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α-MONOFLUOROMETHYL AND α-DIFLUOROMETHYL PUTRESCINE AS ORNITHINE DECARBOXYLASE INHIBITORS: *IN VITRO* AND *IN VIVO* BIOCHEMICAL PROPERTIES

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Abstract—*In vitro*, 5-fluoropentane-1,4-diamine and 5,5-difluoropentane-1,4-diamine are potent enzyme-activated inhibitors of rat liver ornithine decarboxylase (EC 4.1.1.17). The two α-fluoromethyl derivatives of putrescine activate to different degrees *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50). The difluoromethyl derivative differs from the monofluoromethyl derivative in that it is not a substrate of diamine oxidase (EC 1.4.3.6), but is a better substrate of mitochondrial monoamine oxidase (EC 1.4.3.4) than the monofluoromethyl derivative. *In vivo*, a single i.p. injection of 200 mg/kg of 5-fluoropentane-1,4-diamine to rats causes a marked decrease of the ornithine decarboxylase activity in the ventral prostate and to a lesser extent in the thymus, whereas 5,5-difluoropentane-1,4-diamine causes only a slight decrease of this enzyme activity in the prostate and does not affect it in the thymus. Both compounds produce a decrease of 4-aminobutyrate : 2-oxoglutarate aminotransferase (EC 2.6.1.19) activity in the brain. The differences observed between the biochemical properties of the two α-fluoromethyl derivatives of putrescine are discussed in relation to the pK_a value of the α-amino group which decreases from 7.75 for 5-fluoropentane-1,4-diamine to 6.4 for 5,5-difluoropentane-1,4-diamine.

The α-ethynyl analogue of putrescine is a potent enzyme-activated inhibitor of mammalian ornithine decarboxylase (EC 4.1.1.17) [1–3]. The proposed mechanism of inhibition involves abstraction of the propargylic hydrogen of the pseudo-substrate by the enzyme. This is due to the microreversibility of the protonation step in the enzymic decarboxylation of the normal substrate, L-ornithine, to putrescine. This abstraction of hydrogen yields a propargylic carbanion which leads after a prototropic shift to the formation of a Michael acceptor in the enzyme active-site. This electrophilic species can react with a nucleophilic residue within bonding distance to form a covalent bond with the enzyme [1].

By invoking a similar abstraction of hydrogen (Fig. 1) α-halomethyl analogues of putrescine could also be enzyme-activated irreversible inhibitors of ornithine decarboxylase. Such a concept of decarboxylase inhibition has been applied successfully to mammalian L-aromatic amino acid and histidine decarboxylases (EC 4.1.1.26 and EC 4.1.1.22); the α-monofluoromethyl derivatives of dopamine and histamine are enzyme-activated inhibitors of these decarboxylases [4]. The fluorine atom, which is difficult to displace in a direct substitution reaction and which combines a good leaving group ability in elimination reactions [5] with a size comparable to that of a hydrogen atom, appears to be the preferred halogen.

In this publication, we report the *in vitro* and *in vivo* inhibition of rat ornithine decarboxylase by α-monofluoromethyl and α-difluoromethyl putrescine. In addition, these compounds are activators of *S*-adenosylmethionine decarboxylase (EC 4.1.1.50), the other decarboxylase in the polyamine biosynthetic pathway [6]. They are also substrates of the mitochondrial monoamine oxidase (EC 1.4.3.4) and they inhibit 4-aminobutyrate : 2-oxoglutarate aminotransferase (EC 2.6.1.19) *in vivo*, due to their transformation into the corresponding derivatives of 4-aminobutyric acid.

MATERIALS AND METHODS

Chemicals. The following compounds were purchased: DL[1-^{14}C]ornithine (58 Ci/mole) and *S*-adenosyl-L-[carboxyl-^{14}C]methionine (60 Ci/mole) (Radiochemical Center, Amersham, England); DL[1-^{14}C]glutamate (50 Ci/mole) (New England Nuclear Corp., Boston, MA, U.S.A.); L-ornithine, pyridoxal phosphate, ammonium sulfate, reduced glutathione (GSH), thioacetamide, sucrose, potassium nitrate, KOH 0.1 N (Titrisol) and buffer reagents (Merck, Darmstadt, Germany); EDTA tetrasodium dihydrate (Calbiochem, La Jolla, CA, U.S.A.); dithiothreitol, NAD^+, *S*-adenosyl-L-methionine, putrescine dihydrochloride, 4-aminobutyric acid, horseradish peroxidase, hog kidney diamine oxidase (Sigma, St Louis, MO, U.S.A.). Methylglyoxal-bis-(guanylhydrazone) dihydrochloride monohydrate was obtained from Aldrich-Europe (Beerse, Belgium). Scintillators were purchased from Beckman Instruments, Fullerton, CA, U.S.A. L-2-Methylornithine and D-2-methylornithine were synthesized in our laboratories [7].

