Putrescine Catabolism in Mammalian Brain

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(Received 15 May 1974)

In contrast with putrescine (1,4-diaminobutane), which is a substrate of diamine oxidase, monoacetylputrescine is oxidatively deaminated both *in vitro* and *in vivo* by monoamine oxidase. The product of this reaction is *N*-acetyl- γ -aminobutyrate. The existence of a degradative pathway in mammalian brain for putrescine is shown, which comprises acetylation of putrescine, oxidative deamination of monoacetylputrescine to *N*-acetyl- γ -aminobutyrate, transformation of *N*-acetyl- γ -aminobutyrate to γ -aminobutyrate and degradation of γ -aminobutyrate to CO₂ via the tricarboxylic acid cycle.

As a precursor of the polyamines spermidine and spermine, putrescine (1,4-diaminobutane) plays an important role in cell physiology (Bachrach, 1973; Cohen, 1971; Tabor & Tabor, 1964, 1972). Its formation in mammalian tissues has been extensively studied; its catabolism, however, is less well known.

Several years ago it was shown that exogenous putrescine is rapidly oxidized. About 30% of the radioactive carbon of intraperitoneally injected [1,4-¹⁴C]putrescine dihydrochloride is expired from rats (Jänne, 1967) and mice as CO₂, and after the injection of [2,3-³H]putrescine dihydrochloride, a large amount of ³H₂O is detectable in the different organs of the animals (Fischer *et al.*, 1972).

An idea of the degradative pathway of putrescine was obtained by the detection of incorporation of putrescine carbon into γ -aminobutyric acid in rat brain and liver (Seiler *et al.*, 1971), in mouse brain (Seiler & Knödgen, 1971) and liver (N. Seiler & B. Eichentopf, unpublished work) and in fish brain (Seiler *et al.*, 1973a). In these experiments it was shown that the transformation of putrescine into γ -aminobutyric acid occurs by a direct pathway, which does not include glutamic acid as an intermediate. Since the occurrence of γ -aminobutyric acid is not restricted to brain (Dent, 1948; Gordon, 1949; Tallan *et al.*, 1954; Zachmann *et al.*, 1966; Seiler & Wiechmann, 1969), and since the rapid oxidation of γ -aminobutyric acid in non-neural tissue is well established (Wilson *et al.*, 1959), it was assumed that γ -aminobutyrate is a general metabolic intermediate in a pathway which channels putrescine carbon, and possibly also the carbon atoms of the putrescine moiety of spermidine and spermine, into the tricarboxylic acid cycle (Seiler, 1973). The enzymic reactions involved in the transformation of putrescine into γ -aminobutyrate were, however, not known.

As a first step one might assume the oxidative deamination of putrescine by diamine oxidase. However, diamine oxidase activity is low in the brains of

mammals (Burkard *et al.*, 1963), so that this enzyme cannot be exclusively responsible for putrescine degradation in mammalian brain. Moreover, it was shown that the low activity of putrescine oxidation by sheep brain homogenate was inhibited by an inhibitor of monoamine oxidase rather than by a diamine oxidase inhibitor (Kremzner, 1973). Transamination of putrescine in brain tissue with α -oxoglutarate as acceptor has been looked for with sensitive methodology (N. Seiler, unpublished work) but no activity was detected.

The detection of monoacetylputrescine in human brain (Perry *et al.*, 1967) and in brain and other organs of different vertebrates (Seiler *et al.*, 1973b), and the detection of active putrescine acetylation by brain and other tissue preparations (Seiler & Al-Therib, 1974), suggested a pathway on which acetylation represents the first step, followed by the oxidation of monoacetylputrescine.

In the present work experiments are described in which the validity of this hypothesis was tested.

