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## Baclofen enhances [ $^3\text{H}$ ]-GABA release from rat globus pallidus *in vitro*

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The  $\gamma$ -aminobutyric acid (GABA) derivative  $\beta$ -parachlorophenyl-GABA (baclofen, Lioresal-CIBA-GEIGY) is believed to act as a GABA-mimetic in the central nervous system (e.g. Andén & Wachtel, 1977) although this suggestion has been challenged (Davies & Watkins, 1974). We have explored the possibility that baclofen may enhance GABA-like transmission by a presynaptic action on GABA release. *In vitro* release of [ $^3\text{H}$ ]-GABA was studied from rat globus pallidus, a region known to contain large numbers of GABA-like terminals. The methods used were essentially those of Srinivisan, Neal & Mitchell (1969). Pallidal cubes (0.2 mm) were prelabelled with transmitter (1  $\mu\text{Ci/ml}$ ) and superfused at 1.5 ml/min with Krebs-bicarbonate buffer (37°C) containing aminoxyacetic acid ( $10^{-5}$  M). Results are expressed as fractional rate constants calculated from the total radioactivity recovered: significances were determined by Mann-Whitney 'U' test. The effect of depolarizing stimuli (50 mM  $\text{K}^+$ ) and electrical stimulation (20 V, 1 ms, 50 Hz) or test drug (baclofen hydrochloride, DL- $\alpha,\epsilon$ -diaminopimelic acid) on the efflux of radioactivity was studied.

Both electrical stimulation and  $\text{K}^+$  (50 mM) enhanced the efflux of tritium. No change in the efflux of a [ $^{14}\text{C}$ ]-sucrose space marker was seen during electrical stimulation. Electrically evoked increase of radioactivity was markedly reduced in the absence of calcium. Baclofen (400  $\mu\text{M}$ ) significantly increases efflux of radioactivity ( $P < 0.02$ ), although baclofen (100  $\mu\text{M}$ ) alone produces only a small and non-significant enhancement. Aminocyclohexane carboxylic acid (20  $\mu\text{M}$ ), a selective competitive inhibitor of neuronal GABA transport (Bowery, Jones & Neal, 1976), significantly enhanced efflux of radioactivity, and addition of baclofen (400  $\mu\text{M}$ ) produced a further significant

enhancement ( $P < 0.05$ ). In the presence of  $\beta$ -alanine (20  $\mu\text{M}$ ), a competitive inhibitor of glial GABA transport (Iversen & Kelly, 1975), the spontaneous efflux was not significantly different from that produced by baclofen (400  $\mu\text{M}$ ) alone and subsequent addition of baclofen produced no further increase in efflux. Of the two stereoisomers only (+)-baclofen significantly ( $P < 0.02$ ) enhanced radioactive efflux at 200  $\mu\text{M}$ .

At baclofen (400  $\mu\text{M}$ ) also enhanced the efflux of [ $^3\text{H}$ ]- $\beta$ -alanine and [ $^3\text{H}$ ]-diaminobutyric acid, presumed to be from glial and neuronal elements respectively (Iversen & Kelly, 1975).

DL- $\alpha,\epsilon$ -Diaminopimelic acid, a neuronal depressant (Biscoe *et al.*, 1977) unrelated to GABA, had no effect on efflux of [ $^3\text{H}$ ]-GABA at 1 mM.

These results suggest that baclofen may enhance GABA efflux in a specific manner unrelated to the direct neuronal depressant action of baclofen and may help to provide an explanation for the observed GABA-mimetic actions of baclofen.

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## Effects of $\gamma$ -aminobutyric acid on the potassium and tyramine induced release of $[^3\text{H}]\text{-noradrenaline}$ from rat occipital cortex slices

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Both in the peripheral and in the central nervous system the release of noradrenaline (NA) is regulated through a negative feed-back mechanism mediated by presynaptic  $\alpha$ -adrenoceptors (for reviews, see Langer, 1974, 1977; Starke, 1977). In addition the release of central neurotransmitters can be modified by other putative neurotransmitters through presynaptic mechanisms (Jessel & Iversen, 1977; Arbilla & Langer, 1978).

Recently, it was reported by Taube, Starke & Borowski (1977) that exogenous gamma aminobutyric acid (GABA) does not affect the  $\text{K}^+$ -induced release of  $[^3\text{H}]\text{-NA}$  from rat cerebral slices. On the other hand, the turnover of NA in the rat brain is increased after the administration of GABA systemically and intracerebroventricularly (Biswas & Carlsson, 1977a, b). In the present experiments the effects of GABA were re-examined for the release of  $[^3\text{H}]\text{-NA}$  induced by two different concentrations of potassium and by tyramine.

