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INHIBITION OF HISTAMINE SYNTHESIS *IN VITRO* AND *IN VIVO* BY S-α-FLUOROMETHYLHISTIDINE

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Abstract—(*S*)-α-Fluoromethylhistidine (α-FMH) is a K_{cat} or "suicide-substrate" inhibitor of partially purified mammalian histidine decarboxylase; i.e. the agent is converted enzymatically to a more active form which effects a time-dependent, irreversible inhibition. Incubation of a α-FMH[4-^{3}H] with enzyme and pyridoxal phosphate resulted in an apparently irreversible labeling of protein, with no demonstratable formation of free-amine product, suggesting a very low to non-existent turnover ratio. α-FMH was accumulated in isolated mastocytoma cells and effected a time-dependent inhibition of the conversion histidine[^{3}H] → histamine[^{3}H], the latter product having a markedly different distribution between cells and medium than the pre-existing histamine pool. Inhibition of whole-body histidine decarboxylase activity, as specifically measured by α-methylhistidine-^{14}COOH → $^{14}CO_2$, was also time dependent. Concomitant reduction in histamine levels was seen only in the rapidly turning-over pools of stomach and brain. However, over the course of 13 weeks of chronic treatment, depletion of the relatively inert mast-cell histamine pool(s) was seen as well.

α-Fluoromethylhistidine (α-FMH) is one of a novel class of α-halomethyl amino acids designed to be irreversible inhibitors of the enzymatic decarboxylation of the specific natural amino acids of which they are analogs [1]. Mechanistically, such agents are termed K_{cat} or "suicide-substrate" inhibitors, and are characterized by time dependence and a high degree of selectivity [2].

The synthesis and the kinetic properties of five such agents and some of the corresponding amines have been reported [1]. We present here further results with α-FMH *in vitro* and *in vivo*.

MATERIALS AND METHODS

Racemic α-FMH was prepared [1] and resolved as described elsewhere [3]. The absolute stereochemical configuration of the (*S*) antimer was assigned on the basis of single crystal X-ray diffraction (J. Hirschfield and K. Hoogsteen, to be published). (*S*)-α-FMH[4-^{3}H] (48.3 mCi/mg) was synthesized by J. Kollonitsch, G. A. Doldouras, A. Rosegay and H. T. Meriwether (to be published), and racemic α-methylhistidine-^{14}COOH (50.5 μCi/mg) by R. L. Ellsworth. L-Histidine[*carboxyl*-^{14}C] (NEC-493), L-histidine [3-^{3}H] (NET-194) and *S*-adenosylmethionine[*methyl*-^{14}C] (SAM[*me*-^{14}c]) (NEC-363) were obtained from New England Nuclear. Sprague–Dawley rats were obtained from Charles River Laboratories, and CXB6 and W/W^v mice from Jackson Laboratories.

Histidine decarboxylase (HD) was partially purified from fetal rat liver as per Hakanson [4], and the activity and inhibition thereof were based upon measurement of the rate of recovery in CO_2 of the label from L-histidine-^{14}COOH [4]. Histidine decarboxylase activity *in vivo* was estimated directly and indirectly: directly by rate of recovery in expired CO_2 of the label from an i.v. bolus of α-methylhistidine-^{14}COOH (see Results), or by rate of conversion of L-histidine[^{3}H] → histamine[^{3}H]; indirectly by measurement of histamine levels in tissues. These were determined by the enzyme/isotopic method as modified by Beaven [5], employing SAM[*Me*-^{14}C] and histamine-*N*-methyltransferase prepared from guinea pig brain.

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RESULTS

In vitro. Racemic α-fluoromethylhistidine at 0.1 mM had no effect upon DOPA-decarboxylase (hog kidney), glutamate decarboxylase (rat liver), or upon other enzymes mediating the catabolism of histidine or of histamine, viz. histidase, diamine oxidase (hog kidney) and histamine-*N*-methyltransferase (guinea pig brain).

