Biochemical Pharmacology, Vol. 35, No. 2, pp. 159–165, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

MARKED AND PROLONGED INHIBITION OF MAMMALIAN ORNITHINE DECARBOXYLASE *IN VIVO* BY ESTERS OF (E)-2-(FLUOROMETHYL)DEHYDROORNITHINE

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(*Received* 14 *March* 1985; *accepted* 27 *June* 1985)

Abstract—(1) (E)-2-(fluoromethyl)dehydroornithine, a new enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC) is no more effective than α-difluoromethylornithine (DFMO) at inhibiting polyamine biosynthesis in rat hepatoma tissue culture (HTC) cells and in rat organs even though its potency is over 15 times higher than that of DFMO *in vitro*.

(2) The methyl, ethyl, octyl and benzyl esters of (E)-2-(fluoromethyl)dehydroornithine were synthesized as potential prodrugs of the amino acid. When tested at concentration equivalent to the K_i value of the amino acid, they are devoid of ODC-inhibitory property.

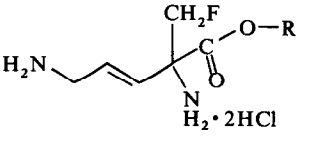
(3) When measured 6 hr after its addition to the HTC cell culture medium, the absorption of methyl ester was 20 times higher than that of the parent amino acid or that of DFMO, and was accompanied by a more marked intracellular accumulation of (E)-2-(fluoromethyl)dehydroornithine than that achieved by the addition of the parent amino acid. The methyl ester used at 10 times lower concentrations is as effective as its parent amino acid or as DFMO at inhibiting polyamine biosynthesis in HTC cells.

(4) Similarly, the methyl and the ethyl esters of (E)-2-(fluoromethyl)dehydroornithine used at 10 times lower doses are as effective as the parent amino acid and as DFMO at inhibiting ODC in the ventral prostate of rat, 6 hr after oral administration. All the esters of (E)-2-(fluoromethyl)-dehydroornithine produce a particularly long duration of ODC inhibition in the ventral prostate and in the testes.

(5) Repeated administration (25 mg/kg given once a day by gavage) of the methyl ester of (E)-2-(fluoromethyl)dehydroornithine for 8 days to rats results in a constant 80% inhibition of ODC over a 24-hr period, accompanied by a 90% decrease of putrescine and spermidine concentrations in the ventral prostate.

Considerable progress in the knowledge of the functional role of polyamines at the cellular level has been achieved with the availability of specific inhibitors of ornithine decarboxylase (EC 4.1.1.17, ODC) the enzyme responsible for the biosynthesis of putrescine (reviewed in [1–3]). Among these inhibitors, α-difluoromethylornithine (DFMO), an enzyme-activated irreversible inhibitor [4] has attracted much attention. Not only has DFMO proven to be a useful tool to study pharmacological consequences of ODC inhibition *in vivo* [5–9], but also it has potential for treatment of diseases characterized by rapid cell proliferation or caused by parasitic protozoa [10–12]. The search for ODC inhibitors more effective and longer-acting than DFMO has been actively pursued. Along this line, we have demonstrated recently that (E)-2-(fluoromethyl)dehydroornithine (MDL 72.246) is a potent enzyme-activated irreversible inhibitor of ODC *in vitro* with a K_I value 14 times lower than that of DFMO [13]. Esters of (E)-2-(fluoromethyl)dehydroornithine were synthesized as pro-drugs (see Scheme 1); none of them produced a significant time-dependent inhibition of ODC *in vitro* at 2.5 μM, a concentration equivalent to the K_I of the parent amino acid [13].

We report now the effects of (E)-2-(fluoromethyl)dehydroornithine and of some of its esters on ODC and S-adenosyl-L-methionine decarboxylase (EC 4.1.1.50, SAM-DC) activities and on polyamine concentrations in cultured cells and in various rat organs. These effects are compared to those of DFMO.



MDL 72403: R = —CH_3
MDL 72430: R = —CH_2—CH_3
MDL 72493: R = —$(CH_2)_7$—CH_3
MDL 72504: R = —CH_2—C_6H_5

Scheme 1. Formulae of the various esters synthesized for the present study.

