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Effects of the enantiomers of 5-hexyne-1,4-diamine on ODC, GAD and GABA-T activities in the rat

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(±)-5-Hexyne-1,4-diamine is a potent enzyme-activated irreversible inhibitor of mammalian ornithine decarboxylase (ODC) (EC 4.1.1.17) [1]. When injected into rats, it causes marked decreases in ODC activity and polyamine concns in several organs [2]. However, this compound is rapidly transformed *in vivo* to 4-aminohex-5-ynoic acid, a potent enzyme-activated irreversible inhibitor of 4-aminobutyrate:2-oxoglutarate aminotransferase (GABA-T) (EC 2.6.1.19) and of glutamate decarboxylase (GAD) (EC 4.1.1.15) [3]. The use of specific inhibitors allowed us to identify mitochondrial monoamine oxidase (MAO) (EC 1.4.3.4) as being involved in this metabolism. When (±)-5-hexyne-1,4-diamine is given at dose sufficient to effectively reduce polyamine concns in rat organs, the consequent inhibition of 4-aminobutyrate (GABA) metabolism in the brain leads to undesirable behavioural changes [3]. The compound is therefore unsuitable as a tool for the investigation of the physiological role of polyamines.

We reported recently the asymmetric synthesis of the two enantiomers of 5-hexyne-1,4-diamine from the corresponding enantiomers of 4-aminohex-5-ynoic acids [4]. *R*-(−)-5-Hexyne-1,4-diamine was the only enantiomer responsible for the inactivation of rat liver ODC [4]. Since the mitochondrial oxidation of 5-hexyne-1,4-diamine concerns only the amino group at the 1-position [3], *R*-(−)-5-hexyne-1,4-diamine should be metabolized to *R*-(−)-4-aminohex-5-ynoic acid which was believed to have no action on GABA metabolism [5]. Therefore *R*-(−)-5-hexyne-1,4-diamine should selectively inactivate ODC. In order to confirm this hypothesis, we have investigated the effects of each of the enantiomers of 5-hexyne-1,4-diamine on the activities of ODC in the ventral prostrate and of GABA-T and GAD in the brain of rats.

Materials and methods

Chemicals. The following compounds were purchased: L-ornithine, pyridoxal-phosphate, ammonium sulfate, reduced glutathione (GSH), sucrose and buffer reagents (Merck, Darmstadt, F.R.G.); *S*-adenosyl-L-methionine, dithiothreitol, NAD^+, 4-aminobutyric acid, L-glutamate, 2-oxoglutarate (Sigma, St. Louis, MO); homovanillic acid, horseradish peroxidase, tetrasodium EDTA (Calbiochem, San Diego, CA); DL-[1-^{14}C]ornithine (sp. radioactivity 58 Ci/mole) (Radiochemical Centre, Amersham, U.K.); DL-[1-^{14}C]glutamate (50 Ci/mole) (New England Nuclear Corp., Boston, MA). (±)-5-Hexyne-1,4-diamine [1] and its pure enantiomers (cross-contamination <0.5%) were synthesized in our laboratories [4].

Animals. Male rats of the Sprague–Dawley strain (200–220 g body wt) were purchased from Charles River, France. Animals had access to standard diet and water *ad lib.* and were kept under a constant 12 hr light/12 hr dark lighting schedule. They were killed by decapitation at about the same time of day to minimize effects due to diurnal fluctuations. Drugs, dissolved in 0.9% saline, were injected intraperitoneally. Rats given saline served as controls.

Assays of enzyme activities and determination of GABA. The assays of MAO, ODC, GABA-T and GAD activities, and the measurements of whole-brain GABA concns were performed as described previously [3].

Results and discussion

As a preliminary experiment, we investigated the *in vitro* oxidation of the two enantiomers of 5-hexyne-1,4-diamine by MAO. The two enantiomers and the racemic mixture of 5-hexyne-1,4-diamine were oxidized at the same maximum velocity by the MAO preparation (not shown). Michaelis constants (K_m) were found to be 1.0 ± 0.1, 0.9 ± 0.1 and 0.8 ± 0.1 mM for *R*-(−)-, *S*-(+)- and *R,S*-(±)-5-hexyne-1,4-diamine respectively. These results suggest that both enantiomers could be oxidized *in vivo* to the corresponding enantiomers of 4-aminohex-5-ynoic acid with retention of configuration as the oxidation does not involve the asymmetric center [2]. Nevertheless *R*-(−)-5-hexyne-1,4-diamine should be a selective inhibitor of ODC *in vivo*, in spite of its possible oxidation, since it has been reported that the *S*-(+)-enantiomer of 4-aminohex-5-ynoic acid was responsible for the inhibition of mammalian GABA-T and GAD [5]. The effect of *R*-(−)-5-hexyne-1,4-diamine on GABA metabolism was investigated in the following experiments.

