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EFFECT OF 5-HEXYNE-1,4-DIAMINE ON BRAIN 4-AMINOBUTYRIC ACID METABOLISM IN RATS AND MICE

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Abstract—1. 5-Hexyne-1,4-diamine (RMI 71696) an enzyme-activated irreversible inhibitor of ornithine decarboxylase (EC 4.1.1.17), induces a rapid, long-lasting, dose-dependent decrease of 4-aminobutyrate: 2-oxoglutarate aminotransferase (EC 2.6.1.19) activity and, to a lesser extent, of glutamate decarboxylase (EC 4.1.1.15) in the brains of rats when injected intraperitoneally. The 4-aminobutyric acid concentration in whole brain increases five-fold over control values. 2. Pretreatment with pargyline, a potent inhibitor of monoamine oxidase (EC 1.4.3.4) abolishes the effects on 4-aminobutyric acid metabolism and potentiates the inhibitory effect of 5-hexyne-1,4-diamine on prostatic ornithine decarboxylase activity. 3. 5-hexyne-1,4-diamine is a substrate of monoamine oxidase but not of diamine oxidase (EC 1.4.3.6) and is oxidized by a mitochondrial suspension to 4-aminohex-5-ynoic acid. This is a known potent inhibitor of 4-aminobutyrate: 2-oxoglutarate aminotransferase and of glutamate decarboxylase. 4. 4-aminohex-5-ynoic acid is formed *in vivo* from 5-hexyne-1,4-diamine by the same mechanism.

5-Hexyne-1,4-diamine was designed as an inhibitor of orithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) [1, 2]. High doses of this compound caused changes in the behaviour of rats, such as sedation, ataxia, piloerection and lacrimation [2]. These central nervous system effects could be due either to the decrease of polyamine levels or to an action of the drug unrelated to polyamine metabolism. A metabolite of the drug might also be responsible.

The behavioural pattern was similar to the one observed in rats treated with high doses of 4-aminohex-5-ynoic acid [3]. 4-Aminohex-5-ynoic acid had been previously shown to be a potent enzyme-activated irreversible inhibitor of 4-aminobutyrate: 2-oxoglutarate aminotransferase (EC 2.6.1.19) and of glutamate decarboxylase (L-glutamate-1-carboxy-lyase, EC 4.1.1.15) [4, 5]. This acid could be expected to be formed from 5-hexyne-1,4-diamine by oxidation of the 1-aminomethylene group.

This paper presents evidence that 4-aminohex-5-ynoic acid is indeed the reaction product of mitochondrial oxidation of 5-hexyne-1,4-diamine and that it is formed *in vivo* in rats and mice.

MATERIALS AND METHODS

Chemicals. The following compounds were purchased: aminoguanidine nitrate (Eastman Kodak Corp., Rochester N.Y.); pargyline hydrochloride (*N*-benzyl-*N*-methylprop-2-ynyl-amine hydrochloride) (Abbott Laboratories, North Chicago, Ill.); benzylamine (Aldrich-Europe); homovanillic acid, horseradish peroxidase, EDTA tetrasodium (Calbiochem, San Diego, Calif.); L-ornithine, pyridoxal-phosphate, ammonium sulfate, reduced glutathione (GSH), sucrose, buffer reagents and silica gel G (Merck, Darmstadt, Germany); dithiothreitol, NAD^+, 4-aminobutyric acid, L-glutamate, 2-oxo-glutarate (Sigma, St Louis, Mo.); 5-aminonaphthalene-1-sulfonic acid (Fluka, Buchs, Switzerland); D, L [1-^{14}C] ornithine (58 Ci/mole) (Radiochemical Center, Amersham, England); D,L [1-^{14}C] glutamate (50 Ci/mole) (New England Nuclear Corp., Boston, Mass.). 5-Hexyne-1,4-diamine, dihydrochloride (RMI 71.696) [1], 4-aminohex-5-ynoic acid (RMI 71.645) [6] and Dansyl chloride (Dns-Cl) [7] were synthetised according to published procedures.

Animals. Male rats of the Sprague–Dawley strain (180–200 g) body weight and CD albino mice (25–30 g) were purchased from Charles River, France. They had access to standard diet and water *ad libitum* and were kept under a constant 12 hr light–12 hr dark lighting schedule. Animals were killed by decapitation at about the same time of day to minimize effects due to diurnal fluctuations. Drugs dissolved in 0.9 per cent saline were injected in a volume of 5 ml/kg body weight intraperitoneally. Control animals received an equivalent volume of saline.

Mitochondria from brain and liver. These were obtained from the rat organs by differential centrifugation of the tissue homogenates in 0.25 M-sucrose, pH 7.4 [8]. The particulate fractions were resuspended in ice-cold 0.1 M Na, K phosphate buffer, pH 7.4.