MATERIALS AND METHODS

Chemicals

The following compounds were purchased: DL-[1-^{14}C]ornithine (58 Ci/mole) and S-adenosyl-L-[carboxyl-^{14}C] (60 Ci/mole) (Radiochemical Centre, Amersham, U.K.); L-ornithine, pyridoxal

phosphate, thioacetamide, sucrose and buffer reagents (Merck, Darmstadt, F.R.G.); EDTA tetrasodium dihydrate (Calbiochem, La Jolla, CA, U.S.A.); dithiothreitol, S-adenosyl-L-methionine (Sigma, St. Louis, MO, U.S.A.). Scintillators were purchased from Beckman Instruments, Fullterton, CA, U.S.A. α-Difluoromethylornithine (DFMO, MDL 71.782) and (E)-2-(fluoromethyl)dehydroornithine [(E)-2,5-diamino-2-fluoromethyl-3-pentenoic acid: MDL 72.246] were synthesized as previously published [4, 13]. The various esters were obtained by *O*-alkylation of *N,N'*-diprotected (E)-2-(fluoromethyl)dehydroornithine as follows.

(E)-2-(fluoromethyl)dehydroornithine was treated with 3 equiv. of di-*tert*-butyldicarbonate in the presence of triethylamine in tetrahydrofurane/water to give (E)-2,5-di-*tert*-butyloxycarbamido-2-fluoromethyl-3-pentenoic acid in 97.6% yield. To a solution of this material in dimethylformamide (20 ml/mmol) was added dicyclohexylamine (1.1 equiv.), an alkylhalide RX (4 equiv.) and sodium iodide (0.2 equiv.). The mixture was stirred for 24 hr, and worked up with diethyl ether to yield the (E)-2,5-di-*tert*-butyloxy-carbamido-2-fluoromethyl-3-pentenoic acid ester. The dihydrochloride salts of the esters were obtained upon treatment of an ice-cold solution of the ester in diethylether (20 ml/mmol) with diethylether saturated previously with anhydrous HCl.

(*E*)-2,5-*diamino*-2-*fluoromethyl*-3-*pentenoic acid*, *methyl ester*, *dihydrochloride* (*MDL* 72.403): RX = CH_3I; mp: 191°; Analysis calculated for $C_7H_{13}FN_2O_2$, 2HCl: C, 33.75; H, 6.07; N, 11.25. Found: C, 33.84; H, 6.12; N, 11.10.

(*E*)-2,5-*diamino*-2-*fluoromethyl*-3-*pentenoic acid*, *ethyl ester*, *dihydrochloride* (*MDL* 72.430): RX = C_2H_5Br; mp: 178°; Analysis calculated for $C_8H_{15}FN_2O_2$, 2HCl: C, 36.52; H, 6.51; N, 10.65. Found: C, 36.45; H, 6.40; N, 10.65.

(*E*)-2,5-*diamino*-2-*fluoromethyl*-3-*pentenoic acid*, n-*octyl ester*, *dihydrochloride* (*MDL* 72.493): RX = n-$C_8H_{17}Br$; mp: 136°; Analysis calculated for $C_{14}H_{27}FN_2O_2$, 2HCl: C, 48.42; H, 8.42; N, 8.07. Found: C, 48.30; H, 8.13; N, 7.87.

(*E*)-2,5-*diamino*-2-*fluoromethyl*-3-*pentenoic acid*, *benzyl ester*, *dihydrochloride* (*MDL* 72.504): RX = C_6H_5-CH_2Br; mp: 185°; Analysis calculated for $C_{13}H_{17}FN_2O_2$, 2HCl: C, 48.01; H, 5.89; N, 8.61. Found: C, 47.99; H, 5.95; N, 8.63.

Cell culture

Rat hepatoma tissue culture (HTC) cells were routinely grown in suspension as previously described [14]. Determination of SAM-DC activity, using cell sonicates, was performed according to a published procedure [14]. For the determination of intracellular polyamines and of the methyl ester of (E)-2-(fluoromethyl)dehydroornithine, cells were disrupted by sonication in 0.1 N HCl and, after precipitation of proteins by 0.2 N perchloric acid, analyses were performed by high performance liquid chromatography according to the method of Seiler and Knödgen [15]. Using a linear gradient from 0% to 100% solvent B within 30 min, the ester eluted at 13.1 min whereas putrescine and 1,7-diaminoheptane, used as an internal standard, eluted at 10.1 and 17.5 min, respectively. DFMO and (E)-2-(fluoromethyl)dehydroornithine were determined on cell perchloric extracts with an amino acid analyser, by using the method of Grove *et al.* [16].