Single doses of 100 mg/kg of each enantiomer or of 200 mg/kg of the racemate of 5-hexyne-1,4-diamine were injected 4 hr before the killing of the animals. As expected

Table 1. Effects of a single dose of the racemic mixture and of the enantiomers of 5-hexyne-1,4-diamine on ODC activity in the ventral prostrate and on whole-brain GABA-T and GAD activities, and GABA concns

Compound injected	ODC activity (% control) (ventral prostate)	GABA-T activity (% control) (brain)	GAD activity (% control) (brain)	GABA (μmoles/g) (brain)
Saline	100 ± 9	100 ± 3	100 ± 5	2.1 ± 0.1
R,S-(±)-5-Hexyne-1,4-diamine (200 mg/kg)	3 ± 1*	9 ± 1*	42 ± 3*	8.8 ± 0.2*
R-(−)-5-Hexyne-1,4-diamine (100 mg/kg)	4 ± 1*	35 ± 2*	97 ± 5	4.7 ± 0.4*
S-(+)-5-Hexyne-1,4-diamine (100 mg/kg)	87 ± 16	7 ± 1*	36 ± 2*	8.9 ± 0.1*

The animals were killed 4 hr after intraperitoneal injection. Each value is the mean ± S.E.M. of five animals. The significance of the differences between treated and control animals was calculated by Student's *t*-test: *$P < 0.001$.

R-(−)-5-hexyne-1,4-diamine caused the same decrease in ODC activity in the ventral prostrate as double the dose of the racemate. Furthermore *S*-(+)-5-hexyne-1,4-diamine did not cause any significant change of prostatic ODC activity when compared with control animals (Table 1).

The effects of *R*-(−)-, *S*-(+)- and *R,S*-(±)-5-hexyne-1,4-diamine on the activities of GABA-T and GAD and on the concns of GABA in the whole brain are reported in Table 1. GABA-T activity was decreased approximately to the same extent after 100 mg/kg *S*-(+)-5-hexyne-1,4-diamine or 200 mg/kg racemate. Unexpectedly however, GABA-T activity was reduced by 65% after 100 mg/kg *R*-(−)-5-hexyne-1,4-diamine. GAD activity, in the same brain extracts, was not reduced by the *R*-(−)-enantiomer but was decreased by 64 and 58% after the injections of 100 mg/kg *S*-(+)-enantiomer and 200 mg/kg racemate respectively. The GABA concn in the brain was increased about 4.5 times over control levels by the *S*-(+)-enantiomer or double the dose of the racemate. The GABA concn was also increased after the injection of the *R*-(−)-enantiomer, presumably due to the observed GABA-T inhibition.

In summary, it was expected from previously published results [2, 5] that the *R*-(−)-enantiomer of 5-hexyne-1,4-diamine would selectively inhibit ODC, having no effect on the brain GABA metabolism *in vivo*, and that the *S*-(+)-enantiomer would selectively inhibit GABA-T and GAD, leaving ODC unaffected. We confirm here that the *S*-(+)-enantiomer does not inhibit ODC and has a marked effect on GABA-T and GAD activities and GABA levels in rat brain. The *R*-(−)-enantiomer inhibits ODC as expected from *in vivo* data but, surprisingly, this compound produces a significant reduction of GABA-T activity and an increase of GABA levels, while GAD activity remains unaffected.

If racemisation of the 5-hexyne-1,4-diamine or of 4-aminohex-5-ynoic acid had occurred one would have expected to find also GAD inhibition [6]. These intriguing results warrant a re-examination of the *in vitro* and *in vivo* effects of the enantiomers of 4-aminohex-5-ynoic acid on GAD and GABA-T. Such studies are currently underway in our laboratory.

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