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Baclofen (β-*p*-chlorophenyl-γ-aminobutyric acid) enhances [^{3}H]γ-aminobutyric acid (^{3}H-GABA) release from rat globus pallidus *in vitro*

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The rat globus pallidus has been investigated as a possible model in which to study pre-synaptic GABA mechanisms *in vitro*. (±)-Baclofen (300 μM–1 mM) significantly enhanced the release of radioactivity from superfused slices of rat globus pallidus prelabelled with ^{3}H-GABA *in vitro*. This releasing action was specific to the (+)-isomer of baclofen: neither the (−)-isomer nor another neuronal depressant DL-α-ε-diaminopimelic acid had any significant effect. The releasing effect of baclofen appeared unrelated to the phenethylamine moiety of its structure as neither β-phenethylamine nor dopamine evoked release of ^{3}H-GABA from pallidal slices. Baclofen increased the efflux of radioactivity from pallidal slices prelabelled with either [^{3}H]β-alanine or [^{3}H]diaminobutyric acid *in vitro*. The use of specific glial and neuronal GABA uptake blocking compounds (β-alanine and (±)-*cis*-1,3-aminocyclohexanecarboxylic acid) did not permit resolution of the elements from which baclofen was evoking [^{3}H]GABA release. Baclofen also inhibited uptake of [^{3}H]GABA into pallidal slices with an IC50 value of 6×10^{-4} M. The GABA-like properties of baclofen may be related to the (+)-isomer while non-specific neuronal depressant actions are an effect of the (−)-isomer. The potential of the (+)-isomer as an antipsychotic agent while (−)-baclofen remains the effective antispastic drug free from unwanted side-effects, is discussed.

Baclofen [β-(*p*-chlorophenyl)-GABA, Lioresal-CIBA-Geigy] is a GABA derivative which, unlike GABA itself, can penetrate the blood-brain barrier. The drug has proved to be of use in the alleviation of spasticity (Birkmayer, 1972). Its locus of action in this respect is spinal, although the drug has been shown to be of use in the treatment of choreic symptoms (Barbeau, 1973) suggesting also a supraspinal site of activity. Much indirect evidence has been reported to propose a GABAmimetic action for baclofen within the central nervous system. For example Andén & Wachtel (1977) observed increases in dopamine concentrations in rat brain following systemically administered baclofen and likened this to the effects of GABA-like drugs. Behaviourally, intranigrally injected baclofen induces circling in rats (Olpe, Schellenberg & Koella, 1977b; Waddington, 1977): an effect which is blocked by the GABA antagonist picrotoxin (Waddington, 1976). Baclofen has also been reported recently as potentiating the cataleptic effect of the neuroleptic agent α-flupenthixol in rats (Davies & Williams, 1978). However not all results support a GABAmimetic action for baclofen as electrophysiological studies have found that this drug is insensitive to the GABA antagonist bicuculline on mammalian preparations (Curtis, Game & others, 1974; Davies & Watkins, 1974).

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To try and clarify this controversy, we have investigated the effects of baclofen on pre-synaptic GABAergic mechanisms. Release and uptake of ^{3}H-GABA in slices of rat globus pallidus has been studied *in vitro*. The globus pallidus is an easily identifiable nucleus known to contain large numbers of GABAergic nerve terminals (Okada, Hassler & others, 1971) and therefore may provide a good model in which to study pre-synaptic GABA mechanisms.

MATERIALS AND METHODS

Female Porton rats, ~200 g, were killed, the brains removed and further dissection carried out over ice. Two transverse cuts were made, one through the caudal aspect of the tuberculum olfactorium and the other midway through the hypothalamus (corresponding to planes A 6860 and 5340 of the atlas of König & Klippel, 1963). The left and right globus pallidi were pooled with those from 3 other animals and the tissue was chopped in two directions at 0·2 mm intervals on a McIlwain tissue chopper. (Each pair of pallidi weighed approximately 25–30 mg, but were inevitably contaminated with adjacent striatal and thalamic tissue).