Measurements of enzyme activities. The assays of glutamate decarboxylase, 4-aminobutyrate: 2-oxoglutarate aminotransferase and ornithine decarboxylase activities in tissue homogenates were those applied previously [2, 5]. Mitochondrial monamine oxidase ([amine:oxygen oxidoreductase (deaminating) (flavin containing)], EC 1.4.3.4) activity of rat brain and liver and diamine oxidase ([amine:oxygen oxidoreductase (deaminating) (pyridoxal-containing)] EC 1.4.3.6) activity of the supernatant of rat small intestine were measured as described by Snyder and Hendley [9], using 5-hexyne-1,4-diamine as substrate.

Determination of whole brain 4-aminobutyric acid concentrations. 4-Aminobutyric acid concentrations of tissues were determined as described previously [5].

Identification of 4-aminohex-5-ynoic acid as its dansyl derivative. Tissue homogenates were prepared with 10 volumes of 0.2 N perchloric acid, and enzymatic reactions were stopped by addition of 0.25 ml 1N perchloric acid. 0.5 ml of the deproteinized acidic solutions were reacted with Dns-Cl as described previously [7].

The mixtures of the dansyl-derivatives were applied in 50 mm long streaks to silica gel plates (200 μm thick layers of silica gel G) and developed in solvent vapour saturated chambers using benzene–cyclohexane–methanol (85 + 15 + 2) as solvent. The zone corresponding with the reference compound was eluted with ethyl acetate and this extract rechromatographed using ethylacetate–cyclohexane (1 + 1) as solvent. Before mass spectrometric identification, the zone comigrating with the dansyl derivative of 4-aminohex-5-ynoic acid was rechromatographed in a two dimensional TLC using benzene-cyclohexane-methanol (85 + 15 + 2) in the first direction and chloroform–triethylamine (10 + 1) in the second direction.

Mass spectra were obtained after elution of the appropriate spot with 100 μl solvent (for details of the extraction method see [10] and [11]) using a Varian CH5 Mass Spectrometer at an electron beam energy of 70 eV and an ion source temperature of 250°; resolution about 2000.

An alternative method for the accumulation of the dansyl derivative of 4-aminohex-5-ynoic acid was that described for the accumulation and purification of *N*-Dns-2-oxopyrrolidine, the reaction product of 4-aminobutyric acid with Dns-Cl [10]. Chromatographic separation of *N*-Dns-2-oxo-4-ethynyl pyrrolidine, the reaction product of 4-aminohex-5-ynoic acid, was achieved in this case by undimensional TLC using benzene–cyclohexane–methanol (85 + 15 + 2) as solvent.

Data analysis. Statistical analysis of *in vivo* data was carried out using Student's *t* test.

RESULTS

Effects of 5-hexyne-1,4-diamine on whole brain 4-aminobutyrate-2-oxoglutarate aminotransferase and glutamate decarboxylase activities and 4-aminobutyrate concentrations.

Following a single dose (200 mg/kg; i.p.) of 5-hexyne-1,4-diamine, whole brain 4-aminobutyrate:2-oxoglutarate aminotransferase activity decreased rapidly in rats during the first 4 hr to a level about 10 per cent of the control (Fig. 1). Thereafter, activity increased slowly to reach 20 per cent of control values 24 hr after injection. Glutamate decarboxylase activity also decreased but only to 50 per cent of the control 4 hr after injection and remained at this level until 24 hr. The decrease of 4-aminobutyrate:2-oxoglutarate aminotransferase activity was accompanied by an increase of brain 4-aminobutyric acid levels. Maximum levels were about 5 times the control levels and were attained between 4 and 6 hr after injection. Twenty-four hours after injection of the drug, the levels of 4-aminobutyric acid were still 2.5 times the control levels ($P < 0.001$). In mice the results were very similar, but the effects less marked. Maximum effects were observed 8 hr after injection of the drug. At this time brain 4-aminobutyrate:2-oxoglutarate aminotransferase and glutamate decarboxylase activities were 40 and 80 per cent of their respective controls, whereas the 4-aminobutyric acid concentration was twice the control value.

When doses of 5-hexyne-1,4-diamine ranging from 12.5 to 200 mg/kg were given to rats 4 hr before sacrifice, brain 4-aminobutyrate:2-oxoglutarate aminotransferase and glutamate decarboxylase activities were decreased in a dose-dependent manner (Fig. 2), the former enzyme being always more inhibited than the latter. Doses which decreased 4-aminobutyrate:2-oxoglutarate aminotransferase and glutamate decarboxylase activities increased whole brain 4-aminobutyric acid levels in a dose-dependent manner.

