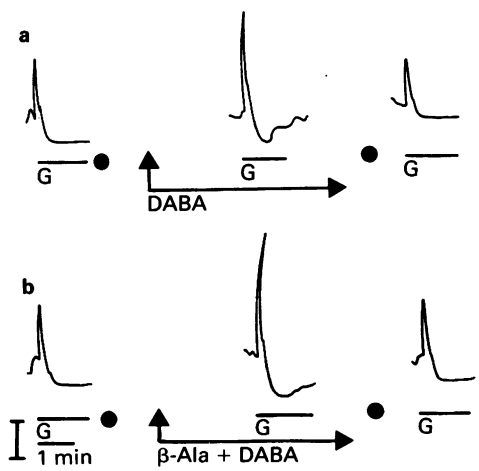

Figure 1 Contractions and delayed 'after-relaxations' induced in guinea-pig isolated ileum by exogenously applied γ-aminobutyric acid (G; 10 μM). The responses were potentiated after exposure to inhibitors of GABA uptake: (a) L-2,4-diaminobutyric acid (DABA; 0.1 mM), and (b) a combination of both DABA (0.1 mM) and β-alanine (β-Ala; 0.1 mM) for 10 min before responses to GABA were elicited, with restoration of the control response following tissue washout. (●) Indicates tissue washout. Vertical bar indicates 10 mN tension.

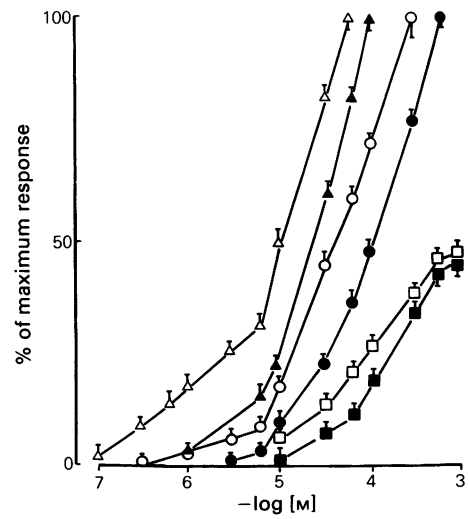

Figure 2 Effect of L-2,4-diaminobutyric acid (DABA) on contractile responses to γ-aminobutyric acid (GABA) in the guinea-pig isolated ileum. Concentration-response curves to GABA in the presence (open symbols) and absence (closed symbols) of DABA (0.1 mM): GABA alone (△, ▲), GABA with bicuculline methochloride (5 μM; ○, ●), GABA with picrotoxinin (10 μM; □, ■). Each point is the mean of the percentage of the maximal contraction induced by GABA; vertical lines represent s.e.mean for at least 6 experiments.

Table 1 Effect of inhibitors of γ-aminobutyric acid (GABA) uptake on contractile responses to GABA and GABA-mimetics in the guinea-pig isolated ileum

GABA agonist + uptake inhibitor	EC_{16} (μM)
GABA	4.9 ± 0.2
+ ACHC	1.0 ± 0.5
β-Ala	2.6 ± 0.3
DABA	0.93 ± 0.04
Nip	1.9 ± 0.5
β-Ala + DABA	0.45 ± 0.03
β-Ala + Nip	1.2 ± 0.4
3APS	2.4 ± 0.5
+ β-Ala	2.2 ± 0.4
DABA	2.4 ± 0.5
Nip	2.3 ± 0.6
Muscimol	2.7 ± 0.4
+ β-Ala	2.8 ± 0.6
DABA	2.9 ± 0.5
Nip	2.6 ± 0.5

Data shown are means ± s.e.mean ($n > 6$). ACHC = (±)-*cis*-3-aminocyclohexane carboxylic acid (10 μM); 3APS = 3-amino-1-propanesulphonic acid; β-Ala = β-alanine (0.1 mM); DABA = L-2,4-diamino-n-butyric acid (0.1 mM); Nip = (±)-nipecotic acid (0.1 mM). Responses were measured at the EC_{16}, using log-probit plots, since this falls within the region of the maximal potentiation with each uptake inhibitor.
The EC_{16} for GABA was significantly lowered ($P < 0.05$) in the presence of the uptake inhibitors, but there was no significant difference using 3APS or muscimol as the agonist.