The methods used to study the release of radioactive GABA from prelabelled tissue were essentially those of Srinivisan, Neal & Mitchell (1969). The pallidal slices were pre-incubated for 20 min in oxygen-bubbled Krebs-Ringer bicarbonate buffer,

pH 7·4, (5 ml) containing 10^{-8} M labelled transmitter (^{3}H-GABA, 1 μCi ml^{-1}, sp. act. 54 Ci mM^{-1}, Radiochemical Centre, Amersham) at 37°. In all experiments the Krebs-Ringer buffer contained 10^{-5} M aminooxyacetic acid (AOAA) to prevent metabolism of GABA. The prelabelled tissue was transferred to superfusion chambers and a half hour washout period was commenced by superfusing with Krebs at a rate of 1·5 ml min^{-1}. After 30 min, 3 ml fractions were collected every 2 min and radioactivity in each fraction was determined before and after addition of 50 mM K^+ (chloride) or test drug perfused for 6 min. Drugs used in this system were (±)-baclofen hydrochloride (0·1–1 mM) and the individual isomers of baclofen (200 μM), dopamine hydrochloride (100 and 500 μM) (Koch-Light Laboratories), β-phenethylamine (1 mM) (Sigma) and DL-α-ϵ-diaminopimelic acid (DAPA) (1 mM) (Sigma). Radioactivity in the efflux was also studied following electrical stimulation of the tissue (rectangular pulse: 5 ms, 20 mA, 20 V, 50 s^{-1} for 30 s). In these latter experiments [^{14}C]sucrose was added to the medium (0·4 μCi ml^{-1}) to monitor extracellular space changes. Also the effect replacing the calcium concentration in the superfusing medium with magnesium chloride (>0·1 mM Ca^{2+}; 1 mM Mg^{2+}) was studied on the release of radioactivity during electrical stimulation.

In an attempt to distinguish between glial and neuronal release, the GABA uptake blocking agents β-alanine (20 μM and 1 mM) (Sigma) or (±)-*cis*-1,3-aminocyclohexane carboxylic acid (ACHC) (20 and 600 μM) were included in both the preincubate and superfusate during some experiments. The release of ^{3}H-GABA by 400 μM (±)-baclofen was studied in the presence of these two agents. In addition, the efflux of radioactivity evoked by 400 μM (±)-baclofen was studied after prelabelling tissue with either [^{3}H]β-alanine (10^{-8} M, sp. act. 49 Ci mM^{-1}) or [^{3}H]diaminobutyric acid (^{3}H-DABA) (10^{-8} M, sp. act. 12 Ci mM^{-1}, Radiochemical Centre, Amersham).

In another series of experiments the effect of (±)-baclofen (10^{-3} – 10^{-6} M) on the uptake of ^{3}H-GABA into slices of globus pallidus was studied. Pallidal slices (5–8 mg) were preincubated for 10 min in 2 ml Krebs-Ringer bicarbonate buffer, with or without (±)-baclofen, at 37°. ^{3}H-GABA was added to each tube (final concentration 10^{-8} M) and the samples incubated for 10 min. Transmitter uptake was terminated by rapid filtration of each sample on millipore filters. Following washing with 12 ml ice-cold buffer adioactivity in each tissue sample was determined by scintillation counting. Blank values were determined by measuring uptake of radioactivity into tissue slices incubated in Krebs in an ice-bath at 0°.

Results of the release experiments are expressed as a rate constant derived from the recovered radioactivity, released into each 2-min fraction, and plotted as either efflux curves to show the effect of electrical and potassium stimulation; or as the amount of radioactivity released during a 6-min superfusion before and after addition of test drug. Results of the uptake experiments are expressed as d min^{-1} mg^{-1} weight wet tissue. Statistical significances between control and drug-treated data were calculated using a Mann-Whitney U-test for non-paired samples.