Materials and Methods

Materials

Reagents. Usual laboratory chemicals were of A grade. With the following exceptions, they were purchased from E. Merck, Darmstadt, Germany. Aminoguanidine sulphate and putrescine dihydrochloride were from Th. Schuchardt, München, Germany; putrescine base was from EGA Chemie, Frankfurt, Germany; spermidine phosphate and spermine phosphate were from Fluka A.G., Buchs, Switzerland; γ -aminobutyric acid was from Serva A.G., Heidelberg, Germany; dithiothreitol was from Sigma Chemical Corp., St. Louis, Mo., U.S.A.; 2,5-diphenyloxazole (PPO) was from Koch-Light Laboratories, Colnbrook, Bucks., U.K.; hydrazine sulphate was from Riedel de Haen, Seelze, Germany;

Marsilid phosphate was from Deutsche Hoffmann-La Roche, Grenzach, Germany. Pargyline hydrochloride was from Abbott Laboratories, North Chicago, Ill., U.S.A.

Monoacetylputrescine hydrochloride. This was synthesized as described by Tabor *et al.* (1964) from putrescine base.

N-Acetyl-γ-aminobutyric acid. This was obtained by reaction of 10mmol of γ-aminobutyric acid with 12mmol of acetic anhydride in 20ml of acetic acid at room temperature overnight, and recrystallization of the residue of the reaction mixture from acetone-water (m.p. 132°C; yield 90%).

Radiochemicals. [1,4-¹⁴C]Putrescine dihydrochloride (sp. radioactivity 23Ci/mol) and γ-amino[1-¹⁴C]butyric acid (sp. radioactivity 3.6Ci/mol) were purchased from New England Nuclear Corp., Boston, Mass., U.S.A.; [2,3-³H]putrescine dihydrochloride was synthesized as described by Fischer *et al.* (1971).

Monoacetyl[2,3-³H]putrescine hydrochloride. This was obtained as follows: 80mg of [2,3-³H]putrescine dihydrochloride was dissolved in 50μl of water. To this solution 200μl of acetic anhydride and 100μl of pyridine were added. After reaction for 1h at room temperature the reaction mixture was adjusted to pH1 with 2M-HCl, and then diluted with 50ml of propan-2-ol. The solution was evaporated nearly to dryness and the residue redissolved in 5ml of propan-2-ol. After storage at +3°C mainly [2,3-³H]putrescine dihydrochloride crystallized from this solution. The propan-2-ol solution was applied to a total of six t.l.c. plates coated with 1mm-thick silica-gel Glayers. The plates were developed with methyl acetate-propan-2-ol-25% NH₃ (9:7:4, by vol.). The radioactive zones were scraped out and eluted with methanol-25% NH₃ (5:1, v/v). The residues of the monoacetylputrescine zones were redissolved in propan-2-ol; pyridine acetate crystallized from this solution. After evaporation of the filtered propan-2-ol solution to about 1ml, 10mg of non-radioactive monoacetylputrescine hydrochloride was added, followed by ethyl acetate, dropwise, until monoacetylputrescine hydrochloride was precipitated. Recrystallization from propan-2-ol yielded about 4mg of monoacetylputrescine hydrochloride (sp. radioactivity, 550Ci/mol). The mother liquor was evaporated to dryness; then 80mg of non-radioactive monoacetylputrescine hydrochloride was added to the residue. This was twice crystallized from propan-2-ol-ethyl acetate (yield 60mg; sp. radioactivity 35Ci/mol).

N-Acetyl-γ-amino[1-¹⁴C]butyric acid. This was prepared by reaction of radioactive γ-aminobutyric acid with acetic anhydride in acetic acid solution overnight at room temperature. The excess of anhydride was decomposed with a few drops of water. This solution was used without further purification.

Experimental animals. Male albino mice (NMRI and Wistar rats (Gesellschaft f. Versuchstierzucht, Hannover, Germany) were used. The animals were kept under standard conditions, having access to standard diet (Altromin, Lage, Germany) and water *ad libitum*.

Methods

Mitochondria from brain and liver. These were obtained from the rat organs by differential centrifugation of the tissue homogenates in 0.25M-sucrose, pH7.0, which contained 1mmol of EDTA/litre (Ragab *et al.*, 1967). The particulate fractions were resuspended either in ice-cold 0.1M-triethanolamine-HCl buffer, pH8.0, which contained 1.7mmol of EDTA and 1mmol of dithiothreitol/litre, or in 0.06M-phosphate buffer, pH7.2 (0.06M-KH₂PO₄ adjusted to pH7.2 with 0.06M-Na₂HPO₄).