5-Fluoropentane-1,4-diamine dihydrochloride (*RMI* 71864). 1-Fluoro-5-phthalimido-pentan-2-one (**1**) was synthesized in 63% yield from 4-

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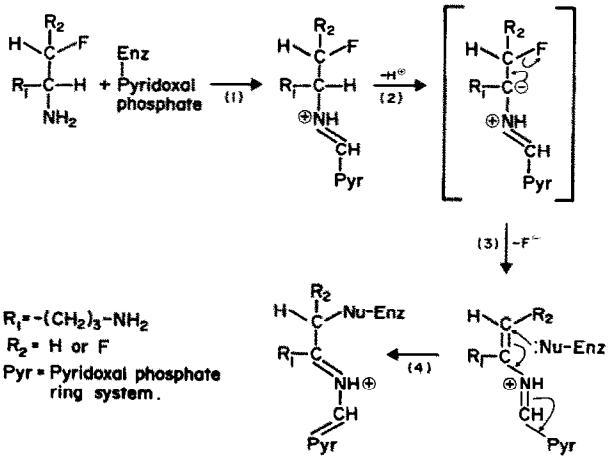


Fig. 1. Proposed mechanism for the enzyme-activated inhibition of ornithine decarboxylase by the α-fluoromethyl derivatives of putrescine. After formation of a Schiff base with pyridoxal phosphate in the enzyme active site (step 1), hydrogen abstraction (step 2) and elimination of one of the fluorine atoms (step 3) leave a highly reactive Michael acceptor which can alkylate some nucleophilic residue (Nu) of the enzyme active site (step 4) to inactivate the enzyme.

phthalimido-1-butyryl chloride [8] according to the method of Olah and Welch [9] [m.p.: 92° (ether–pentane); Anal. calc. for $C_{13}H_{12}FNO_3$: C, 62.65; H, 4.85; N, 5.62. Found: C, 62.72; H, 4.76; N, 5.56%]. Reduction of **1** with 1.5 equiv. of $NaBH_4$ in a 1:1 (v/v) mixture of tetrahydrofuran and methanol at −20° for 15 min gave the corresponding alcohol **2** in 85% yield [m.p.: 85° (ether); Anal. calc. for $C_{13}H_{14}FO_3N$: C, 62.14; H, 5.62; N, 5.57. Found: C, 62.12; H, 5.27; N, 5.50%]. Transformation of the alcohol **2** to 1-fluoro-2,5-diphthalimido-pentane (**3**) was achieved in 60% yield by the method of Mitsunobu *et al.* [10]. [m.p.: 112° (tetrahydrofuran–ether); Anal. calc. for $C_{21}H_{17}FN_2O_4$: C, 66.31; H, 4.50; N, 7.36. Found: C, 65.95; H, 4.63; N, 7.19%]. Hydrolysis of the diphthalimido intermediate **3** in concentrated hydrochloric acid at 100° for 3 days afforded 5-fluoropentane-1,4-diamine dihydrochloride in 85% yield [m.p.: 154° (ethanol); Anal. calc. for $C_5H_{13}FN_2.2HCl$: C, 31.10; H, 7.83; N, 14.51. Found: C, 31.25; H, 7.66; N, 14.22%].

5,5-Difluoropentane-1,4-diamine (MDL 72034). The synthesis of this compound has been described elsewhere [11].

4-Deuterio-5,5-difluoropentane-1,4-diamine. This compound was prepared by using the following sequence: decarboxylation of 2-difluoromethyl-2-ethoxycarbonyl-5-phthalimido-1-pentanoic acid [11] in deuterated acetic acid, followed by hydrolysis of the carboxylic ester under neutral conditions (iodotrimethylsilane [12], 110°, 4 hr) yielded the corresponding acid (85% ^2H, as determined by mass spectrometry); conversion of the acid to the corresponding amine was achieved by the standard Curtius rearrangement sequence; hydrolysis with concentrated HCl, 100°, 48 hr, afforded the dihydrochloride salt of 4-deuterio-5,5-difluoropentane-1,4-diamine with an overall yield of 35% [m.p.: 138° (ethanol)].