RESULTS

Both electrical stimulation and 50 mM potassium stimulates the efflux of radioactivity from slices of rat globus pallidus prelabelled with ^{3}H-GABA (Fig. 1). The effect of electrical stimulation is markedly reduced in a high magnesium (1 mM) low calcium medium (Fig. 1a). No significant increase in [^{14}C]-

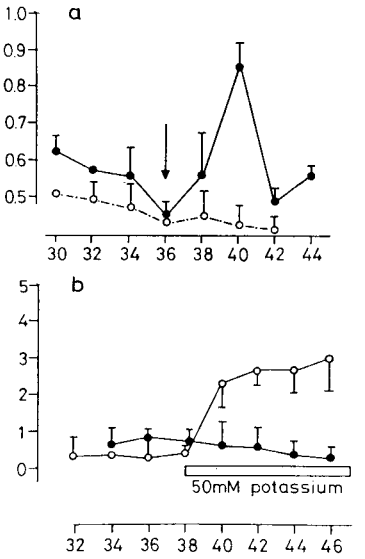

FIG. 1. Release of radioactivity from superfused slices of rat globus pallidus prelabelled with ^{3}H-GABA *in vitro*. (a) Effect of electrical stimulation (↓) (rectangular pulses: 5 ms, 20 mA, 20 V, 50 s^{-1} for 30 s) on the release of radioactivity in the presence of 1 mM Ca^{2+} (●——●) or in the absence of calcium (1 mM Mg^{2+}, ○—·—○). (b) Spontaneous (●——●) and K^+ evoked (○——○) release of radioactivity from superfused slices. The results are shown as a release rate constant derived from the recovered radioactivity and expressed as % of total radioactivity in the tissue at that instant. Each point is the mean of 4–6 determinations: vertical bars represent standard errors of the mean. Ordinates a: % total radioactivity recovered; b: % total radioactivity. Abscissa: Time (min).

sucrose efflux was seen during electrical stimulation. (These results are not shown). At the time of experimentation (after 30 min superfusion) the spontaneous efflux of radioactivity from the tissue had reached a steady state (Fig. 1b).

The racemic mixture (±)-baclofen, in concentrations 300 μM to 1 mM, was able to significantly enhance release of radioactivity from slices of globus pallidus prelabelled with ^{3}H-GABA *in vitro* ($P < 0.05$, $P < 0.01$) (Fig. 2). Lower concentrations of (±)-baclofen (100 and 200 μM), although increasing spontaneous efflux, did not have a significant effect. A typical profile of the release of radioactivity from pallidal slices after superfusion with 300 μM and 1 mM baclofen is shown in Fig. 3. In both cases the peak in radioactivity occurs after the drug has been removed during the subsequent washout phase. Of the two stereoisomers, (+)-baclofen, but not (−)-baclofen, effectively enhanced the release of radioactivity at 200 μM (Fig. 4). DAPA had no significant effect on efflux of radioactivity at 1 mM (Fig. 4). Neither dopamine (100 and 500 μM) nor β-phenethylamine (1 mM) caused any release of radioactivity from superfused slices of globus pallidus prelabelled with ^{3}H-GABA (Fig. 5).

In addition to evoking release of ^{3}H-GABA from slices of globus pallidus, (±)-baclofen (400 μM) significantly enhanced release of radioactivity from tissue prelabelled with either [^{3}H]β-alanine or ^{3}H-DABA (Fig. 6).

Superfusing the tissue with the glial GABA uptake blocking agent β-alanine (20 μM and 1 mM) prevented (±)-baclofen from increasing efflux of radioactivity above that of the spontaneous concentrations (Fig. 7). Addition of a high concentration of ACHC (600 μM) similarly inhibited any releasing effect of (±)-baclofen, although (±)-baclofen was shown to increase radioactivity above that of the spontaneous

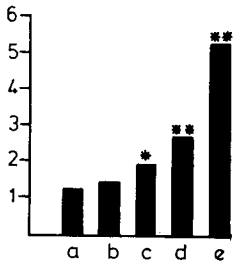

FIG. 2. Effect of (±)-baclofen on release of radioactivity from superfused slices of rat globus pallidus prelabelled with ^{3}H-GABA *in vitro*. Each histobar is the ratio of the release of radioactivity evoked by the drug during a 6-min exposure to the spontaneous radioactivity released from that tissue. * $P < 0.05$, ** $P < 0.01$ (Mann-Whitney U-test). a: 100; b: 200; c: 300; d: 400 μM; e:1 mM.