Oxidative degradation of monoacetylputrescine in vitro. Portions (100μl) of mitochondrial suspension were mixed with 20μl of a solution of 0.2μmol of monoacetyl[2,3-³H]putrescine dihydrochloride (sp. radioactivity 13.5Ci/mol) and with either 20μl of phosphate buffer, pH7.2, or triethanolamine-HCl buffer, pH8.0 (controls), or 20μl of the solutions of the inhibitors (0.1μmol). The final reaction mixtures were 1.4mm with respect to the substrate, and 0.7mm with respect to the inhibitors. The reaction mixtures were shaken in a water bath at 37°C for 45min; then 100μl of 1.5% (w/v) HClO₄ was added to stop the enzymic reaction. Samples (180μl) from the supernatants of the centrifuged (800g; 15min) suspensions were mixed with 20μl of Methyl Orange solution (0.03% in water) and then neutralized with K₂CO₃ solution. To complete the precipitation of KClO₄, 1ml of ethanol was added to the neutralized solutions. After storage overnight at +3°C the mixtures were centrifuged, the clear supernatants were evaporated nearly to dryness in a stream of air, and the residues redissolved in 0.1ml of ethanol. These solutions were applied to 20cm×20cm thin-layer plates (with 200μm thick silica-gel G layers) in 3cm long streaks over a distance of 8.5cm from one plate edge. Separation was achieved by high-voltage electrophoresis. The plates were sprayed with pyridine acetate buffer, pH4.8 (Fischer & Bohn, 1957). Electrophoresis was performed with the thin-layer electrophoresis equipment of Camag (Muttentz, Switzerland) at 20V/cm for 60min and about +5°C. The dried plates were scanned with a radiochromatogram scanner LB 2720 (Berthold, Erlangen, Germany).

Metabolism of intraventricularly injected monoacetyl-[2,3-³H]putrescine and [1,4-¹⁴C]putrescine. At 90min before the administration of the radioactive compounds, the animals were injected intraperitoneally either with 200μl of water, or with 200μl of solu-

tions containing 2mg of inhibitor (Marsilid phosphate, Pargyline hydrochloride or aminoguanidine sulphate). Then 15min before the administration of the radioactive compounds, 200 μ l of a 10% (w/v) solution of chloral hydrate was administered intraperitoneally, to anaesthetize the animals. Next 10 μ l portions of [3 H]monoacetylputrescine hydrochloride solution (0.02 μ mol; 12 μ Ci) were injected into each lateral ventricle (i.e. two injections per animal) through cannulae of appropriate length (Noble *et al.*, 1967; Haley & McCormick, 1957). The same procedure was used for the administration of 0.4 μ mol (10 μ Ci) of [1,4- 14 C]putrescine dihydrochloride.

At various time-intervals animals were decapitated and the brains homogenized in 10 vol. of 0.2M-HClO₄. After storage for 3h at +3°C the homogenates were centrifuged (800g; 15min), and 1ml of the supernatant was used for the determination of the distribution of radioactivity among the metabolites of monoacetylputrescine or of putrescine. The remaining extract was used for the determination of γ -aminobutyric acid concentration and the specific radioactivity of γ -aminobutyrate.

(a) *Distribution of radioactivity among the metabolites.* The procedure was the same as that described above for the determination of the products of oxidative degradation of monoacetylputrescine, except that electrophoresis was carried out for only 45min, and that radioactivity along the length of the electrophoretic path was determined by scraping out 5mm $\times$ 40mm zones, extraction of the adsorbent in each zone with 3ml of methanol- β -phenylethylamine (4:1, v/v) and determination of radioactivity in the extracts by liquid-scintillation counting, after addition of 10ml of PPO scintillator (5g/litre of toluene).