Table 2 Effect of inhibitors of γ-aminobutyric acid (GABA) uptake on the bicuculline-induced antagonism of contractile responses to GABA-mimetics in the guinea-pig isolated ileum

GABA agonist + Bic + uptake inhibitor	A'_B/A_B	A'_B/A
GABA + Bic	—	(4.5)
+ β-Ala	5.0	3.9
DABA	3.5	1.8
Nip	7.0	3.0
β-Ala + DABA	5.7	1.3
β-Ala + Nip	6.4	2
3APS + Bic	—	(17)
+ β-Ala	15	14
DABA	17	17
Nip	16	16
Musc + Bic	—	(5.7)
+ β-Ala	5.5	5.6
DABA	5.6	5.9
Nip	5.4	5.5

Data shown are concentration-ratios calculated from mean EC_{50} values using log-probit plots. Abbreviations as in Table 1 (Musc = muscimol). A = agonist alone, A_B = agonist in presence of uptake inhibitor, A'_B = agonist in presence of uptake inhibitor and bicuculline methochloride (Bic, 5 μM). Control values for agonists in presence of bicuculline alone appear in parentheses. All uptake inhibitors were used at 0.1 mM. Only the responses using GABA as the agonist showed any influence of the uptake inhibitors on the concentration-ratios with bicuculline antagonism ($P < 0.05$).

ponse curves, upon repeated washing with Krebs solution, the post-wash responses did not differ significantly from the control contractions obtained before the application of the uptake inhibitors.

Antagonism of ileal responses to GABA by bicuculline and picrotoxinin in the presence of uptake blockers

Using bicuculline (5 μM) as a GABA antagonist resulted in a rightward shift of the concentration-response curve for GABA (4.5 fold at the EC_{50}). Preincubation with DABA (0.1 mM) reduced this shift to 1.8 fold relative to the control curve for GABA alone, but 3.5 fold relative to the concentration-response curve for GABA in the presence of DABA (Figure 2). As seen in Table 2, on preincubation with both DABA (0.1 mM) and β-alanine (0.1 mM) in combination, the rightward shift due to antagonism of GABA with bicuculline (5 μM) was further reduced relative to the control concentration-response curve for GABA alone (1.3 fold), but was 5.7 fold relative to GABA in the combined presence of these two inhibitors. In the same way, the concentration-ratios, relative to GABA alone, for bicuculline antagonism were also reduced in the presence of β-alanine (0.1 mM) or (±)-nipecotic acid (0.1 mM) as uptake inhibitors, but remained higher relative to GABA in the presence of these inhibitors (Table 2).

When using picrotoxinin (10 μM) as the antagonist (Figure 2, Table 3), there was both a marked depression of the maximum response and a lessened slope of the concentration-response curve for the GABA-induced ileal contractions, indicative of non-competitive antagonism (cf. Ong & Kerr, 1983b). Following preincubation with DABA (0.1 mM), the concentration-response curve for GABA in the presence of picrotoxinin was once again shifted leftwards relative to that with picrotoxinin alone, but the maximum was barely increased (Figure 2). As with bicuculline, there was a lessened rightward shift of the concentration-response curve for picrotoxinin antagonism when the tissues were preincubated with β-alanine (0.1 mM) and DABA (0.1 mM) in combination, but still with little effect on the maximum response in the presence of picrotoxinin. As can be seen from Table 3, lessened

Table 3 Effect of inhibitors of γ-aminobutyric acid (GABA) uptake on the antagonism of contractile responses to GABA-mimetics by picrotoxinin (Pic) in the guinea-pig isolated ileum

GABA agonist + Pic + uptake inhibitor	A'_B/A	Δ *Max*
GABA + Pic	(16)	(55)
+ β-Ala	4	53
DABA	6	52
Nip	6	54
β-Ala + DABA	4	50
β-Ala + Nip	3.6	50
3APS + Pic	(20)	(75)
+ β-Ala	20	75
DABA	25	73
Nip	28	74
Musc + Pic	(9)	(59)
+ β-Ala	9	60
DABA	7	60
Nip	6	62

