might be the result of a change in the pharmacological properties of the nerve ending in the isolated preparations brought about by repeated doses.

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Baclofen: stereoselective inhibition of excitant amino acid release

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Baclofen, β-(p-chlorophenyl)-γ-aminobutyric acid (CIBA 34,647-Ba, Lioresal), is used clinically to reduce spasticity in various neurological disorders (Birkmayer 1972). Although first synthesized as an analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) likely to penetrate the blood brain barrier, baclofen may inhibit the release of excitatory amino acid neurotransmitters at lower concentrations than those needed to influence GABA receptors or GABA release (Waddington & Cross 1979). Several studies have shown that baclofen may reduce primary afferent depolarization in the spinal cord by a depression of transmitter release (Davidoff & Sears 1974; Ault & Evans 1978; Curtis & Lodge 1978; Fox et al 1978; Kato et al 1978), and baclofen, 4 μM, has been shown to depress the electrically evoked release of endogenous glutamate and aspartate from slices of guinea-pig cerebral cortex (Potashner 1979).

The availability of the stereoisomers of baclofen, and the demonstration that the action on spinal reflexes resides with the (−)-isomer (Olpe et al 1978) has enabled the stereoselectivity of various actions of baclofen to be investigated. (−)-Baclofen is over twenty times more potent than the (+)-isomer in depressing synaptic activity in the immature rat isolated spinal cord (Ault & Evans 1978) and greater than two orders of magnitude more potent in its antinociceptive action in rats (Wilson & Yaksh 1978). On the other hand, potentiation of GABA release appears to be specific for the (+)-isomer (Kerwin & Pycock 1978), while both isomers are equally active in weakly inhibiting GABA receptor binding and in a GABA-dependent rotational behavioural test (Cross & Waddington 1978); these results provide further evidence against baclofen acting as a GABA-mimetic in its therapeutic effects.

The present investigations show that (−)-baclofen has a stereoselective action in inhibiting the evoked release of D-aspartate from slices of rat cerebral cortex and cat spinal cord. D-Aspartate is a good substrate for the high affinity acidic amino acid transport system that takes up the excitant amino acid neurotransmitters, L-glutamate and L-aspartate (Davies & Johnston 1976; Takagaki 1978). D-Aspartate appears to enter the presynaptic transmitter pools of L-glutamate and L-aspartate, and the uptake of D-aspartate has been used to study the localization of excitant amino acid-accumulating nerve terminals (Storm-Mathisen 1978). Preloaded D-aspartate can be released from these presynaptic pools in rat brain slices by increased potassium concentrations in a calcium-dependent manner, so that such evoked release of the metabolically stable D-aspartate may be a useful model for the synaptic release of the rapidly metabolized natural excitatory amino acid neurotransmitters (Davies & Johnston 1976).

The release of preloaded labelled D-aspartate and GABA from c.n.s. tissue slices was studied as described in detail previously (Davies et al 1975). The slices (0·1 × 0·1 × ca 2 mm) of rat cerebral cortex (removed after decapitation) or cat spinal cord (removed under

Table 1. Effects of baclofen stereoisomers on stimulated release of D-aspartate from c.n.s. tissue slices

Conditions	Maximum efflux rate constant (min^{-1})	% Control stimulated release
A. Slices of rat cerebral cortex:		
(i) Potassium (40 mM)		
Stimulation Control	0·0055 ± 0·0003 (6)	100 ± 5
(−)-Baclofen 4 μM	0·0039 ± 0·0004 (5)	71 ± 7*
(+)-Baclofen 4 μM	0·0060 ± 0·0007 (5)	109 ± 13
(ii) Protoveratrine (100 μM)		
Stimulation Control	0·0136 ± 0·0006 (10)	100 ± 4
(−)-Baclofen 1 μM	0·0113 ± 0·0017 (3)	83 ± 13
4 μM	0·0073 ± 0·0009 (3)	54 ± 7**
10 μM	0·0088 ± 0·0016 (3)	65 ± 12*
(+)-Baclofen 1 μM	0·0145 ± 0·0008 (3)	107 ± 6
4 μM	0·0121 ± 0·0008 (3)	89 ± 6
10 μM	0·0157 ± 0·0003 (3)	115 ± 2*
B. Slices of cat spinal cord: Protoveratrine (100 μM)		
Stimulation Control	0·0097 ± 0·0005 (6)	100 ± 5
(−)-Baclofen 4 μM	0·0055 ± 0·0004 (3)	57 ± 4**
(+)-Baclofen 4 μM	0·0099 ± 0·0006 (3)	102 ± 6

Values are means ± s.e. of the number of determinations shown in brackets, and those labelled * and ** are significantly different from controls at $P < 0·02$ and 0·005 respectively by Student's *t*-test.