(b) *Specific radioactivity of γ -aminobutyric acid.* Portions (3ml) of the extracts (see above) were evaporated at room temperature in a stream of air to approx. 1ml. The extracts were treated as described previously (Seiler *et al.*, 1973a) with 50mg of dansyl chloride. Dns-2-oxopyrrolidine, the reaction product of γ -aminobutyric acid, was purified by hydrolytic cleavage and reformation of the lactam ring (Seiler & Wiechmann, 1969), and by t.l.c. in the solvent systems benzene-cyclohexane-methanol (170:30:3, by vol.) and cyclohexane-ethyl acetate (7:5, v/v) (one run with both solvents in the same direction). Further purification of the γ -aminobutyric acid derivative, after its extraction with ethyl acetate, was done by two-dimensional t.l.c. in benzene-cyclohexane-methanol (85:15:2, by vol.) (two runs) in the first direction and diethyl ether-cyclohexane (3:1, v/v) (one run) in the second direction (for a detailed description of the procedure see Seiler & Wiechmann, 1970). The spot of the γ -aminobutyric acid derivative was eluted from the adsorbent with 5ml of dioxan. The fluorescence intensity and radioactivity were determined on this solution. Authentic Dns-2-oxo-

pyrrolidine was used as fluorescence standard. For the determination of radioactivity 10ml portions of PPO scintillator were mixed with 4.5ml portions of the dioxan solutions.

Identification of N-acetyl- γ -aminobutyric acid. The reaction product of oxidative deamination of monoacetylputrescine was identified by the following experiments. (a) Comparison of the electrophoretic mobility with authentic N-acetyl- γ -aminobutyric acid. The conditions were the same as those described above. (b) Extraction with methanol of the radioactive spot from the thin-layer electrophoretogram or thin-layer chromatogram [solvent: butan-1-ol-acetic acid-water (4:1:1, by vol.)] and comparison of chromatographic mobility with authentic N-acetyl- γ -aminobutyric acid in the following solvent mixtures: ethanol-water (7:3, v/v); chloroform-methanol-17% NH₃ (2:2:1, by vol.); butan-1-ol-acetic acid-water (4:1:1, by vol.); methyl acetate-propan-2-ol-25% NH₃ (9:7:4, by vol.). In every case 200 μ m-thick silica-gel G layers were used for the separations. (c) Hydrolytic cleavage of the radioactive spot with 1ml of 6M-HCl at 120°C for 2h followed by (i) thin-layer electrophoretic comparison of the hydrolysis product with authentic γ -aminobutyric acid or (ii) reaction of the hydrolysis product with dansyl chloride, and chromatographic and mass-spectrometric identification of the reaction product as Dns-2-oxopyrrolidine. (The thin-layer chromatographic systems were the same as those used for the determination of the specific radioactivity of γ -aminobutyric acid.)

Determination of polyamines and of γ -aminobutyric acid. This was done by dansylation as described in detail previously (Seiler & Lamberty, 1973; Seiler & Wiechmann, 1969, 1970).

Mass spectrometry. A CH5 Varian MAT single-focusing mass spectrometer was used at an electron-beam energy of 70eV. The temperature of the ion source was 250°C [for details of the mass spectrometry of dansyl derivatives see Seiler *et al.* (1970)].

Results

Oxidative deamination of monoacetylputrescine in vitro

Suspensions of brain and liver mitochondria were incubated with monoacetyl-[3 H]putrescine hydrochloride (1.4mM) for 45min at 37°C. The electrophoretically separated incubation mixtures normally showed only one radioactive compound other than monoacetylputrescine. This compound moved at pH 4.8 towards the anode. In suspensions of mitochondria from 750mg of rat liver in 0.1ml of buffer, pH 8.0 (0.1M-triethanolamine-HCl, containing 1.7mmol of EDTA and 1mmol of dithiothreitol per litre), the formation of this compound was nearly