Animals

Male rats of the Sprague–Dawley strain (200–300 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 minimize effects due to diurnal fluctuations. Drugs dissolved in water were given by gavage. Rats given water served as controls.

Measurement of ODC and SAM-DC activities in rat tissues

Immediately after sacrifice, the ventral prostate, thymus and testes of the animals were excised and homogenized. ODC and SAM-DC activities were measured according to a published procedure [5].

Determination of polyamines and decarboxylated-S-adenosylmethionine in rat tissues

Ventral prostate, thymus and testes were removed, freed of fat and connective tissue, weighed and frozen in liquid nitrogen. The organs were homogenized at 4° in 5 ml of 0.2 N perchloric acid and the homogenates were centrifuged at 3000 *g*. The supernatants were filtered through a Millipore membrane (pore size 0.22 μm) before simultaneous measurements of polyamines and decarboxylated-S-adenosylmethionine according to the method of Wagner *et al.* [17].

RESULTS

Absorption of (E)-2-(fluoromethyl)dehydroornithine, its methyl ester and DFMO in HTC cells in culture. Evidence for intracellular hydrolysis of the methyl ester of (E)-2-(fluoromethyl)dehydroornithine

Table 1 compares the intracellular concentrations of DFMO, (E)-2-(fluoromethyl)dehydroornithine and its methyl ester 6 hr after addition of various concentrations of these compounds to HTC cell cultures. After addition of DFMO or of (E)-2-(fluoromethyl)dehydroornithine similar intracellular concentrations of these amino acids were observed. In cells treated with (E)-2-(fluoromethyl)dehydroornithine methyl ester both the unchanged methyl ester and the free parent amino acid can be detected in a proportion of 2 to 1. The free amino acid concentration is about 10 times higher than that achieved by addition of (E)-2-(fluoromethyl)-dehydroornithine to culture. This result demonstrates that the methyl ester is better absorbed than the parent amino acid and is intracellularly hydrolyzed to the ODC-inhibitory amino acid.

Effects of the methyl ester of (E)-2-(fluoromethyl)-dehydroornithine on polyamine metabolism in HTC cells. Comparison with the parent amino acid and DFMO

A high density HTC cell-culture was diluted with

Table 1. HTC cell content of DMFO, (E)-2-(fluoromethyl)dehydroornithine (MDL 72246) and its methyl ester (MDL 72403)

Addition		Intracellular content (nmol/10^6 cells) DFMO	A MDL 72246	B MDL 72403	Total (A + B)
DFMO	0.1 mM	0.035			
	1.0 mM	0.33			
MDL 72246	0.1 mM		0.029		
	1.0 mM		0.32		
MDL 72403	0.01 mM		ND*	0.08	0.08
	0.1 mM		0.21	0.42	0.63
	1.0 mM		2.25	4.52	6.77

HTC cell cultures (1×10^5 cells/ml) were incubated in the presence of the compounds at the indicated concentrations; 6 hr later, perchloric acid extracts were prepared and the intracellular content of the compounds was determined as described in Materials and Methods.
* ND: Not detectable.

fresh medium [14] containing different concentrations of DFMO, (E)-2-(fluoromethyl)dehydroornithine or the methyl ester of (E)-2-(fluoromethyl)dehydroornithine. Polyamine concentrations and SAM-DC activity were determined 24 hr later. The three compounds decreased the putrescine and spermidine contents of the cells and increased SAM-DC activity in a dose-dependent manner (Fig. 1). The elevation of SAM-DC activity has been demonstrated to reflect the extent of spermidine deficiency [14, 18, 19]. (E)-2-(Fluoromethyl)dehydroornithine was no more effective than DFMO. Thus, both amino acids reduced the spermidine content of the cells and increased SAM-DC activity in a similar concentration-dependent manner. The methyl ester of (E)-2-(fluoromethyl)dehydroornithine required 10 times lower concentration to exert identical effects on these parameters. Thus, it appears that the ester was about 10 times more effective than its parent amino acid or DFMO.