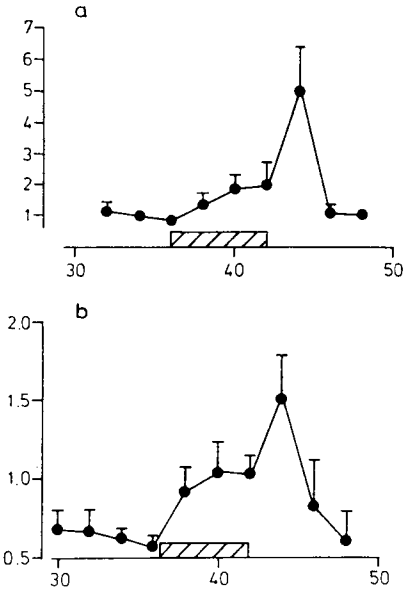

FIG. 3. Profiles of release of radioactivity from superfused slices of rat globus pallidus prelabelled with ^{3}H-GABA *in vitro* evoked by a 6 min exposure to (±)-baclofen at concentrations of 1 mM (upper trace) and 300 μM (lower trace). The results are shown as a release rate constant derived from the recovered radioactivity and expressed as % of total radioactivity in the tissue at that instant. Each point is the mean of 4 determinations: vertical bars denote standard errors of the mean. Ordinate: % total radioactivity. Absicissa: Time (min).

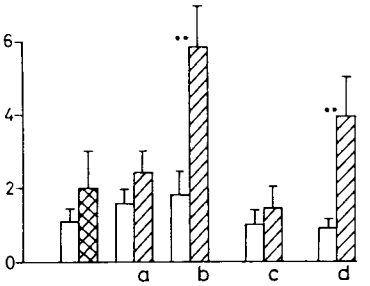

FIG. 4. Effect of DAPA (1 mM) (cross-hatched columns) and baclofen hydrochloride (hatched columns) on spontaneous efflux (open columns) of radioactivity from superfused slices of rat globus pallidus prelabelled with ^{3}H-GABA *in vitro*. Baclofen was added as either (±)-baclofen (100 (a) and 400 μM (b)) or as the individual stereoisomers (—) (c) and (+)-baclofen (d) (200 μM). Each histobar is the % of tissue radioactivity released in a 6 min superfusion and represents the mean ±s.e.m. of 4–6 determinations. ** $P < 0.02$ (Mann-Whitney U-test).

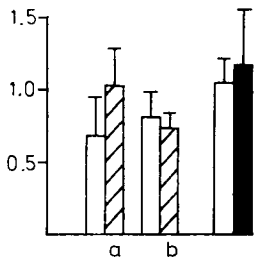

FIG. 5. Effect of dopamine (hatched columns) (100 (a) and 500 μM (b)) and β-phenethylamine (solid columns) (1 mM) on spontaneous efflux (open columns) of radioactivity from superfused slices of rat globus pallidus prelabelled with ^{3}H-GABA *in vitro*. Each histobar is the % of tissue radioactivity released in a 6 min superfusion and represents the mean $\pm$ 1 s.e.m. of 4–6 determinations.

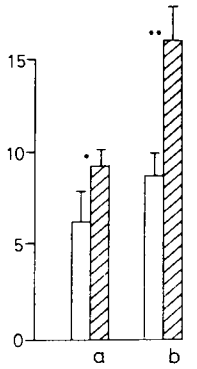

FIG. 6. Effect of ($\pm$)-baclofen (hatched columns) (400 μM) on the spontaneous efflux (open columns) of radioactivity from superfused slices of rat globus pallidus prelabelled with either [^{3}H]β-alanine (a) or ^{3}H-DABA (b) *in vitro*. Each histobar is the % of tissue radioactivity released in a 6 min supefusion and represents the mean $\pm$ s.e.m. of 4–6 determinations. *P <0·05, ** P <0·02 (Mann-Whitney U test).

concentrations in the presence of a low concentration of ACHC (20 μM) (Fig. 7).

($\pm$)-Baclofen, in concentrations greater than 10^{-5} M, significantly inhibited the uptake of labelled GABA into pallidal slices (Table 1). The IC50 value of ($\pm$)-baclofen on GABA uptake was calculated as 6×10^{-4} M.