With partially purified fetal rat liver histidine decarboxylase, only the (*S*)-enantiomer of α-fluoromethylhistidine (hereafter designated α-FMH) was active. Inhibition was enhanced with time, and further so by inclusion of pyridoxal phosphate in the preincubation mixture (Fig. 1). The latter effect was in marked contrast to that of added pyridoxal phosphate upon HD inhibition by α-hydrazinohistidine and NSD-1055, taken as evidence that these agents act primarily via direct interaction with the cofactor [6]. Respective I_{50} concentrations of α-FMH were 8×10^{-6} M with no preincubation, 2.5×10^{-6} M after 30 min preincubation of enzyme and inhibitor, and 8×10^{-7} M when enzyme, inhibitor and pyridoxal phosphate were preincubated, the last effect suggesting the formation of a ternary complex among enzyme, inhibitor and co-factor analogous to that leading to irreversible inhibition of DOPA-decarboxylase by α-fluoromethyl DOPA[7].

To demonstrate that covalent attachment of inhibitor to enzyme occurs, enzyme was incubated with

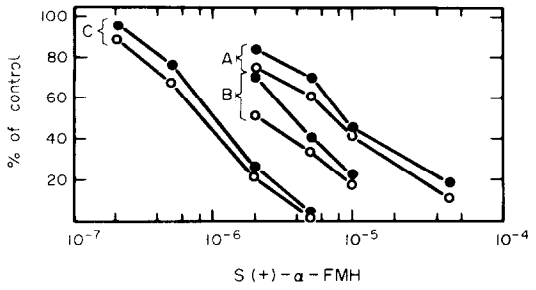

Fig. 1. Inhibition of histidine decarboxylase by *S*-(+)-α-fluoromethylhistidine alone (○) and in the presence of its *R*(-) enantiomer (●). Reaction mixture consisted of partially purified fetal rat liver enzyme, ~1.0 mg; L-histidine-^{14}COOH, 2×10^{-4} M; pyridoxal phosphate, 5 μg; inhibitor(s) at concentrations indicated in a total volume of 1.0 ml of 0.1 M phosphate buffer, pH 7.0, at 37°. Condition A, reaction started by addition of enzyme; condition B, enzyme and inhibitor preincubated for 30 min prior to addition of co-factor and substrate; condition C, enzyme, inhibitor and pyridoxal phosphate preincubated for 30 min prior to addition of substrate.

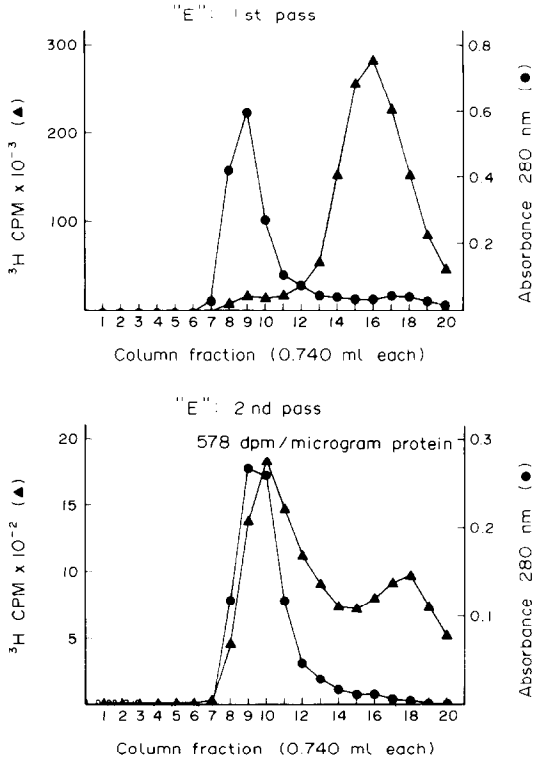

Fig. 2. Covalent product of α-FMH for ^{3}H and histidine decarboxylase. An aliquot of reaction mixture D (see Table 1) was applied to a 1.3 × 17 cm column of Sephadex G-25 and developed with 0.1 M phosphate buffer, pH 7.4, at 1.0 ml/min. Absorbance was measured at 280 nm; then 200 μl of each fraction was counted. Specific activity refers to weighted mean for fraction numbers 8–10, second pass.