5-Pentane-1,4-diamine (MDL 71823). 1,4-Dibromopentane (15 g), prepared by treatment of α-methyltetrahydrofuran (10 g) with 40% hydrobromic acid (60 ml) and concentrated sulfuric acid (22 ml) for 3.5 hr at 100°, was reacted with potassium phthalimide (20.4 g) and sodium iodide (20.4 g) in dimethylformamide (165 ml) at 160° for 8 days. After concentration *in vacuo*, extraction of the residue with chloroform and purification by chromatography on silica gel (eluant: tetrahydrofuran–hexane 1:9), 1,4-diphthalimido pentane (3.5 g) was obtained which, upon hydrolysis with concentrated hydrochloride acid (100 ml, 3 days, 100°), afforded, after recrystallization from methanol–ether, 1.2 g of α-methylputrescine dihydrochloride [m.p.: 143°; Anal. calc. for $C_5H_{14}N_2$, 2HCl: C, 34.28; H, 9.23; N, 16.00. Found: C, 34.27; H, 8.98; N, 15.79%].

Determination of the pK_a values. The measurements were carried out by titration at 37° essentially as described by Wagner *et al.* [13], using a semi-automatic titration system consisting of the Mettler modules DK 10, 12, 13 and 15 connected to a DV II burette, and equipped with a combined glass electrode Metrohm EA 125. A 2 ml quantity of 5×10^{-3} M amine solution in water containing an excess of HCl were titrated at constant ionic strength (0.1 M KNO_3) with 0.1 N KOH.

The pK_as were determined by comparison of the calculated and the experimental titration curves until the best fit between the two curves was obtained by using a Hewlett–Packard HP 9820 calculator fitted with a HP 9862 plotter. The concentrations of the different ion species for a given pH were calculated from the pK_a values, by using a computer program (P. Vitali and J. Wagner, unpublished).

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 libitum* 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 minimise effects due to diurnal fluctuations. Drugs dissolved in 0.9% saline, were injected intraperitoneally. Rats given saline served as controls.

Assay of time-dependent inhibition of ornithine decarboxylase (in vitro). The enzyme preparation

was obtained from the liver of rats which had been injected with thioacetamide (150 mg/kg of body wt) 18 hr before sacrifice, and was purified about 10 fold by acid treatment at pH 4.6 as described by Ono *et al.* [14]. The specific activity of this preparation was 0.2 nmoles of CO_2/min/mg of protein. The kinetic constants of the time-dependent inhibition were determined essentially as described previously [1].

Measurement of ornithine decarboxylase activity (ex vivo). Immediately after sacrifice, the ventral prostate, testis and thymus of the animals were excised and homogenized. Ornithine decarboxylase activity was measured according to a published procedure [2].

Assay of S-adenosyl-L-methionine decarboxylase (in vitro). This enzyme was prepared from the liver of rats which had been injected with methylglyoxal-bis-(guanylhydrazone).2HCl. The purification steps were steps 1 and 2 (105,000 *g* centrifugation and $(NH_4)_2SO_4$ precipitation) described by Pegg [15]. The specific activity of this precipitate was 0.3 nmoles of CO_2/min/mg of protein. The enzymic activity was measured according to Pegg and Williams-Ashman [16]. Measurement of the activation constants was performed as described for putrescine by Pösö *et al.* [17] at 0.2 mM *S*-adenosyl-L-methionine concentration in 30 mM sodium phosphate buffer, pH 7.1.

Assay of mitochondrial monoamine oxidase and of diamine oxidase (in vitro). Mitochondria from rat liver were obtained by differential centrifugation of the tissue homogenates in 0.25 M sucrose [18]. The particulate fraction was resuspended in ice-cold 0.1 M phosphate buffer, pH 7.4 and served as a source of monoamine oxidase. The activities of mitochondrial monoamine oxidase and of diamine oxidase were measured at pH 7.8 as described by Snyder and Hendley [19], using the putrescine derivatives as substrates.

Assay of glutamate decarboxylase, 4-aminobutyrate:2-oxoglutarate aminotransferase and determination of whole brain 4-aminobutyric acid concentration. The activities of the two enzymes and the concentration of 4-aminobutyric acid were estimated as described previously [20].

Data processing. Kinetic constants were calculated by using a least-squares fit of the data points with a Hewlett–Packard 9820 calculator. *Ex vivo* values of enzyme activities and of 4-aminobutyric acid concentrations were the means ± S.E.M. of five animals. The significance of the differences between controls and treated animals was calculated by Student's *t* test with the above-described calculator.