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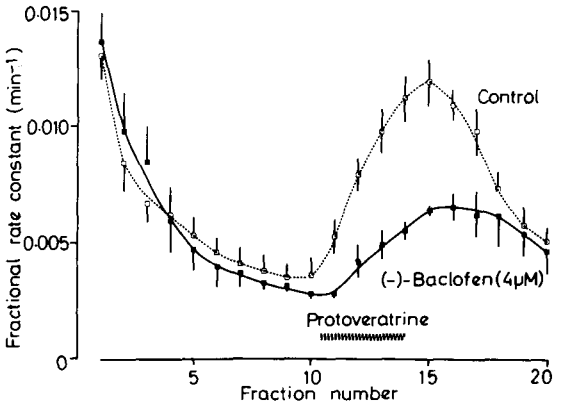

FIG. 1. Efflux of ^{3}H-D-aspartate from slices of rat cerebral cortex into perfusing medium. Solid curve and symbols: perfusion with medium containing 4 μM (—)-baclofen. Broken curve and open symbols: control. Fractions of perfusate were collected every 2·8 min. During the period indicated 100 μM protoveratrine was added to the perfusing medium. Each point and error bar represents the mean and standard error from 3 experiments.

pentobarbitone anaesthesia) were preloaded in batches of 50 mg with 0·5 μCi of D-[2,3-^{3}H]aspartate and/or [U-^{14}C]GABA for 20 min at 37 °C. In all experiments with labelled GABA, 20 μM amino-oxyacetic acid was used to inhibit GABA catabolism. Fractions of perfusate were collected every 2·8 min directly into scintillation vials and counted after addition of scintillant. Release was stimulated by increasing the potassium chloride concentration in the buffer from 4·75 to 44·75 mM ('potassium stimulation') or by the addition of 100 μM protoveratrine A ('protoveratrine stimulation'). Previous studies have shown that essentially no metabolism of D-aspartate or GABA occurs under the above conditions (Davies & Johnston 1976).

The stereoisomers of baclofen were a generous gift from Dr J. Gelzer and D. K. Scheibli (CIBA-GEIGY, Basle, Switzerland). Protoveratrine A was purchased from Sigma Chemical Company (St. Louis, USA) and was dissolved in an equivalent amount of 0·1 M-HCl before addition to the perfusion buffer. D-[2,3-^{3}H]-Aspartic acid, specific activity 16·1 Ci mmol^{-1}, was a gift from Dr J. Storm-Mathisen (Kjeller, Norway). [U-^{14}C]GABA, 228 mCi mmol^{-1} was purchased from the Radiochemical Centre (Amersham, England).

Effects of the stereoisomers of baclofen on the stimulated release of D-aspartate are summarized in Table 1. (−)-Baclofen, 4 μM, inhibited by approximately 50% the release of preloaded ^{3}H-D-aspartate from slices of rat cerebral cortex (illustrated in Fig. 1) and cat spinal cord evoked by protoveratrine, whereas (+)-baclofen was inactive under these conditions, and at 10 μM weakly potentiated D-aspartate release. These results are consistent with the observations that 6 μM (±)-baclofen produced 50% depression of dorsal root evoked synaptic activity in the immature rat isolated spinal cord, and that the (−)-isomer of baclofen was over twenty times more potent than the (+)-isomer in this preparation (Ault & Evans 1978).

The inhibition of D-aspartate release by (−)-baclofen is unlikely to be due to a GABA-mimetic action, since GABA, 10–100 μM, did not influence D-aspartate release. Neither isomer of baclofen influenced ^{3}H-GABA release at 10 μM, although higher concentrations of the (+)-isomer are known to potentiate GABA release (Kerwin & Pycock 1978).

The present findings are consistent with the anti-spasticity effect of baclofen resulting in part from the (−)-stereoisomer inhibiting the release of excitatory amino acid neurotransmitters. Furthermore, they emphasise the usefulness of metabolically stable D-aspartate for labelling central excitant amino acid transmitter stores.

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