Dose- and time-dependent inhibition of ODC in rat organs by (E)-2-(fluoromethyl)dehydroornithine and its prodrugs. Comparison with DFMO

Figure 2 compares the dose-dependent inhibition of ODC in ventral prostate 6 hr after administration of DFMO, or of (E)-2-(fluoromethyl)dehydroornithine or of the methyl and ethyl esters of (E)-2-(fluoromethyl)dehydroornithine. The two esters were equipotent and almost 10 times more effective than the parent amino acid. In spite of its higher potency *in vitro* [13] (E)-2-(fluoromethyl)de-

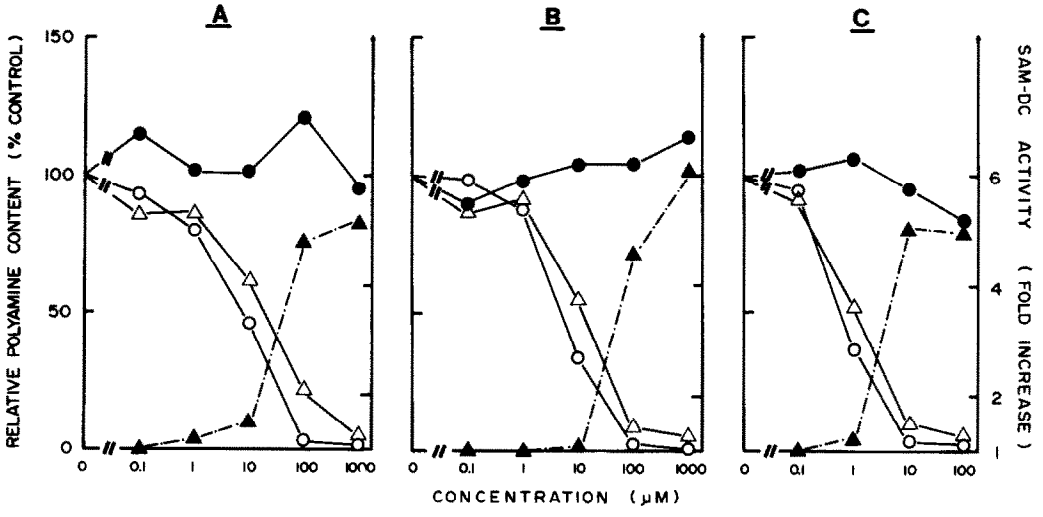


Fig. 1. Concentration-dependent effects of DFMO (A), (E)-2-(fluoromethyl)dehydroornithine (B), and (E)-2-(fluoromethyl)dehydroornithine methyl ester (C). High density cell cultures were centrifuged and the cell pellets were resuspended in fresh medium supplemented with 10% horse serum at a cell density of $1.0–1.2 \times 10^5$ cells/ml. The cultures were then incubated in the presence or absence of increasing concentrations of the compounds. SAM-DC activity and polyamine content were measured 24 hr after addition of the drugs. Results are the averages of two separate experiments and are expressed as increase relative to control SAM-DC activity (1.2 nmol $\times$ hr^{-1} $\times$ mg $protein^{-1}$) and as percentages of control values for putrescine (0.91 nmol/10^6 cells), spermidine (3.10 nmol/10^6 cells) and spermine (2.45 nmol/10^6 cells). SAM-DC activities (▲), putrescine (○), spermidine (△), spermine (●).

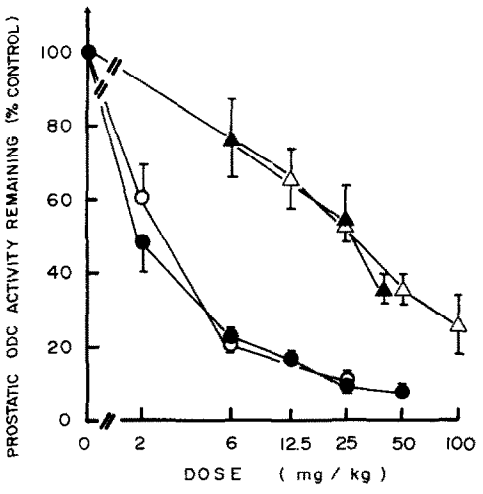


Fig. 2. Comparison of the effects of a single oral administration of DFMO (△), (E)-2-(fluoromethyl)dehydroornithine (▲), (E)-2-(fluoromethyl)dehydroornithine methyl ester (●), and (E)-2-(fluoromethyl)dehydroornithine ethyl ester (○) on ODC activity in ventral prostate. ODC activity was measured 6 hr after administration of the compounds to rats.

hydroornithine produced an inhibition of ODC similar to that caused by DFMO.