RESULTS

Effects of α-fluoromethyl derivatives of putrescine on ornithine decarboxylase activity in vitro. The two α-fluoromethyl derivatives of putrescine were tested as time-dependent irreversible inhibitors of ornithine decarboxylase. Liver from thioacetamide-treated rats was used as the source of the enzyme for the *in vitro* experiments. Incubation of the enzyme preparation with 5-fluoropentane-1,4-diamine and 5,5-difluoropentane-1,4-diamine resulted in both cases in a time-dependent loss of enzyme activity which followed pseudo first-order kinetics for about two half-lives as shown in Fig. 2. Over longer periods of time, the semilogarithmic plot deviated from linearity analogously to the inactivation of ornithine decarboxylase by α-ethynyl putrescine (or 5-hexyne-1,4-diamine) or by α-difluoromethylornithine [1]. The kinetic constants of inactivation (Table 1) of rat liver ornithine decarboxylase by 5-fluoropentane-1,4-diamine and by 5,5-difluoropentane-1,4-diamine were determined by plotting the time of half-inactivation of the enzyme as a function of the reciprocal of the inhibitor con-

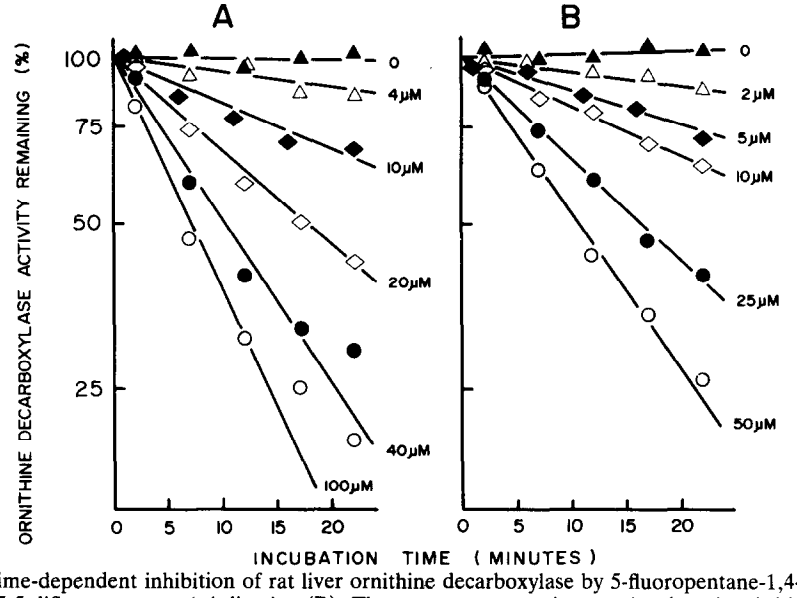
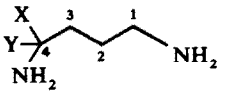


Fig. 2. Time-dependent inhibition of rat liver ornithine decarboxylase by 5-fluoropentane-1,4-diamine (A) and 5,5-difluoropentane-1,4-diamine (B). The enzyme preparation was incubated at 37° in 30 mM pyridoxal phosphate with different concentrations of inhibitor. At given time intervals 50 μl aliquots were assayed for remaining enzyme activity using DL-[1-^{14}C]ornithine according to a published procedure [1].

Table 1. pK_a values of the amino groups of the 4-fluoromethyl derivatives of 1,4-diaminobutane and kinetic constants of ornithine decarboxylase inhibition and *S*-adenosylmethionine decarboxylase activation by these compounds

	pK_a values of amino groups		Ornithine decarboxylase		*S*-Adenosylmethionine decarboxylase activation	
	4-NH_2	1-NH_2	K_I (μM)	$t_{\frac{1}{2}}$ (min)	K_A (μM)	(V_{max}/v) (*x*-fold)
X = CH_3 Y = H	9.15	10.3	520 ± 50	No time-dependent inhibition	38 ± 11	5.2
X = CH_2F Y = H	7.75	10.0	56 ± 12	4.4 ± 0.4	60 ± 6	4.8
X = CHF_2 Y = H	6.40	10.1	30 ± 4	7.4 ± 0.8	280 ± 20	4.9
X = CHF_2 Y = ^2H	—	—	90 ± 6	7.3 ± 0.6	—	—

For the inhibition of ornithine decarboxylase, K_I is the apparent dissociation constant and $t_{\frac{1}{2}}$ is the time of half enzyme-inactivation extrapolated at infinite concentration of inhibitor. For the activation of *S*-adenosylmethionine decarboxylase, K_A is the activation constant and V_{max}/v is the ratio of reaction velocity extrapolated at infinite concentration of activator to reaction velocity measured without activator, in the presence of 0.2 mM *S*-adenosylmethionine. All constants reported in this table were measured at 37° as described in Materials and Methods.

centration (Fig. 3), according to the method of Kitz and Wilson [21].