Data shown are concentration-ratios calculated from mean EC_{25} values using log-probit plots. Abbreviations as in Table 1. A = agonist alone, A'_B = agonist in presence of uptake inhibitor and picrotoxinin (Pic, 10 μM). Δ Max = % depression of maximal response. Control values for agonists in presence of picrotoxinin alone appear in parentheses. In the presence of the uptake inhibitors, all at 0.1 mM, the concentration-ratios for picrotoxinin antagonism of GABA were significantly lowered ($P < 0.05$), but not with 3APS or muscimol (Musc) as the agonists.

shifts of the concentration-response curve for GABA antagonism with picrotoxinin (10 μM) were in turn found when using β-alanine (0.1 mM) or (±)-nipecotic acid (0.1 mM), also without greatly affecting the maximum; β-alanine (0.1 mM) and (±)-nipecotic acid (0.1 mM) in combination shifted the concentration-response curve further towards that for GABA alone, but again without affecting the maximum.

Effects of uptake blockers on ileal responses induced by 3-amino-1-propanesulphonic acid or muscimol

Muscimol and 3APS elicited only transient, dose-dependent, contractile responses in the guinea-pig ileal preparations (cf. Ong & Kerr, 1983a,b). The potencies of muscimol and 3APS were approximately equal but some 2 fold greater than that of GABA; these contractile effects were also atropine-, bicuculline- and TTX-sensitive. In contrast to the effects seen with GABA as the agonist, none of the GABA uptake inhibitors, β-alanine, ACHC, (±)-nipecotic acid or DABA, caused any significant shift of the concentration-response curves for ileal contractions induced by muscimol or 3APS (Table 1), neither did they influence the rightward shift of these curves in the presence of bicuculline (5 μM) or picrotoxinin (10 μM). However, 3APS was more sensitive to antagonism by bicuculline than was GABA or muscimol (Table 2), and picrotoxinin was more effective in depressing the maximum response to 3APS than to GABA or muscimol (Table 3).

Discussion

In the guinea-pig isolated ileum, inhibitors of GABA uptake evidently potentiate GABA-induced contractions. This suggests that exogenously applied GABA is removed from its sites of action in the myenteric plexus by an active transport mechanism. At the concentrations employed here, (±)-nipecotic acid and β-alanine appeared less potent than DABA or ACHC in potentiating GABA-induced ileal contractions, the most potent inhibitor being ACHC which at 10 μM was essentially equipotent with DABA at 100 μM. If this potentiation were solely due to inhibition of GABA uptake, then these apparent relative potencies are in contrast to those found in the CNS where nipecotic acid > DABA > ACHC (albeit each at 1 mM) whilst β-alanine actually increases GABA uptake (Brown *et al.*, 1980). In the CNS, DABA and ACHC are relatively selective inhibitors of neuronal uptake, with minimal GABA-mimetic actions (Beart *et al.*, 1972; Harris *et al.*, 1973; Bowery *et al.*, 1976). However, nipecotic acid is said to be less specific since it inhibits both glial and neuronal GABA uptake (Krogsgaard-Larsen, 1980), whereas β-alanine is a specific inhibitor of glial uptake (Schon & Kelly, 1975). On the other hand, in the ENS, the neuronal uptake of [^{3}H]-GABA, can be prevented by ACHC, DABA or nipecotic acid, but the behaviour of the enteric glial uptake system is less clear since DABA but not β-alanine prevents some presumptive labelling of glia (Kerr & Krantis, 1983; Saffrey *et al.*, 1983; Krantis *et al.*, 1986). Nevertheless, in the present experiments, there was a greater potentiation of GABA-induced ileal contractions if β-alanine was added simultaneously with DABA or nipecotic acid, suggesting that β-alanine does inhibit a component of GABA uptake, presumably glial, in the ENS. It follows that the combined inhibition of both glial and neuronal uptake would allow a greater accumulation of exogenous GABA at the relevant receptor sites, resulting in the observed potentiations. Similarly, such an accumulation of endogenous GABA is also likely to be responsible for the GABA-mimetic properties of the uptake inhibitors when applied at the highest concentrations, as suggested for nipecotic acid by Krogsgaard-Larsen *et al.* (1975). The more marked leftward shift of the concentration-response curves at lower concentrations of GABA, in the