α-FMA[4-^{3}H], dialyzed, and chromatographed on Sephadex G-25 (Table 1 and Fig. 2). The fractions comprising the minor radioactive peak under the protein absorption band after a single pass were combined and rechromatographed, resulting in superimposition of the protein and ^{3}H peaks, and an average specific activity of 578 dpm/μg protein, i.e. 580 pmoles/mg protein. An additional aliquot of the original reaction mixture was boiled and then reacted with SAM[*me*-^{14}C] and histamine-*N*-methyltransferase (HNMT) to measure any free α-fluormethylhistamine which might have been liberated in the course of reaction of α-FMH with the enzyme. In the absence of other substrates for HNMT, the ratio of ^{3}H : ^{14}C in apparent amine product(s) should be predictable from the respective activities of α-FMH[^{3}H] and SAM[^{14}C]; in the present instance,

Table 1. Demonstration of lack of free amine product for reaction of α-FMH with histidine decarboxylase*

	Reaction I		Reaction II			Total dpm per sample		Amine products (pmoles)	
Sample No.	α-FMH[^{3}H] (μg)	HD (ml)	SAM[^{14}C] (μg)	HNMT (ml)	α-FM histamine (pmoles)	^{14}C	^{3}H	by ^{14}C	by ^{3}H
STD 1			1.3	0.1	0	25			
STD 2			1.3	0.1	70	2825		66.3	
STD 3			1.3	0.1	140	6345		151.3	
STD 4			1.3	0.1	210	8520		203.7	
A	0	0.1	1.3	0.1		1485		34.0	
A′	0	0.3	1.3	0.1		4715		112	
B	1.0	0	1.3	0.1			2530		(0)
C	1.0	0.1	1.3	0.1		86	2725	0.17	0.2
D	1.0	0.3	1.3	0.1		1195	2550	27.0	0.02
E	1.0	0.5	1.3	0.1		2550	2575	59.7	0.04

* Reaction I consisting of labeled α-FMH (9.9×10^5 dpm/nmole) and partially purified histidine decarboxylase at indicated concentrations was incubated for 30 min at 37°; a 50% aliquot was boiled and then treated with *S*-adenosylmethionine[*methyl*-^{14}C] (7.7×10^4 dpm/nmole) and histamine-*N*-methyltransferase (Reaction II) under conditions which convert α-fluoromethylhistamine (α-FM-histamine) to *N*-methyl-α-fluoromethylhistamine [an average of 54% (STDs 1–4)]. The remaining material from Reaction I was subjected to successive fractionation on Sephadex G-25 (Fig. 2).

Table 2. Effect of α-FMH upon histamine synthesis in CXB6 mastocytoma cells*

Sample no.	[α-FMH]	Preincubation (min)	Incubation (min)	Total histamine (ng)	Total histamine C/M†	Histamine [^{3}H] dpm/ng	Histamine [^{3}H] C/M	Histamine [^{3}H] % Con‡
1	0		30	141	90	416	3.5	(100)
			120	154	74	740	3.5	(100)
2	1×10^{-4}	0	30	147	77	24.4	1.5	5.9
			120	170	70	32.9	3.4	2.6
3	1×10^{-5}	0	30	168	88	80.3	15.4	19.3
			120	138	50	86.2	7.9	1.8
4	1×10^{-5}	60	30	146	55	21.9	2.0	5.3
			120	149	36	28.3	4.9	1.9

* CXB6 mastocytoma were harvested from four CXBG mice at 24 days post-transplant, pooled, and digested with collagenase to yield a total of ~80 μl of packed cells. These were suspended at 1% (v/v) in 1.0-ml aliquots of Tyrode's buffer containing 7.14×10^6 dpm of L-histidine[^{3}H], and α-FMH at concentrations indicated. In Sample no. 4, the otherwise complete reaction mixture was preincubated for 60 min at 37° prior to addition of labeled histidine. At the times indicated, cells and medium were separated, immersed in boiling water for 5 min, and then incubated with SAM[*Me*-^{14}C] and histamine-*N*-methyltransferase as per Materials and Methods for simultaneous determination of total histamine (^{14}C-label) and newly synthesized histamine (^{3}H-label).

† C/M denotes the cell : medium concentration gradient based upon a nominal cell density of 1% (v/v).

‡ Con refers to percent inhibition of reaction for the respective increments 0–30 and 30–120 min.