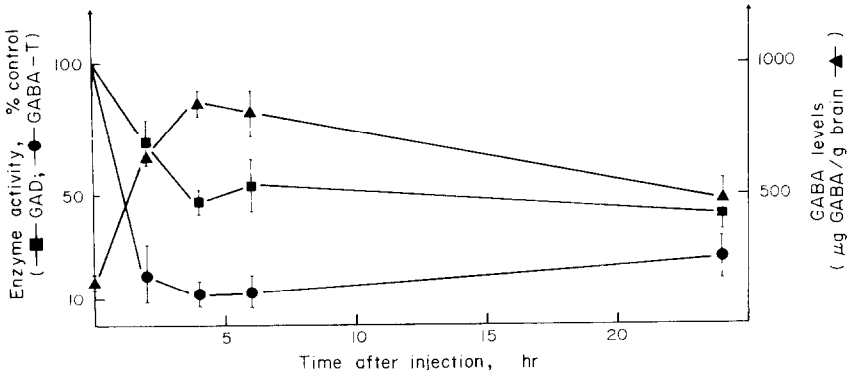

Fig. 1. Effect of a single dose of 5-hexyne-1,4-diamine on rat brain 4-aminobutyrate-2-oxoglutarate aminotransferase and glutamate decarboxylase and 4-aminobutyric acid as a function of time. Rats were injected intraperitoneally at time 0 with 200 mg/kg of 5-hexyne-1,4-diamine. At given intervals, the animals were killed and brain 4-aminobutyric acid (GABA) levels (▲), 4-aminobutyrate-2-oxoglutarate aminotransferase (GABA-T) (●) and glutamate decarboxylase (GAD) (■) activities were determined as described in Materials and Methods. The enzyme activities are expressed as per cent of control. Each value is the mean ± S.E.M. of 5 animals. The activity of 4-aminobutyrate-2-oxoglutarate aminotransferase from control animals was 67 ± 3 μmol/g/hr (mean ± S.E.M., $n = 5$). The activity of glutamate decarboxylase from control animals was 44 ± 1 μmol/g/hr (mean ± S.E.M., $n = 5$).

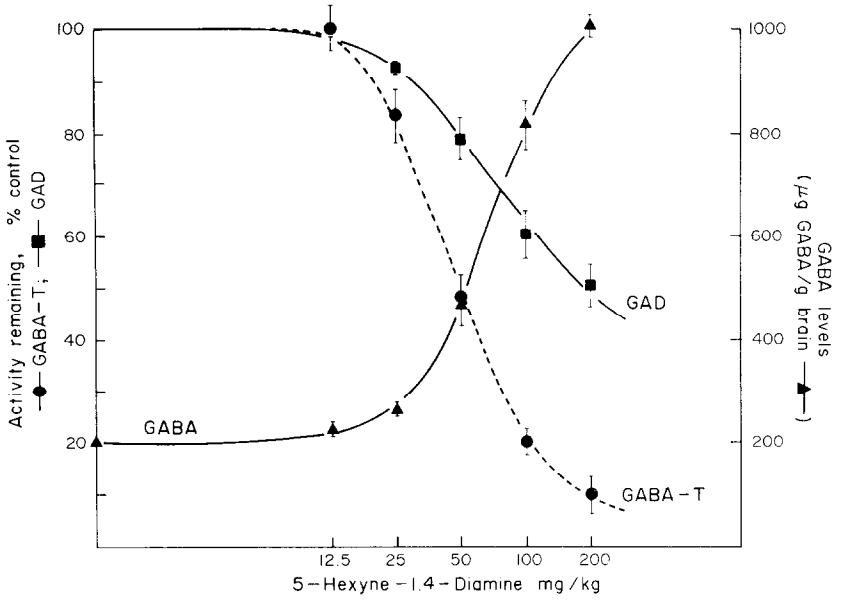

Fig. 2. Dose–effect relationship between a single dose of 5-hexyne-1,4-diamine and whole brain 4-aminobutyrate-2-oxoglutarate aminotransferase and glutamate decarboxylase activities and 4-aminobutyrate levels. Rats were injected intraperitoneally with a single dose of 5-hexyne-1,4-diamine ranging from 12.5 to 200 mg/kg. Four hours after injection the animals were killed and 4-aminobutyric acid (GABA) levels (▲), 4-aminobutyrate-2-oxoglutarate aminotransferase (GABA-T) (●) and glutamate decarboxylase (GAD) (◆) activities were measured in brain as described in the Materials and Methods. The enzyme activities are expressed in per cent control. Each value is the mean ± S.E.M. of 5 animals. Values for control animals are given in legend of Fig. 1.

Effects of pretreatment with aminoguanidine and pargyline on 5-hexyne-1, 4-diamine induced inhibition of 4-aminobutyrate:2-oxoglutarate aminotransferase and glutamate decarboxylase activities and of 4-aminobutyric acid levels. As is shown in Fig. 3, pretreatment with pargyline, a potent inhibitor of monoamine oxidase [12], prevented the decrease of brain 4-aminobutyrate:2-oxoglutarate aminotransferase and glutamate decarboxylase activities and the increase of 4 aminobutyric acid levels by 5-hexyne-1,4-diamine. On the other hand aminoguanidine, a potent inhibitor of diamine oxidase [13, 14] was without effect. The protection by pargyline was dose-dependent and complete only when pargyline was injected at a dose of 100 mg/kg (Fig. 4).