presence of the uptake inhibitors, further suggests that a degree of saturation occurs in the uptake mechanism at higher concentrations of GABA. The nature and properties of the relevant uptake mechanisms for GABA in the ENS require better definition with kinetic studies, but the alterations in the concentration-response curves for GABA in the presence of the uptake inhibitors clearly resemble those for catecholamines where saturable uptake has been demonstrated (Langer & Trendelenburg, 1969).

Ileal contractile responses to 3APS or muscimol were not affected by ACHC, β-alanine, DABA or nipecotic acid. Evidently there is no significant uptake of 3APS or muscimol into neurones or glia of the myenteric plexus by a transport system sensitive to inhibitors of GABA uptake, although muscimol is weakly transported in the CNS (Johnston *et al.*, 1978). Thus ileal responses to 3APS or muscimol do not appear to be limited by uptake, unlike the responses to GABA where this does occur. Indeed, when both neuronal and glial uptake of GABA was inhibited then, over much of the concentration-response curve, GABA appeared more potent than 3APS or muscimol in eliciting ileal contractions. This reinforces the conclusion by Brown *et al.* (1980) that concentrations of bath applied GABA achieved at the receptor may bear little relationship to the external concentration in the face of active uptake. The fact that ileal contractile responses to 3APS or muscimol were not potentiated by any of the uptake inhibitors also argues against a contribution of GABA release, or of an accumulation of endogenous GABA, to the observed potentiation of GABA-induced responses. Were this so, then the potentiation should have been apparent not only with GABA but also with 3APS and muscimol in the presence of ACHC, DABA or nipecotic acid. However, release of endogenous GABA by these agents could contribute to their GABA-mimetic actions in the intestine at higher doses (1 mM), since they all release GABA from slices of rat olfactory cortex (Brown *et al.*, 1980), and release of GABA from glial cells has been seen with β-alanine (Bowery *et al.*, 1976).

When bicuculline was used as a GABA antagonist in the ileum, there was a rightward shift of the GABA concentration-response curve without any effect on the maximum response (cf. Krantis & Kerr, 1981b); here, in the presence of uptake inhibitors, the rightward shift was less relative to the control curve for GABA alone. This is an example of the interaction between antagonism and potentiation by uptake inhibition as discussed by Kenakin (1982; cf. his Figure 3), where simultaneous potentiation and antagonism cause a lesser rightward shift than would occur in the absence of concomitant uptake inhibition. However, the concentration-ratio for bicuculline antagonism with GABA alone (in the absence of uptake inhibitor) was almost the same as that when inhibitor was present both in the GABA control and with bicuculline. In the same way, there was also a rightward shift of the concentration-response curve for GABA, although with a lowered slope and reduced maximum, when picrotoxinin was used as the GABA antagonist. This rightward shift, relative to the control concentration-response curve for GABA, was again less in the presence of any of the uptake inhibitors. However, despite this potentiation, the maximum response was not increased towards the control level in the way that is seen with barbiturate potentiation of GABA-induced responses in the intestine, where picrotoxinin antagonism is reversed by barbiturate (Ong & Kerr, 1984).

It is concluded, from such use of GABA uptake inhibitors, that GABA actions in the ENS are most likely terminated predominantly by an active, saturable uptake process into ENS neurones, but with some uptake into glial cells sensitive to β-alanine. Inhibition of GABA uptake allows a greater accumulation of exogenous GABA at the $GABA_A$-receptor sites responsible for the GABA-induced contractions, although substrate saturation probably limits this potentiating effect at higher GABA concentrations. Evidently this preparation may prove useful in examining the selectivity and potency of potential inhibitors of GABA uptake in relation to GABA-induced responses.

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