Fig. 1. Oxidative degradation of monoacetylputrescine *in vitro*

Thin-layer electrophoretograms (20 V/cm; 60 min; pH 4.8; +5°C) of incubation mixtures (pH 8.0) of 1.4 mM-monoacetyl[2,3-³H]putrescine hydrochloride with rat liver mitochondria (a) without inhibitor, (b) with 0.7 mM-Marsilid phosphate and (c) with 0.7 mM-aminoguanidine sulphate. Peaks: 1, monoacetylputrescine; 2, *N*-acetyl- γ -aminobutyric acid. The units on the ordinate are radioactivity relative to recorded voltage.

quantitative within 60 min. Monoamine oxidase inhibitors (Marsilid phosphate, Pargyline hydrochloride at 0.7 mM concentration) inhibited the formation of the degradation product of monoacetylputrescine completely, whereas aminoguanidine sulphate, an effective inhibitor of diamine oxidase (Webb, 1966), had no influence on the degradation of monoacetylputrescine at 1 mM concentration (Fig. 1).

Essentially the same results were obtained with liver and brain homogenates, but in some experiments an additional small radioactive peak was observed near the origin, and another radioactive peak at a distance from the origin identical with that of putrescine.

The identity of the reaction product of mitochondrial oxidation of monoacetylputrescine with *N*-acetyl- γ -aminobutyrate was established by the following criteria. (1) Identical electrophoretic mobility at pH 4.8 with authentic *N*-acetyl- γ -aminobutyrate. (2) Identical t.l.c. properties with *N*-acetyl- γ -aminobutyrate in the following solvents: (a) butan-1-ol-

acetic acid-water (4:1:1, by vol.); (b) ethanol-water (7:3, v/v); (c) chloroform-methanol-17% NH₃ (2:2:1, by vol.); (d) methyl acetate-propan-2-ol-25% NH₃ (9:7:4, by vol.). (3) Hydrolysis of the reaction product with 6M-HCl and identification of γ -aminobutyric acid by (a) identical electrophoretic mobility at pH 4.8 with γ -aminobutyrate, and (b) reaction of the hydrolysis product with dansyl chloride, and identification of the γ -aminobutyric acid derivative by t.l.c. and mass spectrometry.

Metabolism of monoacetylputrescine in mouse brain in vivo

N-Acetyl- γ -aminobutyric acid was not only formed *in vitro* by mitochondrial oxidation, but also *in vivo* in mouse brain, after the intraventricular injection of monoacetylputrescine. At 1 h after the injection of 0.02 μ mol of monoacetyl[³H]putrescine hydrochloride into the lateral ventricles of chloral hydrate-anesthetized mice about 50% of the acid-soluble radioactive material present in brain (disregarding water and other volatile compounds, which evaporate during the electrophoretic procedure) was represented by acetylputrescine, approx. 30% of the radioactivity was in the *N*-acetyl- γ -aminobutyrate zone of the electrophoretogram, and about 6% in the putrescine zone (Fig. 2). (The remaining radioactive compounds, except for γ -aminobutyric acid, were not identified.) With increasing time the proportion of radioactivity in the acetylputrescine zone decreased very considerably, whereas the proportion of radioactivity present in putrescine and its metabolites, spermidine and spermine, was relatively increased. The decrease in the amount of radioactive *N*-acetyl- γ -aminobutyrate appeared to be slower than that of monoacetylputrescine, since 19 h after administration of monoacetylputrescine about 20% of the radioactivity was still found in the *N*-acetyl- γ -aminobutyrate zone, but somewhat less in the acetylputrescine zone (Fig. 2).

Pretreatment of the animals with 100 mg of the monoamine oxidase inhibitors, Marsilid and Pargyline, per kg body weight effected a dramatic decrease in the radioactivity in the *N*-acetyl- γ -aminobutyrate zone. Pargyline was the more effective compound; however, in neither case was the oxidative deamination of monoacetylputrescine totally inhibited, despite the high doses of the monoamine oxidase inhibitors given (Fig. 2).