In vitro oxidation of 5-hexyne-1,4-diamine by rat mitochondrial monoamine oxidase and small intestine

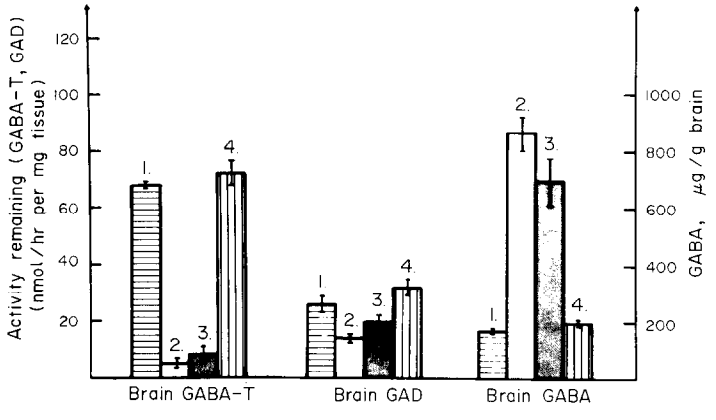

Fig. 3. Effects of previous administration of aminoguanidine and pargyline on the inhibition of 4-aminobutyrate-2-oxoglutarate aminotransferase and glutamate decarboxylase activities and on the increases of brain 4-aminobutyric acid levels induced by 5-hexyne-1,4-diamine. Rats were injected intraperitoneally as follows: group 1 (control) was injected with saline 6 hr and 4 hr before killing; groups 2, 3 and 4 were injected with 5-hexyne-1,4-diamine (200 mg/kg) 4 hr before killing; 6 hr before killing group 2 was injected with saline, group 3 with aminoguanidine (100 mg/kg) and group 4 with pargyline (100 mg/kg). 4-Aminobutyrate levels (GABA), 4-aminobutyrate-2-oxoglutarate aminotransferase (GABA-T) and glutamate decarboxy-lase (GAD) activities were measured as described in Materials and Methods.

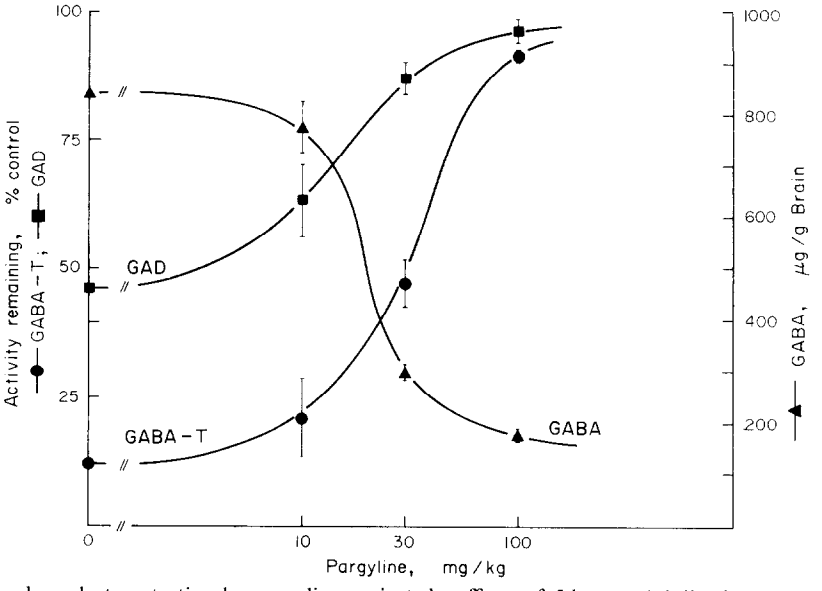

Fig. 4. Dose-dependent protection by pargyline against the effects of 5-hexyne-1,4-diamine. Rats were injected intraperitoneally 6 hours before sacrifice with saline or a single dose of pargyline ranging from 10 to 100 mg/kg and 2 hours later with a single dose of 5-hexyne-1,4-diamine (200 mg/kg). 4-aminobutyrate 2-oxoglutarate aminotransferase (GABA-T) (●) and glutamate decarboxylase (GAD) (■) activities and GABA levels (▲) were measured in brain as described in Materials and Methods. Enzyme activities are expressed as per cent control ± S.E.M., $n = 5$. The control values are given in legend of Fig. 1.

diamine oxidase. 5-Hexyne-1,4-diamine was found to be a substrate of mitochondrial monoamine oxidase preparations from liver and brain. The Michaelis constant (K_m) for both enzymes was 0.8 mM. These preparations were devoid of diamine-oxidase activity as checked by the lack of putrescine oxidation. 5-hexyne-1,4-diamine was not appreciably oxidized by a diamine oxidase preparation from small intestine. It has been previously shown [1] that this compound is not a time-dependent inhibitor of diamine oxidase.