The times of half-inactivation of ornithine decarboxylase produced by 4-protio-5,5-difluoropentane-1,4-diamine and its 4-deuterio analogue were compared (Fig. 3B) at various concentrations. No kinetic isotope effect on the minimum time of half inactivation, i.e. on the inactivation rate constant was found, but a primary kinetic isotope effect on the apparent dissociation constant was measured ($K_{I,^2H}/K_{I,H}$ = 3.0).

Protective effects of ornithine analogues against inactivation of ornithine decarboxylase caused by 5-fluoropentane-1,4-diamine and 5,5-difluoropentane-1,4-diamine were investigated (Table 2). L-Ornithine and L-2-methylornithine, which have a fair affinity for ornithine decarboxylase [7], protected effectively against enzyme inactivation. D-2-Methylornithine which is practically devoid of affinity for ornithine decarboxylase, did not modify significantly the inactivation of ornithine decarboxylase by either inhibitor.

Incubation of the ornithine decarboxylase preparation with 0.1 mM 5-fluoropentane-1,4-diamine or 5,5-difluoropentane-1,4-diamine resulted in 92% and 91% inactivation after 30 min respectively. Prolonged dialysis (24 hr) of the inactivated enzyme against a buffer solution containing sodium phosphate (30 mM, pH 7.1), pyridoxal phosphate (0.1 mM) and dithiothreitol (5 mM) (conditions

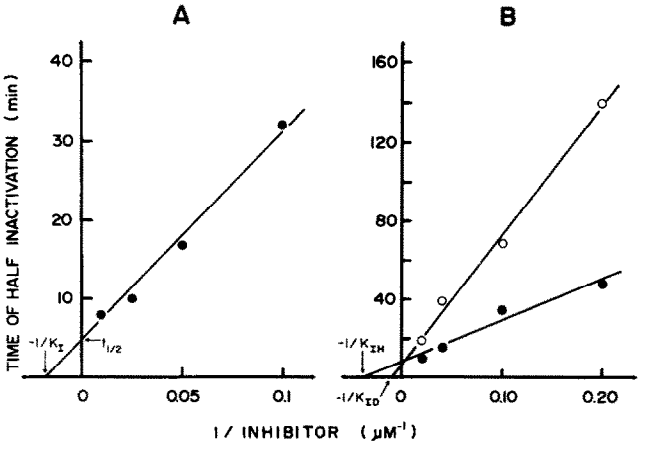


Fig. 3. Effect of inhibitor concentration [A, 5-fluoropentane-1,4-diamine; B, 5,5-difluoropentane-1,4-diamine (●) a 4-deuterio-5,5-difluoropentane-1,4-diamine (○)] on the time of half-inactivation of ornithine decarboxylase.

Table 2. Effects of preincubation with different effectors on the time of half-inactivation of ornithine decarboxylase by the α-fluoromethyl derivatives of putrescine

Addition to incubation media	Time of half-inactivation of ornithine decarboxylase (min)	
	5-Monofluoropentane-1,4-diamine (25 μM)	5,5-Difluoropentane-1,4-diamine (25 μM)
None	20	14
1 mM L-ornithine	132	100
1 mM L-2-methylornithine	108	168
1 mM D-2-methylornithine	30	18

under which the native enzyme is stable) led to a minor recovery of enzyme activity, from 8% and 9% of control before dialysis to 16% and 11% of control after dialysis, respectively.

Effects of the α-fluoromethyl derivatives of putrescine on ornithine decarboxylase activity (ex vivo). Five hours after an intraperitoneal injection of 50 and 200 mg/kg of 5-fluoropentane-1,4-diamine, ornithine decarboxylase activity was significantly decreased in the ventral prostate while only the 200 mg/kg dose caused a significant inhibition of ornithine decarboxylase activity in the thymus. The results are shown in Table 3. No inhibition of testis ornithine decarboxylase activity was observed after either dose. 5,5-Difluoropentane-1,4-diamine did not cause any significant decrease of ornithine decarboxylase activity in the organs studied, except in the ventral prostate when injected at 200 mg/kg.