Occipital cortex slices of 0.3 mm thickness were obtained from male rats (150 to 200 g) and prelabelled with  $[^3\text{H}]\text{-NA}$  (Farah, Adler-Graschinsky & Langer, 1977). The percentage of total tissue radioactivity released by a 1 min exposure to 20 mM  $\text{K}^+$  in the controls was  $4.03 \pm 0.65$  ( $n = 8$ ) during the first period of stimulation ( $S_1$ ) and  $4.74 \pm 1.10$  ( $n = 8$ ) for the second stimulation ( $S_2$ ) obtained 45 min after  $S_1$ . The ratio  $S_2/S_1$  was  $1.09 \pm 0.10$  ( $n = 8$ ). Under these conditions the release of  $[^3\text{H}]\text{-NA}$  elicited by  $\text{K}^+$  was entirely calcium-dependent (Arbilla & Langer, 1978). When exogenous GABA was added prior to  $S_2$  there was a concentration-dependent increase in the release of  $[^3\text{H}]\text{-NA}$  induced by  $\text{K}^+$ . In the presence of 100  $\mu\text{M}$  GABA the ratio  $S_2/S_1$  was  $1.47 \pm 0.13$  ( $n = 6$ ,  $P < 0.02$ ) and for 300  $\mu\text{M}$  GABA the ratio  $S_2/S_1$  was  $2.22 \pm 0.12$  ( $n = 8$ ,  $P < 0.001$ ). During exposure to

GABA (1 mM) the release of  $[^3\text{H}]\text{-transmitter}$  was also increased ( $S_2/S_1 = 2.77 \pm 0.45$ ,  $n = 6$ ,  $P < 0.005$ ). The enhancement in  $[^3\text{H}]\text{-NA}$  release obtained in the presence of GABA (300  $\mu\text{M}$ ) was not antagonized by bicuculline (1 and 10  $\mu\text{M}$ ) or by picrotoxin (10  $\mu\text{M}$ ) under conditions in which the blocking agents did not by themselves affect the  $\text{K}^+$ -induced release of  $[^3\text{H}]\text{-NA}$ .

When  $[^3\text{H}]\text{-NA}$  release was elicited by a 1 min exposure to a higher concentration of  $\text{K}^+$ : 35 mM, the fraction of the total tissue radioactivity released was  $32.3 \pm 1.3\%$  ( $n = 6$ ). The release of the labelled neurotransmitter elicited by  $\text{K}^+$  (35 mM) was not affected by either 300  $\mu\text{M}$  or 1 mM GABA.

In contrast to the  $\text{K}^+$ -induced depolarization, tyramine displaces NA from vesicular storage sites through a calcium-independent mechanism. Consequently, it was considered of interest to examine the effects of exogenous GABA on  $[^3\text{H}]\text{-NA}$  release induced by tyramine. In the controls, exposure to tyramine (0.6  $\mu\text{M}$ ) produced a release of  $8.5 \pm 1.5\%$  of total tissue radioactivity in  $S_1$  ( $n = 4$ ). The release of  $[^3\text{H}]\text{-NA}$  induced by tyramine was not enhanced by either 300  $\mu\text{M}$  or 1 mM GABA.

The effects of exogenous GABA were also studied on the release of total radioactivity induced by nerve stimulation in a peripheral tissue, the isolated nerve-muscle preparation of the cat nictitating membrane, as described by Langer & Luchelli-Fortis (1977). Nerve stimulation was applied at 4 Hz for 5 min and with supramaximal voltage. In the controls the ratio of  $[^3\text{H}]\text{-transmitter}$  overflow between two consecutive periods of nerve stimulation,  $S_2/S_1$  was  $1.06 \pm 0.10$  ( $n = 13$ ). Exogenous GABA (10, 30, 100 and 300  $\mu\text{M}$ ) added 15 min before  $S_2$  did not modify either the spontaneous or the stimulation-induced release of the tritiated neurotransmitter.

In summary, GABA enhances the  $\text{K}^+$ -induced release of  $[^3\text{H}]\text{-NA}$  from the rat occipital cortex while it fails to modify the release of the labelled transmitter induced by tyramine. Recently, Stoof & Mulder (1977) reported a similar effect of endogenous GABA on the  $\text{K}^+$ -induced release of dopamine from the rat striatum. In contrast to the results obtained in the central nervous system, GABA did not affect NA release elicited by nerve stimulation in the isolated nerve-muscle preparation of the cat nictitating membrane.