ODC activity was then determined in ventral prostate, thymus and testes in the rat after a single oral administration (by gavage) of 25 mg/kg of the various esters of (E)-2-(fluoromethyl)dehydroornithine, or of the parent amino acid. Results presented in Table 2 show that the various esters studied inhibited ODC much more effectively than the parent amino acid. The methyl- and the ethyl esters appeared to be the most effective compounds on a mg per kg basis. However, on a molar basis the increased effectiveness of these esters relative to the octyl and benzyl esters might not be significant, in particular with regard to the dose-response curve of ODC inhibition (Fig. 2).

It should be noted that the esters produced an ODC inhibition of particularly long duration at least in the ventral prostate and in the testes.

Effects of a single and repeated administration of the methyl ester of (E)-2-(fluoromethyl)dehydroornithine on polyamine metabolism in ventral prostate, thymus and testes

As shown in Fig. 3A, greater than 80% inhibition of prostatic ODC activity was observed between 3 and 24 hr after a single oral dose of 25 mg/kg of the methyl ester of (E)-2-(fluoromethyl)dehydroornithine; the maximum inhibition occurred at about 12 hr. Thereafter, enzyme activity in the ventral prostate increased slowly and returned to control values within 48 hr. An inverse pattern was observed for SAM-DC activity which was elevated about four-fold over control values between 24 and 36 hr and then returned to control values 48 hr after drug administration.

Repeated oral administration of 25 mg/kg of the methyl ester every 24 hr for eight consecutive days resulted in a constant 80% inhibition of prostatic ODC activity over the 24 hr following the last dose, as shown by Fig. 3B. SAM-DC activity remained elevated during the first 12 hr following the last administration, at levels about four-fold higher than control values. This activity is still three-fold over control values at 24 hr. Thereafter, SAM-DC activity declined to reach control values at 48 hr (Fig. 3B).

Table 2. Effect of a single dose of (E)-2-(fluoromethyl)dehydroornithine and its esters on ODC activity in ventral prostate, thymus and testis

	Dose (mg/kg)	Dose (μmol/kg)	Time after administration (hr)	ODC activity remaining (% control) Ventral prostate	Thymus	Testis
(E)-2-(fluoromethyl)-dehydroornithine (MDL 72246)	25	126	6	54 ± 10*	63 ± 6*	ND†
			12	92 ± 10	127 ± 10	89 ± 13
			24	92 ± 5	117 ± 17	111 ± 16
Methyl ester (MDL 72403)	25	100	6	10 ± 1*	38 ± 2*	33 ± 2*
			12	11 ± 1*	61 ± 7*	23 ± 2*
			24	21 ± 3*	78 ± 5	29 ± 3*
Ethyl ester (MDL 72430)	25	95	6	12 ± 1	31 ± 3*	16 ± 1*
			12	9 ± 1	43 ± 3*	20 ± 3*
			24	23 ± 1*	83 ± 9	22 ± 1*
Octyl ester (MDL 72493)	25	72	6	9 ± 2*	51 ± 4*	41 ± 4*
			12	16 ± 3*	71 ± 6*	44 ± 5*
			24	36 ± 4*	81 ± 1	34 ± 2*
Benzyl ester (MDL 72504)	25	77	6	13 ± 1*	48 ± 6*	39 ± 6*
			12	22 ± 3*	56 ± 6*	39 ± 2*
			24	39 ± 6*	127 ± 9	40 ± 2*

Each compound was given by gavage at time zero. At given intervals, animals were killed and enzyme activities were immediately measured. Each value is the mean ± S.E.M. of five animals. The significance of the differences between controls and treated animals was calculated by Student's *t*-test.

* $P < 0.05$.

† ND: Not determined.