Effects of α-fluoromethyl analogues of putrescine on S-adenosylmethionine decarboxylase activity in vitro. Putrescine is, *in vitro*, an activator of *S*-adenosylmethionine decarboxylase [6, 16, 17, 22]. It was found that the two α-fluoromethyl and the α-methyl derivatives of putrescine activated this enzyme similarly to putrescine. The activation constant (concentration of activator which causes half of the maximum increase of the enzyme-reaction velocity), and the maximum activation (ratio of enzyme-reaction velocity extrapolated at infinite concentration of activator to enzyme-reaction velocity in the absence of activator) of *S*-adenosylmethionine decarboxylase were calculated for each activator. The results are given in Table 1 along with the pK_a values of the two amino groups of the derivatives of putrescine. The percentage of diprotonated species at pH 7.1 of the α-fluoromethyl putrescines and of α-methylputrescine were calculated from the pK_a values. A direct correlation ($r = 0.97$) exists between the reciprocal of the activation constant (i.e. the apparent affinity constant) of the analogues of putrescine and the concentration of the diprotonated species (Fig. 4). The maximum activation of *S*-adenosylmethionine decarboxylase by each of the three putrescine analogues is very similar (Table 1).

In vitro *oxidation of the α-fluoromethyl derivatives of putrescine by rat liver mitochondrial monoamine oxidase and hog kidney diamine oxidase*. The two α-fluoromethyl analogues of putrescine were found to be substrates of a preparation of mitochondrial monoamine oxidase obtained from rat liver. This preparation was devoid of diamine oxidase activity as assessed by the lack of oxidation of putrescine. The Michaelis constants (K_M) and the maximum velocities (V_{max}) extrapolated to infinite concentration of substrate were calculated (Table 4) for the two α-fluoromethyl amines. As shown in Table 4, the K_M decrease and the V_{max} values increase with the concentration of the monoprotonated species at pH 7.8.

5,5-Difluoropentane-1,4-diamine which exists at pH 7.8 essentially as the monoprotonated species is a very poor substrate of hog kidney diamine oxidase. Only 5-fluoropentane-1,4-diamine is significantly oxidized by diamine oxidase (see Table 4).

Table 3. Effect of single doses of 5-fluoropentane-1,4-diamine or of 5,5-difluoropentane-1,4-diamine, given intraperitoneally to rats 5 hr before sacrifice on ornithine decarboxylase activities in ventral prostate, testis and thymus

Inhibitor	Dose (mg/kg)	Remaining ornithine decarboxylase activity (% control)		
		Ventral prostate	Thymus	Testis
Saline (control)		100 ± 15	100 ± 7	100 ± 4
5-Fluoropentane-	50	60 ± 6**	92 ± 9	120 ± 6
1,4-diamine	200	22 ± 2***	56 ± 5***	95 ± 3
5,5-Difluoropentane-	50	77 ± 3	97 ± 13	92 ± 2
1,4-diamine	200	60 ± 13*	104 ± 8	96 ± 4

Each value is the mean ± S.E.M. of five animals. Ornithine decarboxylase activities of control animals were 315 ± 47, 37 ± 3 and 26 ± 1 nmoles of CO_2/hr/g of wet tissue in prostate, testis and thymus, respectively. The significance of the differences between controls and treated animals was calculated by Student's *t* test: *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$.

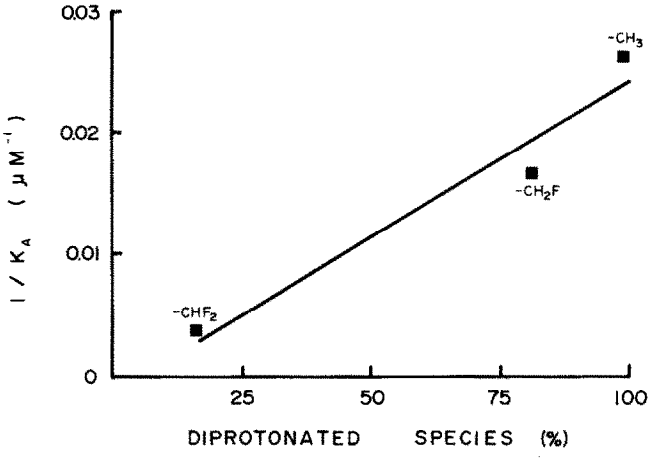


Fig. 4. Effect of the proportion of diprotonated species at pH 7.1, corresponding to each α-fluoromethyl derivative of putrescine on the reciprocal of the activation constant (K_A) of these compounds for *S*-adenosylmethionine decarboxylase, *in vitro*. The percentage of diprotonated species at pH 7.1 was calculated as described in Materials and Methods from the pK_a values of each of the amino groups of the putrescine derivatives (Table 1). The straight line has been drawn using least-squares analysis of the experimental points.