The reaction product of 4-aminohex-5-ynoic acid with 5-dimethylaminonaphthalene1-sulfonyl chloride (Dns-Cl). 4-Aminohex-5-ynoic acid reacted with an excess of Dns-Cl to form a γ-lactam (*N*-Dns-2-oxo-5-ethynylpyrrolidine) as was expected in analogy with 4-aminobutyric acid [10] and other 4-aminoacids [15]. This structure was evident from the similarity of the chromatographic properties with those of *N*-Dns-2-oxopyrrolidine in several solvents, from the finding that the lactam could be hydrolysed in alkaline solution to *N*-Dns-4-aminohex-5-ynoic acid and reformed by reaction with acetic anhydride [10], and from the mass spectrum (Fig. 5). This mass spectrum showed the expected molecular ion at m/e = 342, the typical peak of *N*-Dns-γ-lactams at $(M - 64)^+$ (m/e = 278) which is due to elimination of SO_2 [7] and the peak at m/e = 170 which is due to the characteristic fragment of all dansyl derivatives.

Identification of 4-aminohex-5-ynoic acid as a reaction product of the mitochondrial oxidation of 5-hexyne-1,4-diamine. Mitochondria from one rat liver prepared as described in Materials and Methods were resuspended in 3 ml of ice cold 0.1 M Na, K phosphate buffer, pH 7.4.

0.95 ml samples of this preparation were incubated for 5 min at 37° with 50 μl of water (sample A) or with 50 μl of a 2 mM pargyline solution (samples B and C). Then 10 mg of 5-hexyne-1,4-diamine were added to A and B and incubation was continued for two hours. After reaction of the acidified supernatants with Dns Cl and extraction of the dansyl derivatives along the procedure described previously for the determination of low concentrations of 4-aminobutyric acid [10], a spot comigrating on the TLC with authentic *N*-Dns-2-oxo-5-ethynylpyrrolidine, the reaction product of 4-aminohex-5-ynoic acid with excessive Dns-Cl, was observed (Fig. 6). Beside this spot, *N*-Dns-2-oxo-pyrrolidine, the dansyl derivative of 4-aminobutyric acid, was the most prominent spot of the chromatograms. Bis-Dns-5-hexyne-1,4-diamine, even if not completely removed by the accumulation procedure for the γ-lactam, remains at the origin under the chromatographic conditions (see Materials and Methods and the legend to Fig. 6). 0.1 mM pargyline in the reaction mixture completely prevented the formation of 4-aminohex-5-ynoic acid.

Identification of 4-aminohex-5-ynoic acid in brain and liver of mice after administration of 5-hexyne-1,4-diamine. Brains and livers were removed from mice killed 1 hr after intraperitoneal injection of 400 mg/kg of 5-hexyne-1,4-diamine. The organs were homogenized in 0.2 N perchloric acid. After reaction of the extracts with Dns-Cl, the dansyl derivatives were separated by repeated chromatography (see Materials and

