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STEREOSPECIFICITY OF 2,4-DIAMINOBUTYRIC ACID WITH RESPECT TO INHIBITION OF 4-AMINOBUTYRIC ACID UPTAKE AND BINDING

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S(+)-2,4-Diaminobutyric acid is at least 20 times more potent than the R(−)-stereoisomer as an inhibitor of the sodium-dependent uptake of 4-aminobutyric acid (GABA) in rat brain slices. Both isomers, however, are equipotent as inhibitors of sodium-independent binding of GABA to membranes from rat brain. The latter finding may be relevant to the reported neurotoxicity in rats of both isomers of 2,4-diaminobutyric acid after intracisternal injection.

Introduction 2,4-Diaminobutyric acid (DABA) is a neurotoxic amino acid found in various species of *Lathyrus* and *Vicia* (Johnston, 1974) which appears to reduce selectively the sodium-dependent neuronal uptake of the inhibitory transmitter 4-aminobutyric acid (GABA) in slices of rat cerebral cortex (Iversen & Kelly, 1975) and to potentiate the depressant action of GABA on neuronal firing when administered microelectrophoretically (Curtis, Game & Lodge, 1976). The inhibition of GABA uptake by another amino acid, nipecotic acid, is to some degree stereospecific, since R(−)-nipecotic acid is approximately 5 times more potent than the S(+)-isomer (Johnston, Krogsgaard-Larsen, Stephanson & Twitchin, 1976), and earlier experiments with DABA also indicated stereospecificity since the S(+)-isomer was approximately twice as potent as the (±)-racemate (Iversen & Johnston, 1971). We have prepared R(−)-DABA and compared it to commercially available S(+)-DABA with respect to inhibition of sodium-dependent GABA uptake in rat brain slices and to inhibition of sodium-independent binding of GABA to membranes from rat brain.

Methods R(−)-DABA was prepared as the dihydrochloride, m.p. 234–237°C, $[\alpha]_D^{25} - 19.5°$ (c, 2.0 in water), from R(−)-glutamic acid as described by Adamson (1939) but isolating the product by ion exchange column chromatography. S(+)-DABA monohydrochloride was purchased from Calbiochem, San Diego. The sodium-dependent uptake of GABA (10^{-8} M exogenous concentration) was studied in 'minislices' of rat cerebral cortex at 25°C as described by Beart, Johnston & Uhr (1972). The sodium-independent binding of GABA (5.8×10^{-9} M) to membranes isolated from rat cerebral cortex was studied at 4°C in Tris-citrate buffer as described by Enna & Snyder (1975) using 10^{-3} M GABA to correct for nonspecific binding. [2,3-^{3}H]-GABA, specific activity 43 Ci/mmol, was purchased from New England Nuclear, Boston. Percent inhibition values are means ± s.e.m. of quadruplicate experiments.

Results R(−)-DABA, 10^{-3} M preincubated with the tissue for 15 min, had no significant effect on GABA uptake. This isomer was thus at least 20 times less potent than S(+)-DABA which inhibited GABA uptake under the same conditions by 50% at 5×10^{-5}M (Iversen & Johnston, 1971). Both isomers inhibited GABA binding to a similar extent; 35 ± 6% inhibition by R(−)-DABA and 32 ± 2% by S(+)-DABA at 10^{-4} M. From experiments using inhibitor concentrations of 10^{-4} M, 5×10^{-4} and 10^{-3} M, it was calculated by log-probit analysis (Balcar, Johnston & Stephanson, 1976) that the concentration producing 50% inhibition (IC_{50}) of binding was $2.0 \pm 0.4 \times 10^{-4}$ M for R(−)-DABA and $2.3 \pm 0.3 \times 10^{-4}$ M for S(+)-DABA. These results differ from those of Enna & Snyder (1975) who found an IC_{50} of greater than 10^{-3} M for DABA, presumably the S(+)-isomer, as an inhibitor of GABA binding, but in the same study the IC_{50} of 2.6×10^{-4} M found for DABA inhibition of GABA uptake was appreciably higher than that reported by other workers (5×10^{-5} M, Iversen & Johnston, 1971; 6.6×10^{-5}M, Harris, Hopkin & Neal, 1973). It is known that S(+)-DABA has a relatively weak depressant action (1/4 to 1/20 as potent as GABA) on the firing of cat spinal neurones (Curtis & Watkins, 1960; Curtis *et al.*, 1976), but this could be due to an indirect effect mediated by release of GABA from presynaptic terminals by heteroexchange as has been suggested for the depolarization of sympathetic ganglion cells by β-alanine (Bowery, Brown, Collins, Galvan, Marsh & Yamini, 1976) since S(+)-DABA appears to be a substrate for the neuronal GABA transport system (Simon & Martin, 1973; Iversen & Kelly, 1975). The present experiments indicate that DABA is some 500 times less active than GABA in displacing radioactive GABA bound to rat brain membranes in the absence of sodium ions.

Discussion It has been suggested that the neurotoxic actions of S(+)-DABA may be related to inhibition of GABA uptake (Iversen & Johnston,

1971). This suggestion now seems unlikely since Chen, Flory & Koeppe (1972) observed 'typical neurotoxicity symptoms' in rats after intracisternal injection of *either isomer* of DABA provided the DABA reached a concentration of at least 10^{-3} M in the brain. At such concentrations *in vitro* we have noted non-stereospecific inhibitions of sodium-independent GABA binding, a process considered to reflect the binding of GABA to postsynaptic receptors (Enna & Snyder, 1975). It is thus possible that some of the neurotoxic effects of intracisternal DABA are related to interaction with postsynaptic receptors for GABA. Activation of inhibitory receptors might underlie the 'prostrating' effect of intracisternal DABA (Chen *et al.*, 1972) and the 'hypoactivity and sedation' produced by intraperitoneal S(+)-DABA (Shuter & Robins, 1974), but the symptoms of hyper-irritability and convulsions which are delayed in onset (Vivanco, Ramos & Jimenez-Diaz, 1966; Chen *et al.*, 1972) may reflect much more complex events.

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