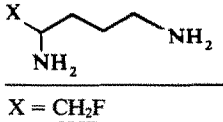
Effects of the α-fluoromethyl derivative of putrescine on whole brain 4-aminobutyrate:2-oxoglutarate aminotransferase and glutamate decarboxylase activities and 4-aminobutyric acid concentrations. 4-Aminobutyrate:2-oxoglutarate aminotransferase activity was found to be reduced by 50% and 38% when measured *ex vivo* 5 hr after a single i.p. injection of 200 mg/kg of 5-fluoropentane-1-4-diamine or of 5,5-difluoropentane-1,4-diamine, respectively. Glutamate decarboxylase activity was not affected by either inhibitor. The concentration of 4-aminobutyric acid was increased three-fold in the brain of rats injected with 200 mg/kg of 5-fluoropentane-1,4-diamine. It was checked that neither 5-fluoropentane-1,4-diamine, nor 5,5-difluoropentane-1,4-diamine were inhibitors of 4-aminobutyrate:2-oxoglutarate aminotransferase or glutamate decarboxylase preparations from rat brain *in vitro*.

DISCUSSION

5-Fluoropentane-1,4-diamine and 5,5-difluoropentane-1,4-diamine produce, *in vitro*, a time-dependent inhibition of rat liver ornithine decarboxylase which follows pseudo first-order kinetics. The lack of any significant recovery of the enzyme activity after prolonged dialysis of the inhibited enzyme suggests the irreversibility of the inactivation process. Moreover, the kinetics demonstrate a saturation effect indicative of the formation of a complex between the enzyme active site and the inhibitor which is not rate-limiting for the overall enzyme inactivation. That the inhibition of ornithine decarboxylase is active-site directed is confirmed by the protective effects of the natural substrate L-ornithine and of the competitive inhibitor L-2-methyl ornithine. The presence of 5 mM dithiothreitol in the preincubation medium and the absence of a lag-time before the onset of inhibition rule out the possibility of inhibition via an affinity labelling mode by a diffusible alkylating species [23]. Thus, the data suggest that these compounds are effective enzyme-activated inhibitors [24] of ornithine decarboxylase, which presumably act according to the mechanism outlined in Fig. 1.

Comparison of the rate of inhibition of ornithine decarboxylase produced by 4-protio-5,5-

Table 4. *In vitro* oxidation of α-fluoromethyl derivatives of putrescine by amine oxidases

Putrescine analogue	Mitochondrial monoamine oxidase		Diamine oxidase		Monoprotonated species at pH 7.8 (%)
	K_M (nM)	V_{max} (F.U.)	K_M (mM)	V_{max} (F.U)	
X = CH_2F	2.1	3.2	0.3	1.5	53
CHF_2	1.2	8.4	—	<0.2	96

The kinetic constants were measured as described in Materials and Methods, using the fluorescence assay of Snyder and Hendley [19]. The maximum velocities (V_{max}) are expressed in arbitrary fluorescence units (F.U)/20 min/50 μl of enzyme preparations. Under similar conditions the rate of oxidation of 0.1 mM benzylamine was 10 F.U/20 min/50 μl of monoamine oxidase preparation and the rate of oxidation of 0.1 mM putrescine was 19 F.U./20 min/50 μl of diamine oxidase preparation.