The elimination rate of monoacetylputrescine from the brains of monoamine oxidase-inhibited animals seemed to be comparable with that in control animals; the same seemed to be true for the formation of putrescine, whereas a somewhat higher proportion of radioactivity was found in spermidine in the monoamine oxidase-inhibitor-pretreated animals. Although this latter observation is not statistically

Fig. 2. Products of monoacetylputrescine metabolism in mouse brain *in vivo*

Thin-layer electrophoretograms (20 V/cm; 45 min; pH 4.8; +5°C) of the HClO_4 extracts of the brains of control animals (a) and of animals pretreated with 100 mg of Marsilid phosphate (b) or Pargyline hydrochloride (c) per kg before the intraventricular injection of 0.02 μmol of monoacetyl[^3H]putrescine hydrochloride. Peaks: 1, monoacetylputrescine; 2, *N*-acetyl- γ -aminobutyric acid; 3, putrescine; 4, spermidine; 5, spermine. Actually measured radioactivity in the 1 h control, 25 500 d.p.m.

Table 1. Influence of monoamine oxidase inhibition on the formation of γ -aminobutyric acid in mouse brain from intraventricularly injected monoacetyl[2,3- ^3H]putrescine hydrochloride (12 μCi ; 0.02 μmol) and [1,4- ^{14}C]putrescine dihydrochloride (10 μCi ; 0.4 μmol)

Measured activity in the 1 h control after monoacetylputrescine injection: 913 d.p.m./0.145 μmol of γ -aminobutyrate. Intraperitoneal injection of Pargyline hydrochloride (100 mg/kg) or aminoguanidine sulphate (100 mg/kg) was given 90 min before the administration of the radioactive precursor. For details see the text.

γ -Aminobutyrate precursor	Pretreatment	No. of animals	Time after injection (h)	Specific radioactivity of γ -aminobutyrate	
				[(d.p.m./mol) $\pm$ S.E.M.]	(% of control)
Monoacetylputrescine	Control	6	1	6330 $\pm$ 440	
	Pargyline	6	1	1720 $\pm$ 140	27
	Aminoguanidine sulphate	2	1	5690 $\pm$ 300	90
	Control	3	5	2320 $\pm$ 210	
	Pargyline	3	5	1040 $\pm$ 110	45
Putrescine	Control	5	1	3410 $\pm$ 30	
	Pargyline	5	1	1250 $\pm$ 30	36
	Control	2	3	3470 $\pm$ 200	
	Pargyline	2	3	960 $\pm$ 200	28
	Control	3	1	3680 $\pm$ 50	
	Aminoguanidine sulphate	3	1	3488 $\pm$ 50	92

verified at present, it may, nevertheless, be significant, since the same tendency was observed in analogous experiments with [1,4- ^{14}C]putrescine.

Since the proportion of radioactivity present in γ -aminobutyrate at any time-interval was relatively low, after the administration of either radioactive putrescine or monoacetylputrescine, the incorpora-

tion of radioactivity into this key metabolite of putrescine was measured in separate experiments. For this purpose γ -aminobutyric acid was modified by reaction with dansyl chloride. The derivative was very carefully purified and the specific radioactivity was determined. In preceding experiments it was shown that neither brain γ -aminobutyrate concentrations,

nor the concentrations of putrescine and the polyamines spermidine and spermine, were altered by administration of Pargyline. Further, we convinced ourselves that the injection of monoacetylputrescine and of putrescine did not alter the endogenous γ -aminobutyrate concentration.

Our results are summarized in Table 1. Monoamine oxidase inhibition decreases the incorporation of radioactivity into γ -aminobutyric acid both from putrescine and from monoacetylputrescine to approximately the same extent, although the endogenous pools of these compounds differ considerably, and the injected amounts of the γ -aminobutyrate precursors were also quite different. Pretreatment of the animals with aminoguanidine influenced the incorporation of radioactivity from monoacetylputrescine and putrescine only slightly (Table 1).

Discussion

From our experimental results the following facts can be derived. (1) In contrast with putrescine, which is a diamine oxidase substrate par excellence, monoacetylputrescine is a substrate of monoamine oxidase. Although a thorough kinetic study has not been performed yet to characterize its substrate properties, this is evident from the rapid oxidation of this compound by liver and brain mitochondria,

from the complete inhibition of the oxidative deamination by typical monoamine oxidase inhibitors, and from the ineffectiveness of even high concentrations of an effective inhibitor of diamine oxidase.