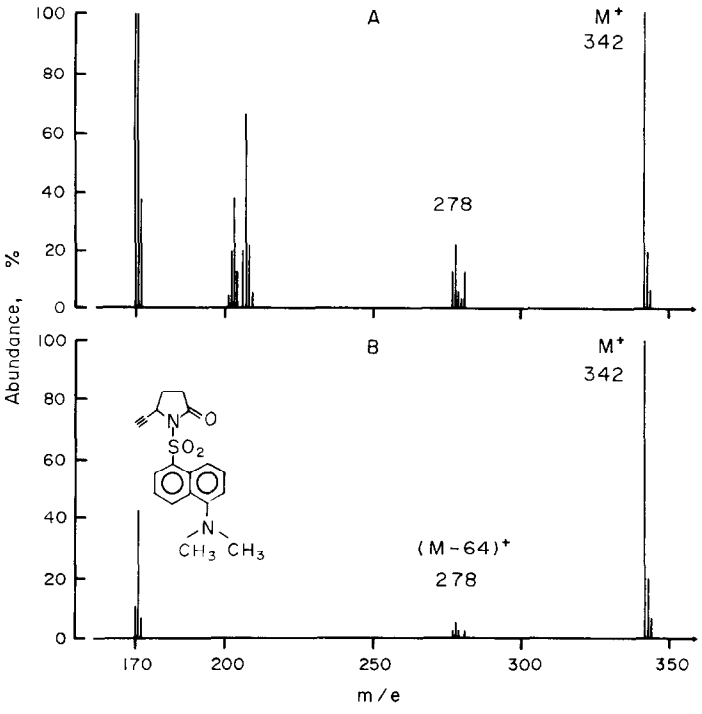

Fig. 5. Mass spectra. A. Dansyl derivative of the metabolite of 5-hexyne-1,4-diamine, isolated from mouse brain 1 hr after the i.p. administration of 400 mg/kg of the drug. B. *N*-Dns-2-oxo-5-ethynylpyrrolidine. The spectra were prepared from nmol amounts of the derivatives by flash evaporation from the direct probe of a Varian CM5 single focusing mass spectrometer. Electron beam energy 70 eV; ion source temperature 250°. Prior to mass spectrometry, the compounds were purified by two-directional TLC, using benzene–cyclohexane–methanol (85 + 15 + 2) in the first, and chloroform–triethylamine (10 + 1) in the second dimension.

Methods). A fluorescent spot isolated from brains and livers, and absent in the organs of drug-free animals, comigrated in the following solvents with authentic *N*-Dns-2-oxo-5-ethynyl pyrrolidine: 1. benzene–cyclohexane–methanol (85 + 15 + 2), 2. ethylacetate–cyclohexane (1 + 1), 3. chloroform–triethylamine (10 + 1). The mass spectra of this spot showed the expected molecular ion of *N*-Dns-2-oxo-5-ethynyl-pyrrolidine (m/e = 342) and the peak at $(m - 64)^+$ m/e = 278) which is typical for *N*-Dns-γ-lactams [15]. However, the peaks at m/e = 170 and 171 were relatively too high as compared with the spectrum of the authentic compound, as was the other typical fragment of Dns-derivatives at m/e = 203 [15]; moreover the spectra showed additional peaks at m/e = 497, 433 and 421, which were not identified. Obviously even the multiple chromatography under the conditions described in Materials and Methods did not completely remove an unknown dansyl-derivative from *N*-Dns-2-oxo-5-ethynylpyrrolidine. However the available analytical information allows the conclusion that 4-aminohex-5-ynoic acid is formed in the living organism.

Effects of pargyline on the inhibition of ornithine decarboxylase activity by 5-hexyne-1,4-diamine. Since 5-hexyne-1,4-diamine was oxidized by monoamine oxidase, inhibition of this enzyme by pargyline was expected to increase its effective concentration *in vivo*. Dose–effect relationships between a single dose of 5-hexyne-1,4-diamine and rat ventral prostrate ornithine decarboxylase activity were studied after previous injections of pargyline or saline. As is shown in Fig. 7, 100 mg/kg pargyline injected two hours before 5-hexyne-1,4-diamine decreased prostatic ornithine decarboxylase activity much more effectively than the ornithine decarboxylase inhibitor alone.

DISCUSSION

Our results demonstrate clearly that 5-hexyne-1,4-diamine is oxidized *in vivo* to 4-aminohex-5-ynoic acid by a mitochondrial pathway involving monamine oxidase.

Recently it has been established that putrescine can be converted into 4-aminobutyric acid by two different pathways in mammals [16]. In one pathway putrescine is first acetylated to monoacetylputrescine. Monoacetylputrescine is oxidized by monoamine oxidase to *N*-acetyl-4-butyraldehyde which, by oxidation to *N*-acetyl-4-aminobutyric acid and hydrolysis, yields 4-aminobutyric acid. In the second pathway putrescine is oxidized directly by diamine oxidase to aminobutyraldehyde which is converted by an aldehyde dehydrogenase to 4-aminobutyric acid. The results presented in this study show that the oxidative degradation of 5-hexyne-1,4-diamine *in vivo* is different from that of putrescine,