DISCUSSION

The globus pallidus is an area of the brain rich in GABA terminals and was chosen to study the *in vitro* release of this transmitter. Indeed, depolarizing stimuli (high extraneuronal potassium concentration and electrical pulses) evoke a significant increase in radioactivity in the superfusate of slices of globus

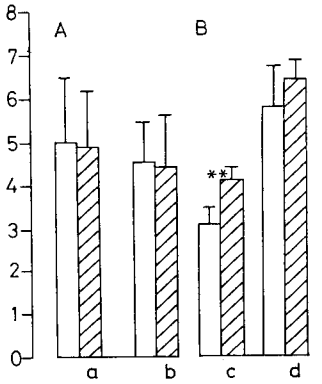

FIG. 7. Effect of A: β-alanine (20 μM (a) and 1 mM (b)) or B: ACHC (20 (c) and 600 μM (d)) on the ability of ($\pm$)-baclofen (hatched columns) (400 μM) to increase the spontaneous efflux (open columns) of radioactivity from superfused slices of rat globus pallidus prelabelled with ^{3}H-GABA *in vitro*. β-Alanine or ACHC were present in both the prelabelling and superfusing periods. Each histobar is the % of tissue radioactivity released in a 6 min superfusion and represents the mean $\pm$ 1 s.e.m. of 4 determinations. ** P <0·02 (Mann-Whitney U-test).

pallidus prelabelled with ^{3}H-GABA. The release is calcium-dependent as substitution of this ion for magnesium prevents electrically-induced release of this radioactivity. Since physiological release of transmitter is believed to be sensitive to calcium (Rubin, 1970), these results suggest that such slices of globus pallidus provide a good model in which to study a quasi-physiological release of GABA *in vitro*.

The results demonstrate that the racemic mixture of baclofen is able to evoke release of radioactivity from slices of rat globus pallidus prelabelled with ^{3}H-GABA *in vitro* in a dose-dependent manner. This releasing effect of baclofen appears specific to the (+)-isomer as 200 μM (+)-baclofen significantly

Table 1. *Effect of* ($\pm$)*baclofen hydrochloride on* ^{3}*H-GABA uptake into slices of rat globus pallidus.* For experimental detail see text. Uptake is expressed as d min^{-1} mg^{-1} wet weight tissue. Each result is the mean $\pm$ s.e.m. of 6 determinations.

Baclofen concn (M)	d min^{-1} mg^{-1} tissue	% uptake inhibition
0	84972 $\pm$ 10049	0
10^{-3}	19154 $\pm$ 1829**	77·5
10^{-4}	45612 $\pm$ 7826*	46·0
10^{-5}	52280 $\pm$ 5643*	38·5
10^{-6}	59839 $\pm$ 9207	30·0

IC50 from dose response curve = 6×10^{-4} M.
* P<0·05; ** P<0·002 Mann-Whitney U test.

evoked release of radioactivity but (−)-baclofen was without effect. The (−)-isomer has been reported to be a neuronal depressant as it, but not the (+)-isomer, decreases the spontaneous firing of nigral neurons in the rat: the depressant action not being antagonized by bicuculline (Olpe, Koella & others, 1977a). Similarly in our system another neuronal depressant, DAPA (Biscoe, Davies & others, 1977), was unable to significantly enhance release of radioactivity from slices of globus pallidus prelabelled with ^{3}H-GABA. These results suggest that the increase in ^{3}H-GABA efflux observed in these experiments is not the non-specific consequence of neuronal depression.

A puzzling point was denoted by the radioactive profiles seen after perfusion with (±)-baclofen, where the peak of radioactivity appeared after drug removal. At present we have no ready explanation for such an effect unless it is related to the neuronal depressant action of the (−)-isomer inhibiting maximal release in the presence of the drug.

Since baclofen is as much a phenethylamine derivative as it is a GABA derivative the possibility that baclofen was acting through dopamine receptors to stimulate release of GABA was tested. It is known that dopamine can stimulate release of GABA from slices of rat substantia nigra (Reubi, Iversen & Jessell, 1977) and that the globus pallidus has been demonstrated to contain dopaminergic nerve terminals (Ungerstedt, 1971). However, our results show that neither dopamine nor the parent β-phenethylamine were able to evoke release of ^{3}H-GABA from slices of rat globus pallidus. Thus it seems unlikely that baclofen stimulates GABA release through dopaminergic receptors.

