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Piperazic acid and related compounds as inhibitors of GABA uptake in rat brain slices

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Cellular uptake of GABA by specific transport processes may have an important physiological role at synapses which use GABA as a neurotransmitter. There is, therefore, a continuing interest in inhibitors of GABA uptake which may be useful pharmacological agents. Two relatively selective inhibitors that have been studied extensively are 2,4-diamino-butyric acid and nipecotic acid (Iversen & Kelly, 1975; Krogsgaard-Larsen & Johnston, 1975). As illustrated in Fig. 1, these inhibitors are structurally related to piperazic acid (hexahydropyridazine-3-carboxylic acid), derivatives of which occur in the monamycins, a family of cyclodepsipeptide antibiotics obtained from cultures of *Streptomyces jamaicensis* (Hassall, Morton & others, 1969; 1971). The optical isomers of piperazic acid, and some related compounds, have been examined as inhibitors of GABA uptake in rat brain slices and *S*(−)-piperazic acid found to be a potent inhibitor.

The sodium-dependent, "high-affinity" uptake of GABA (10 nM exogenous concentration) in "minislices" of rat cerebral cortex (0·1 × 0·1 × 2·0 mm) at 25° and the transamination of GABA catalysed by extracts of rat cerebral cortex were studied as described in detail elsewhere (Beart, Johnston & Uhr, 1972a; Beart, Uhr & Johnston, 1972b). The uptakes of L-glutamate (10 nM), β-alanine (0·3 nM) and L-proline (6 nM) were studied by similar procedures (Balcar & Johnston, 1972; Balcar, Johnston & Stephanson, 1976; Johnston & Stephanson, 1976). Unless stated otherwise, potential inhibitors were preincubated with the brain slices for 15 min.

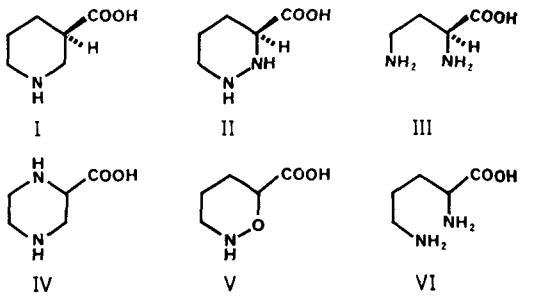

FIG. 1. Some structurally-related inhibitors of GABA uptake. I—*R*(−)-Nipecotic acid, II—*S*(−)-piperazic acid, III—*S*(+)-2,4-diamino-butyric acid, IV—(±)-piperidazine-2-carboxylic acid, V—perhydro-1,2-oxazine-6-carboxylic acid, VI—(±)-ornithine.

S(−)- and *R*(+)-Piperazic acid, and derivatives thereof, were gifts from Dr C. H. Hassall, Roche Products Ltd., Welwyn Garden City. (±)-Piperazine-2-carboxylic acid was prepared by catalytic hydrogenation of pyrazine-2-carboxylic acid (Felder, Maffei & others, 1960); it had a m.p. of 285° and analysed correctly for C, H, N.

The effects of *S*(−)- and *R*(+)-piperazic acid and of (±)-piperazine-2-carboxylic acid on GABA uptake are summarized in Table 1 and compared with the effects of some structurally related substances. *S*(−)-Piperazic acid was approximately 25 times as potent as the *R*(+)-stereoisomer, and was intermediate in potency between *R*(−)-nipecotic acid and *S*(+)-2,4-diamino-butyric acid. The 1-benzyloxycarbonyl derivative of *S*(−)-piperazic acid did not significantly inhibit GABA

* Correspondence.