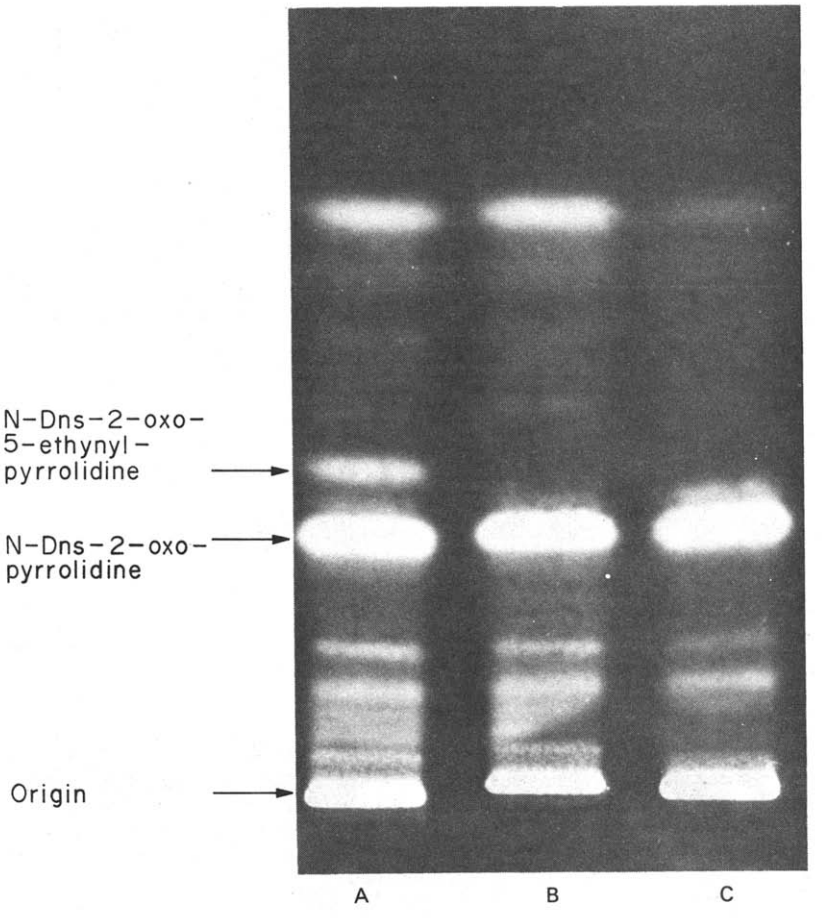

Fig. 6. Thin layer chromatograms of dansyl derivatives. These dansyl derivatives were formed by incubation of mitochondrial suspensions with 5-hexyne-1,4-diamine, reaction with Dns-Cl [7] and isolation of the pyrrolidine derivatives by hydrolytic cleavage of the γ-lactam ring, extraction of the Dns–amine derivatives, and ring closure by reaction with acetic anhydride [10]. 200 μm thick silica gel G plate; solvent: benzene–cyclohexane–methanol (85 + 15 + 2). A. Mitochondrial suspension with 55 mM 5-hexyne-1,4-diamine. B. Mitochondrial suspension with 55 mM 5-hexyne-1,4-diamine plus 0.1 mM pargyline: C. Mitochondrial suspension with 0.1 mM pargyline.

in that 5-hexyne-1,4-diamine is only a substrate of monoamine oxidase but not of diamine oxidase.

Monoamine oxidase is known to oxidize aliphatic diamines only if the two amino groups have a sufficiently large distance from each other, i.e. at least corresponding to 7 methylene groups [17, 18]. In contrast, diamine oxidases are less specific and oxidize both short and long-chain diamines, and even monoamines [19]. Apparently one of the positive centers of the diamines is bound to a negative binding site of monoamine oxidases, so that the short-chain diamines arrange themselves in parallel to the enzyme surface [18], and the second amino group cannot occupy the enzyme active site correctly. However, if the distance between the two positive charges of the diamine is large enough, the terminal amino group can occupy the enzyme active site. If the interaction of the diamine with the negative binding site of monoamine oxidase is impaired either by steric hindrance, or by removal of the positive charge from the second amino group, the compound becomes a substrate, irrespective of the length of the aliphatic chain.

In the case of monoacetylation of putrescine, formerly the second positive charge of the diamine has been completely removed [16]. In the case of 1,2′-aminoethylbenzimidazole, the pK of one of the amino groups has been decreased [20], but steric hindrance may also be involved in the impairment of the interaction of the second amino group with the negative binding site of monoamine oxidase. A comparable example seems to be 5-hexyne-1,4-diamine. The introduction of the α-ethynyl group lowered the pK of the adjacent amino group from 9.6 in putrescine [21] to 7.5 (P. Vitali and J. Wagner, personal communication). The ethynyl group may in addition decrease the interaction with the negative binding site by steric hindrance.

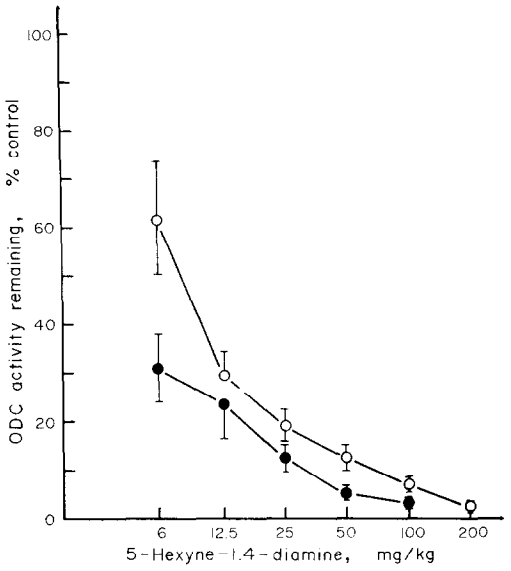

Fig. 7. Effect of pretreatment with pargyline on the inhibition of ventral prostate ornithine decarboxylase activity by 5-hexyne-1, 4-diamine. Rats were injected intraperitoneally at time 0 with a single dose of pargyline (100 mg/kg) (●) or saline (○) and at 2 hr with a single dose of 5-hexyne-1,4-diamine ranging from 6 to 200 mg/kg of body weight. At 7 hr, animals were killed and ornithine decarboxylase activities were measured as described in Materials and Methods. The enzyme activities are expressed as per cent of control injected twice with saline. Each value is the mean ± S.E.M of five animals. Ornithine decarboxylase activities were respectively 271 ± 71 and 297 ± 40 nmol.hr^{-1}/g of wet ventral prostate for control animals and animals injected only with pargyline.