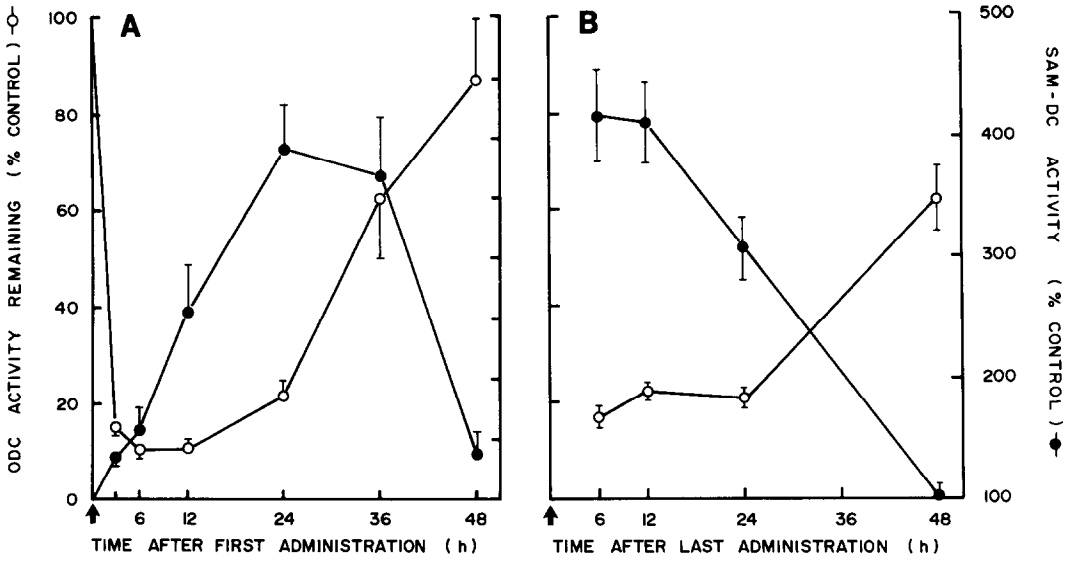


Fig. 3. Effect of a single or repeated administration of (E)-2-(fluoromethyl)dehydroornithine methyl ester on ODC and SAM-DC activities in ventral prostate. Rats were given (E)-2-(fluoromethyl)dehydroornithine methyl ester (25 mg/kg) once a day by gavage. At given intervals after a single administration (Chart A) or after the eighth daily administration (Chart B), the animals were killed and enzyme activities were measured.

Polyamine and decarboxylated S-adenosylmethionine concentrations and ODC activities were measured in ventral prostrate, thymus and testes of rats 24 hr after a single or after the last of eight repeated daily doses of 25 mg/kg of the methyl ester of (E)-2-(fluoromethyl)dehydroornithine. Results given in Table 3 point out differential effects of the compound on the three organs studied. In the ventral prostate, putrescine and spermidine concentrations were already decreased by about 50% 24 hr after a single dose of the compound. These decreases reached over 90% after the eighth daily administration whereas spermine concentration was not significantly affected at this time. Decarboxylated S-adenosylmethionine, which usually accumulates in cells and organs deprived of spermidine [5, 14, 19, 20], was increased by 40-fold and 200-fold in the ventral prostate after one and after the eighth administration of the methyl ester, respectively. In the testes, extents of ODC inhibition at days 1 and 8 were similar to those observed in the ventral prostate (Table 3). Reduction of the testis putrescine concentration at day 8 was also comparable to that seen in the prostate. However, in contrast to the prostate, the marked inhibition of the testis ODC and the profound decrease of putrescine concentration did not result in more than a 20% reduction of the spermidine concentration. As expected from the small decrease of spermidine, there was no accumulation of decarboxylated S-adenosylmethionine. In the thymus, the compound produced a lesser inhibition of ODC than in the two other organs studied and caused a moderate but significant reduction of putrescine and spermidine concentrations at day 8. Under these conditions, levels of decarboxylated S-adenosylmethionine were too low to be detected in this organ.

DISCUSSION

(E)-2-(Fluoromethyl)dehydroornithine is a new enzyme-activated irreversible inhibitor of ODC. *In vitro*, it is about 15 times more potent than DFMO [13]. However, in cultured cells and in animal tissues the two compounds prove to be equipotent as inhibitors of polyamine synthesis. At least in cells, this result most probably cannot be explained by a difference in intracellular concentrations of both inhibitors since they are present in equivalent amounts 6 hr after their addition to the culture medium (Table 1). It must be noted, however, that racemic mixtures are used and that our analytical method does not allow us to measure whether one of the isomers is preferentially taken up. We had demonstrated previously that only (−)-DFMO inactivates ODC *in vitro* [4]; the inactivation by (E)-2-(fluoromethyl)dehydroornithine is presumably also stereoselective. Furthermore, it cannot be excluded that the intracellular compartmentations of the two ODC inhibitors are different.