difluoropentane-1,4-diamine and its 4-deuterio analogue shows no kinetic isotope effect on the inactivation rate constant, but a primary kinetic isotope effect on the apparent dissociation constant (K_I) (Fig. 3B). Comparable isotopic effects have been observed in the inactivation of ornithine decarboxylase by α-deuterio-α-ethynylputrescine [1] and of glutamate decarboxylase by γ-deuterio-γ-ethynyl-γ-aminobutyric acid [25]. The isotope effect on the apparent dissociation constant has been discussed by Jencks [26]. It can be accounted for by the effect of deuterium on the rate constant of the hydrogen abstraction (or catalytic constant) since K_I is a function of the catalytic constant [27]. The absence of a kinetic isotope effect on the inactivation rate constant suggests that the hydrogen abstraction is not rate limiting. For the inhibition of ornithine decarboxylase by 5-hexyne-1,4-diamine, allene formation [3] or covalent linkage of the activated inhibitor to the enzyme [1] has been proposed as the rate-limiting step. In the inactivation of ornithine decarboxylase by mono- and difluoromethyl putrescine, the elimination of fluoride could be rate-determining as in the case of the transformation of β-haloaminobutyrates to ketobutyrates by cystathionine γ-synthetase [28]. It is known that the carbon–fluorine bond labilization triggered by hydrogen abstraction at the α-carbon atom decreases with the number of fluorine atoms substituting the methyl group [5]. Therefore, the higher rate of ornithine decarboxylase inactivation observed with the monofluoromethyl putrescine as compared with difluoromethyl putrescine is consistent with the fluoride elimination being the rate-determining step in the inactivation process. The difference in the maximum rate of inactivation of ornithine decarboxylase by α-monofluoromethyl ornithine ($t_{\frac{1}{2}} = 1.6$ min) and α-difluoromethyl ornithine ($t_{\frac{1}{2}} = 3.1$ min) [29] corroborates this suggestion.

Both 5-fluoropentane-1,4-diamine and 5,5-difluoropentane-1,4-diamine cause a decrease of ornithine decarboxylase activity *in vivo* (Table 3). The marked difference in the potency of the two inhibitors *in vivo* is surprising since their *in vitro* kinetic constants are rather similar. This may be related to differences in the cell-membrane permeability due to the different proportion of diprotonated vs monoprotonated species (see Table 1 for the pK_a values).

Like other analogues of putrescine [2, 30] the α-methyl and the two α-fluoromethyl derivatives of putrescine are activators of *S*-adenosylmethionine decarboxylase *in vitro*. A good correlation exists between the reciprocal of the activation constants for this enzyme and the percentages of diprotonated species for the three derivatives of putrescine, i.e. the activation constants expressed in terms of the concentration of activators in their diprotonated form are very similar for the three compounds.

The α-difluoromethyl derivative of putrescine is a better substrate of the mitochondrial monoamine oxidase than the α-monofluoromethyl derivative (Table 4). The difference in pK_a of the amino groups adjacent to the fluorinated methyl substituent can account for this finding. At pH 7.8, the α-difluoromethyl derivative exists essentially as a monoprotonated species, i.e. as a monoamine, whereas the monoprotonated species of the monofluoromethyl analogue amounts to only 50%. Conversely, the monofluoromethyl analogue, in contrast to the difluoromethyl analogue, is significantly oxidized by diamine oxidase, presumably because it exists as a diprotonated species at pH 7.8 (Table 4). Similar reasoning can explain the fact that 5-hexyne-1,4-diamine, the α-ethynyl derivative of putrescine, is a substrate of monoamine oxidase but not of diamine oxidase in contrast to putrescine [31].

Based on these findings, it is not surprising that 5-difluoropentane-1,4-diamine and 5,5-difluoropentane-1,4-diamine are *in vivo* inhibitors of 4-aminobutyrate : 2-oxoglutarate aminotransferase. Such a result can be explained by the *in vivo* oxidation of these amines by the combined action of an amine oxidase and an aldehyde dehydrogenase to the corresponding fluoromethyl derivatives of 4-aminobutyric acid. Similar oxidation has been previously demonstrated for 5-hexyne-1,4-diamine [31]. Moreover, 4-monofluoromethyl-4-aminobutyric acid and 4-difluoromethyl-4-aminobutyric acid have been recently [32] reported to be potent enzyme-activated inhibitors of 4-aminobutyrate : 2-oxoglutarate aminotransferase *in vitro* and *in vivo* and to have no effect on glutamate decarboxylase.

In conclusion, as demonstrated for aromatic amino acid decarboxylase [4], ornithine decarboxylase is susceptible *in vitro* to enzyme-activated inhibition by analogues of its product which incorporate a fluoromethyl substituent on the α-carbon atom. These putrescine analogues are also inactivators of ornithine decarboxylase *in vivo*. Furthermore, due to their resemblance to putrescine, they share several properties of this diamine. In particular, they activate *S*-adenosylmethionine decarboxylase, they are substrates of amine oxidases and, like putrescine which is converted *in vivo* to 4-aminobutyric acid [33], they are most likely oxidized to the corresponding analogues of 4-aminobutyric acid.

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