In the case of hog kidney diamine oxidase a parallel arrangement of the substrate on the enzyme surface seems to be necessary for the correct interaction with the enzyme active site [19]. Most probably the steric hindrance by the ethynyl group is of greater importance for the impairment of the oxidative deamination of 5-hexyne-1,4-diamine by diamine oxidase, than the decrease of pK of the second amino group.

There are several examples of amines not normally found in the body which are metabolized by monoamine oxidase [18]. The reported oxidation of 5-hexyne-1, 4-diamine represents additional evidence for a detoxicating role of this enzyme. Both, as a function of time and dose (in mole/kg body weight) 4-aminohex-5-ynoic acid [5] and 5-hexyne-1,4-diamine increase brain 4-aminobutyric acid levels similarly. This shows that 5-hexyne-1,4-diamine is rapidly metabolized to 4-aminohex-5-ynoic acid *in vivo* to a great extent. This is further proven by the potentiation of the effect of 5-hexyne-1,4-diamine on prostatic ornithine decarboxylase activity by pargyline. It is not yet known if 5-hexyne-1,4-diamine enters the brain or if its metabolism occurs only in the periphery. 4-Aminohex-5-ynoic acid is known to enter the brains [5].

In conclusion, 5-hexyne-1,4-diamine given to animals after a pretreatment with pargyline may allow to study the effects of a potent inhibitor of polyamine biosynthesis. However, pargyline may add effects to those of 5-hexyne-1,4-diamine since monoamine oxidase is implicated in putrescine catabolism. Thus, interpretation of the effects of this drug combination might be complicated. Work to find an 5-hexyne-1,4-diamine analogue which is not a substrate for monoamine oxidase is in progress.

REFERENCES

1. B. W. Metcalf, P. Bey, C. Danzin, M. J. Jung, P. Casara and J. P. Vevert, *J. Am. chem. Soc.* **100**, 2551 (1978).
2. C. Danzin, M. J. Jung, B. W. Metcalf, J. Grove and P. Casara, *Biochem. Pharmac.* **28**, 627 (1979).
3. P. J. Schechter, Y. Tranier, M. J. Jung and P. Böhlen, *Eur. J. Pharmac.* **45**, 319 (1977).
4. M. J. Jung and B. W. Metcalf, *Biochem. biophys. Res. Commun.* **67**, 301 (1975).
5. M. J. Jung, B. Lippert, B. W. Metcalf, P. J. Schechter, P. Böhlen and A. Sjoerdsma, *J. Neurochem.* **28**, 717 (1977).
6. B. W. Metcalf and P. Casara, *Tetrahedron Lett.* **38**, 3337 (1975).
7. N. Seiler, *Meth. biochem. Analysis* **18**, 259–337 (1970).
8. T. M. Brody and J. A. Bain, *J. biol. Chem.* **195**, 685 (1952).
9. S. H. Snyder and E. D. Hendley, *Meth. Enzym.* **17B**, 741 (1971).
10. N. Seiler and M. Wiechmann, *Hoppe–Seyler's Z. physiol. Chem.* **350**, 1493 (1969).
11. N. Seiler and B. Knödgen, *J. Chromat.* **131**, 109 (1977).
12. J. D. Taylor, A. A. Wykes, Y. C. Gladish and W. B. Martin, *Nature, Lond.* **187**, 941 (1960).
13. W. Schuler, *Experientia* **8**, 230 (1952).
14. M. J. C. Crabbe, R. E. Childs and W. G. Bardsley, *Eur. J. Biochem.* **60**, 325 (1975).
15. N. Seiler, H. Schneider and K. D. Donnenberg, *Z. Analyt. Chem.* **252**, 127 (1970).
16. N. Seiler and B. Eichentopf, *Biochem. J.* **152**, 201 (1975).
17. H. Blaschko and J. Hawkins, *Br. J. Pharmac.* **5**, 625 (1950).
18. H. Blaschko, *Pharmac. Rev.* **4**, 415 (1952).
19. W. G. Bardsley, J. S. Ashford and C. M. Hill, *Biochem. J.* **122**, 557 (1971).
20. H. Blaschko, *Rev. Physiol. Biochem. Pharmac.* **70**, 83 (1974).
21. L. G. Sillen and A. E. Martell, *Chem. Soc. Spec. Publ.* No. 17 (1964).