Table. 1. *Inhibition of GABA uptake in rat brain slices.*

Inhibitor	% Inhibit. at 500 μM	IC50 (μM)
R(−)-Nipecotic acid	99 ± 1	5 ± 1
S(−)-Piperazic acid	95 ± 1	21 ± 3
S(+)-Nipecotic acid	93 ± 1	30 ± 2
S(+)-2,4-Diaminobutyric acid	91 ± 1	35 ± 3
(±)-Perhydro-1,2-oxazine-6-carboxylic acid	70 ± 2	380 ± 17
(±)-Piperidazine-2-carboxylic acid	29 ± 2	>500
(±)-Ornithine	26 ± 4	>500
R(+)-Piperazic acid	22 ± 9	>500

The tissue slices were preincubated with the inhibitor for 15 min at 25°. % Inhibition figures are means ±s.e.m. of quadruplicate experiments. IC50 values are means ±s.e.m. calculated from the results of quadruplicate experiments at each of 3 inhibitor concentrations as described by Balcar & others (1976). Values for *R*(−)- and *S*(+)-nipecotic acid, *S*(+)-2,4-diaminobutyric acid, (±)-perhydro-1,2-oxazine-6-carboxylic acid and (±)-ornithine are from Johnston & others (1976a), Lodge, Johnston & Stephanson (1976), Krogsgaard-Larsen & Johnston (1975), and Iversen & Johnston (1971) respectively.

uptake at 500 μM, while (±)-2-trifluoroacetylpiperazic acid inhibited by 54 ± 3 % at this concentration. The stereoisomers of piperazic acid did not significantly influence at 1 mM the transamination of GABA catalysed by an extract of rat cerebral cortex during 30 min at 37° (no preincubation), or at 500 μM the uptake of L-glutamate and L-proline. *S*(−)-Piperazic acid inhibited the uptake of β-alanine by 63 ± 4 % at 500 μM, but the *R*(+)-stereoisomer was ineffective at this concentration.

Three compounds illustrated in Fig. 1, structurally related to piperazic acid, were weak inhibitors of GABA uptake: piperidazine-2-carboxylic acid which is isomeric with piperazic acid, perhydro-1,2-oxazine-6-carboxylic acid which contains an oxygen in place of N(2) in piperazic acid, and ornithine which is an open chain analogue of piperazic acid.

The absolute configurations of the stereoisomers of piperazic acid have been determined by reduction of *R*(+)-piperazic acid to *R*(−)-ornithine (Hassall & others, 1969), and are in agreement with the present results on inhibition of GABA uptake. There is an obvious similarity (Fig. 1) with respect to the asymmetric centre in the stereoisomer of nipecotic acid, piperazic acid and 2,4-diaminobutyric acid that is more potent as an inhibitor of GABA uptake. There is evidence to indicate that *R*(−)-nipecotic acid and *S*(+)-2,4-diaminobutyric acid interact with the GABA transport system as substrate-competitive inhibitors which are taken up by the tissue (Simon & Martin, 1973; Johnston, Stephanson & Twitchin, 1976b; Johnston, Krogsgaard-Larsen, & others, 1976a). *S*(−)-Piperazic acid is likely to act in a similar way since its effects on GABA uptake are influenced by preincubation: 20 μM-*S*(−)-piperazic acid inhibits GABA uptake by 30 ± 1 % if added at the same time as GABA and by 49 ± 3 % if preincubated with the tissue for 15 min before the addition of GABA. Similar preincubation effects with *R*(−)-nipecotic acid and *S*(+)-2,4-diaminobutyric acid have been interpreted on the basis of the inhibitors being taken up by the tissue during the preincubation period and inhibiting GABA uptake intracellularly as well as extracellularly (Simon & Martin, 1973; Johnston & others, 1976b).

The piperazic acid derivatives that occur in the monamycins have the 3*R* configuration (Hassall & others, 1969, 1971). *R*(+)-Piperazic acid was found to be relatively only a weak inhibitor of GABA uptake in rat brain slices which is unlikely to be a factor to be considered in any pharmacological application for the monamycins. *S*(−)-Piperazic acid, on the other hand, is a relatively potent inhibitor which may be a useful pharmacological agent with which to investigate the physiological role of GABA uptake.

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