In order to improve the efficacy of (E)-2-(fluoromethyl)dehydroornithine, esters were synthesized as potential prodrugs (for a review, see [21]) of this new inhibitor. The use of ester derivatives is based on the ubiquitous distribution of non-specific esterases [22]. Our findings clearly demonstrate an absorption of the methyl ester better than that of the parent amino acid in HTC cells (Table 1). In addition absorption of the methyl ester was accompanied by a marked intracellular accumulation of the parent amino acid, about 10 times higher than that measured when (E)-2-(fluoromethyl)dehydroornithine was added to cells. This result convincingly demonstrates that the ester is hydrolyzed intracellularly to liberate the ODC-inhibitory amino acid. Comparison of the

Table 3. Effect of administration to rats of the methyl ester of (E)-2-(fluoromethyl)dehydroornithine (25 mg/kg given once a day by gavage) on ODC activity and concentration of polyamines and decarboxylated S-adenosylmethionine in ventral prostate, thymus and testes

	Ventral prostate			Thymus			Testes		
Day of treatment	0	1	8	0	1	8	0	1	8
ODC activity (nmol CO_2/hr/g)	321 ± 26	67 ± 9*	67 ± 6*	74 ± 4	58 ± 3	53 ± 4*	40 ± 3	12 ± 2*	8 ± 1*
Putrescine (nmol/g)	260 ± 30	130 ± 30*	<10*	145 ± 20	101 ± 14	51 ± 6*	21 ± 2	3 ± 1*	2 ± 1*
Spermidine (nmol/g)	8800 ± 700	3600 ± 400*	850 ± 90*	2710 ± 110	2580 ± 160	1960 ± 100*	220 ± 10	200 ± 10	170 ± 5*
Spermine (nmol/g)	3640 ± 270	4300 ± 400	4400 ± 400	740 ± 60	770 ± 20	890 ± 20	570 ± 50	510 ± 30	480 ± 10
Decarboxylated S-adenosylmethionine (nmol/g)	3 ± 1	130 ± 10*	580 ± 20*	<2	<2	<2	3 ± 1	2 ± 1	3 ± 1

The various parameters reported in this table were measured 24 hr after the first and the eighth administration of the drug. Each value is the mean ± S.E.M. of five animals. The significance of the differences between controls (day 0) and treated animals was calculated by Student's *t*-test.
* $P < 0.01$.

concentrations of the methyl ester and of the parent amino acid required to produce similar decreases of putrescine and spermidine in HTC cells indicates that the methyl ester used at 10 times lower concentration is as effective as its parent amino acid or as DFMO to inhibit polyamine biosynthesis.

In the same way, the various esters used at 10 times lower dose inhibited ODC *in vivo* to the same extent as did (E)-2-(fluoromethyl)dehydroornithine. By analogy with what has been observed in HTC cells, increased effectiveness of the esters most probably results from their better organ absorption and their efficient hydrolysis *in situ* into the active ODC inhibitor.

DFMO exerts its maximum effect on ODC 6 hr after its administration to animals and repeated administration every 12 hr fails to establish a constant inhibition of the enzyme [23]. As already discussed [23], duration of ODC inhibition is dependent on the turn-over rate of ODC in a particular organ and on the pharmacokinetics of the drug. A single administration of the methyl ester of (E)-2-(fluoromethyl)dehydroornithine produces in ventral prostate and testes an inhibition of ODC which remains nearly constant between 3 and 24 hr. This long duration of action is confirmed by the constant inhibition observed over a 24 hr period following eight daily doses. The daily administration schedule of the methyl ester results in an effective reduction of putrescine and spermidine concentrations in the ventral prostate and, to a lesser extent, in the thymus and the testes. No simple explanation can be furnished at present to account for the fact that the profound reduction of ODC activity and of putrescine concentration in the testes is not accompanied by a more substantial reduction of the spermidine concentration.

Based on their high potency and their long duration of action we consider the esters of (E)-2-(fluoromethyl)dehydroornithine to constitute a new class of inhibitors of polyamine biosynthesis, some of which may prove to be superior to DFMO in envisioned therapeutic applications. Preliminary data indicate that the methyl ester of (E)-2-(fluoromethyl)dehydroornithine and DFMO appear to have biological profiles [12, 25] different from that of the putrescine analogue (2R,5R)-6-heptyne-2,5-diamine, another new very potent ODC inhibitor [19, 24, 26].

Acknowledgements—The authors wish to thank Dr J. Wagner for the determination of polyamines and decarboxylated-S-adenosylmethionine in animal tissues, Dr J. Grove for the determination of (E)-2-(fluoromethyl)-dehydroornithine in HTC cells and J. Barth, M. C. Chanal, N. Claverie and M. Siat for valuable technical assistance.

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