Baclofen stimulated release of radioactivity from pallidal slices pre-incubated with either [^{3}H]β-alanine or ^{3}H-DABA. β-Alanine is reported to be taken up into glial cells while DABA is predominantly a marker of neuronal tissue (Iversen & Kelly, 1975). The release of GABA from glial elements has been reported (Bowery & Brown, 1971) although the exact functional role of such mechanisms has yet to be assessed. However baclofen evoked release of both agents and it must be concluded that the drug has no preference for either glial or neuronal elements.

In a further attempt to distinguish between neuronal and glial release, pallidal slices were pre-incubated with ^{3}H-GABA in the presence of either β-alanine, an inhibitor of glial GABA uptake (IC50 60–400 μM) (Iversen & Kelly, 1975) or ACHC, an inhibitor of neuronal GABA uptake (IC50 60 μM) (Bowery, Jones & Neal, 1976). At concentrations greater than their IC50 values both drugs prevented baclofen-induced release of radioactivity. However these uptake blocking compounds themselves evoked a large efflux of radioactivity (compare Figs 4 and 7) which might be a maximal effect in our system, thereby explaining baclofen's inability to release GABA in their presence. Thus this experiment was unable to distinguish between neuronal and glial GABA release. Perhaps a better approach might be to repeat the observation in either rat cerebral cortex (a tissue which selectively accumulates GABA into neuronal elements, Iversen & Kelly, 1975) or rat retina (a glial marker tissue for GABA) in order to resolve this problem. However in view of the widely differing GABA kinetics in these other tissue regions it would not be possible to meaningfully extrapolate such results to our study on the globus pallidus.

Baclofen was also able to inhibit the uptake of ^{3}H-GABA into tissue slices, with an IC50 value of 600 μM However it is not possible to conclude from the present data whether baclofen enhances efflux of radioactivity solely by its uptake inhibitory capacity or by a releasing action which is either a specific presynaptic effect or a homoexchange mechanism for GABA such as that seen by Roberts (1976): although an increased amount of GABA in the synaptic cleft would result in each case. The effect is, however, apparently specific to the (+)-isomer.

The isomer which is active iontophoretically is the (−)-isomer, although this compound is not believed to be GABA-like as it is not antagonized by bicuculline (Davies & Watkins, 1974; Olpe & others, 1977a). It is possible that the neuronal depression shown by the (−)-isomer represents activation of a post-synaptic phenethylamine receptor (Curtis & others, 1974) which may in turn reflect the increased catecholamine concentrations observed by Andén & Wachtel (1977). In a recent study, Waldmeier & Maitre (1978) recorded that increase in dopamine metabolite concentrations following acute systemic injection of baclofen was entirely confined to the (−)-isomer. It is possible that this increase in dopamine metabolite concentrations is the effect of neuronal depression in a similar manner as is seen with γ-hydroxybutyrate treatment (Walters, Roth & Aghajanian, 1973). Examination of Waldmeier & Maitre's data shows that (+)-baclofen decreased striatal homovanillic acid levels by 22%. This result would fit in with the observed decreases in dopamine turnover associated with elevation of cerebral GABA concentrations following inhibition of GABA transaminase (Huot, Lippert & others, 1977; Pycock &

Horton, 1978). Thus the observed GABAmimetic effects of baclofen may be explained in terms of an enhancement of GABA release by an effect specific to the (+)-isomer.

Clinically, it is possible that the various psychotropic and motor side-effects of baclofen therapy are related to GABA release by the (+)-isomer, whereas it is the (−)-isomer of the drug which, by neuronal depression, is the effective antispastic agent. Furthermore it is suggested that baclofen may have potential as an antischizophrenic agent (Frederiksen, 1975) where both an overactive dopamine system (Matthysse, 1973) and lesions of inhibitory GABAergic pathways (Fuxe, Ljungdahl & others, 1975; Bird, Barnes & others, 1977) have been suspected.

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