(2) *N*-Acetyl- γ -aminobutyric acid can be formed not only from monoacetylputrescine *in vitro*, but also in the brain *in vivo*. Although the experimental conditions were not really physiological and the injected amounts of monoacetylputrescine were above the assumed concentrations (Seiler *et al.*, 1973b) of this compound *in vivo*, there is little doubt that the oxidative deamination of monoacetylputrescine can take place under physiological conditions as well. It is therefore assumed that *N*-acetyl- γ -aminobutyric acid is a natural metabolite of brain metabolism.

(3) The transformation of both putrescine and of monoacetylputrescine to γ -aminobutyrate was influenced by a specific monoamine oxidase inhibitor to the same extent. This finding is consistent with the assumption that a major portion of putrescine is physiologically degraded in mammalian brain via monoacetylputrescine $\rightarrow$ *N*-acetyl- γ -aminobutyric acid $\rightarrow$ γ -aminobutyric acid $\rightarrow$ CO₂ (Scheme 1). Under the experimental conditions the transformation of monoacetylputrescine and putrescine to γ -aminobutyrate was not inhibited completely by Pargyline. There may be several explanations for this finding. It cannot be excluded that some of the

Scheme 1. Scheme of putrescine catabolism via γ -aminobutyric acid

1, Acetyl-CoA-1,4-diaminobutane *N*-acetyltransferase; 2, monoamine oxidase; 3, aldehyde dehydrogenase; 4, diamine oxidase; 5, γ -aminobutyrate deacetylating enzyme; 6, γ -aminobutyrate-2-oxoglutarate aminotransferase; 7, succinate semialdehyde dehydrogenase; 8, glutamate decarboxylase; 9, ornithine decarboxylase.

putrescine is oxidized by diamine oxidase, despite the low activity of this enzyme in mammalian brain (Burkard *et al.*, 1963), and the failure of aminoguanidine to inhibit the incorporation of putrescine carbon into the skeleton of γ -aminobutyric acid. Although it has been shown that aminoguanidine inhibits the degradation of aliphatic diamines *in vivo* in mice (Schayer *et al.*, 1954), it is not clear whether this compound penetrates the blood-brain barrier in amounts sufficient to cause inhibition of diamine oxidase in brain.

(4) There are no methods now available for the determination of small amounts of monoacetylputrescine and *N*-acetyl- γ -aminobutyrate. It is not known therefore to what extent monoacetylputrescine and *N*-acetyl- γ -aminobutyrate concentrations are altered if brain monoamine oxidase is inhibited. The relatively rapid disappearance of the radioactivity from the monoacetylputrescine zone of the electrophoretograms (Fig. 2) seems to suggest that monoacetylputrescine may be a major excretion product under these conditions. There are, however, other possibilities. Labelled putrescine was found in the brains after the administration of monoacetyl-[³H]putrescine in considerable amounts; therefore there may exist either a hydrolytic reaction or an acetyltransferase activity in the brain, which liberates putrescine from monoacetylputrescine. Since the putrescine and polyamine concentrations were not significantly altered after Pargyline administration, but instead an increased proportion of radioactivity was detectable in spermidine after the administration of either putrescine or monoacetylputrescine, the turnover rate of the polyamines could have been influenced by monoamine oxidase inhibition.

(5) Our experiments do not allow us to make suggestions as to the mechanism of the transformation of *N*-acetyl- γ -aminobutyrate to γ -aminobutyrate. This step was therefore formulated in Scheme 1 only as elimination of the acetyl moiety from *N*-acetyl- γ -aminobutyrate.

Many details of the degradative pathway of putrescine remain to be clarified. However, it is evident from the present findings that at least in mammalian brain a pathway exists in which a typical diamine is deaminated by monoamine oxidase. These experiments show further possible metabolic interrelations between the arylethylamines, which have been exclusively recognized as natural substrates of monoamine oxidase, and the polyamines. It is a future task to disclose or refute functional interrelations between these groups of biogenic amines.

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