12.9. That the observed ratio was highly variable, and an inverse function of enzyme concentration, while total extractable ^{3}H was more or less constant over the range of enzyme concentrations (0 to 0.5 ml/sample), suggests that most if not all of the ^{14}C-labeled product represented material other than α-fluoromethylhistamine and, further, that the extractable ^{3}H, which corresponded to less than 0.05% of substrate, represented unchanged α-FMH. That is, little or no α-fluoromethylhistamine was liberated in the course of the apparently irreversible (K_{cat}) reaction of α-FMH with the enzyme.

Histamine synthesis in isolated mast cells. The murine mastocytoma, CXB6, was selected as a model for examining inhibitor effects upon histamine synthesis and subsequent disposition in a closed system (Table 2). In untreated cells, label from a pulse of high specific activity L-histidine[^{3}H] appeared in histamine at a decreasing rate through 2 hr. α-FMH added simultaneously with the labelled substrate effected a greater inhibition over the interval 30–120 min than with respect to the first 30 min. Preincubation of enzyme and α-FMH prior to introduction of substrate confirmed that, as with soluble enzyme, inhibition was time dependent. By separate analysis of cells and medium, a striking difference was noted in the cell : medium concentration gradient of the newly-formed [^{3}H]histamine relative to that of the pre-existing endogenous pool, indicating that only *ca.* 3.5% of endogenous histamine was incorporated into the storage pool. That the observed decrease in histamine[^{3}H] was a function of enzyme inhibition, and not of possible effects upon histidine[^{3}H] uptake, was apparent from the lack of effect by α-FMH upon the cell : medium gradient for total ^{3}H. A similar lack of effect upon histamine uptake has been demonstrated in human white cells (H. Zweerink, unpublished observations).

Histidine decarboxylase in vivo*; direct measurement.* As an estimate of whole-body histidine decarboxylase activity, recovery of label from L-histidine-^{14}COOH is expired CO_2 is not a valid index of direct decarboxylation (as is, for example, L-DOPA-^{14}COOH for aromatic amino acid decarboxylase), since the greater part of CO_2 labelling reflects pathways initiated by reductive deamination or by transamination. The blocking of both these reactions by substitution on the α-carbon should, in theory, provide a substrate from which recovery of $^{14}CO_2$ will be a specific index of direct decarboxylation to histamine.

Accordingly, α-methylhistidine was tested and

Table 3. Decarboxylation of α-methylhistidine in mice as a function of dose

Dose (mg/kg, i.v.) DL-α-Methyl histidine-^{14}COOH	L-α-Methyl histidine	Total L-isomer	α-Hydrazino histidine	$^{14}CO_2$ (%/hr)	Decarboxylation (μg/kg/hr)
1.3	0	0.65		2.02	13.7
1.3	1.0	1.65		0.94	15.5
1.3	3.14	3.79		0.46	17.4
1.3	1.0	1.65	0.5	0.42	7.0
1.3	1.0	1.65	1.0	0.17	2.8

Table 4. Duration of histidine decarboxylase inhibition by racemic α-fluoromethylhistidine*

Time (hr)	% of Control
0	65.7
0.33	22.9
1	11.4
3	22.8
6	22.7
24	54.8

* α-Fluoromethylhistidine (0.83 mg/kg, i.p.) was given at indicated intervals before injection of α-methylhistidine[1-^{14}C]. All experimental values are relative to mean $^{14}CO_2$ recovery for all untreated mice tested concurrently.

found to be a substrate for histidine decarboxylase with an affinity ($K_m = 6.7 \times 10^{-5}$ M) about twice that of L-histidine, of which it is a competitive inhibitor ($K_i = 1.22 \times 10^{-4}$ M). *In vivo*, recovery of carboxyl label from α-methylhistidine in CO_2 was dose dependent and inhibited by L-α-hydrazinohistidine (Table 3), a known inhibitor of histidine decarboxylase *in vivo* [6]. Thus, by all available criteria, decarboxylation of α-methylhistidine-^{14}COOH appears to be a valid index of histidine decarboxylase activity *in vivo*, and one which affords the unique advantage of repeated measurements in the same animal, whether at intervals after a given treatment or in crossover designs.

In mice, the ED_{50} for α-FMH with respect to inhibition of α-methylhistidine-^{14}COOH decarboxylation was *ca.* 0.5 mg/kg, i.p., the log-dose vs inhibition curve being quite flat (Fig. 3). Duration of inhibition was examined by injecting labeled substrate simultaneous with, and at increasing intervals after, treatment with α-FMH (Table 4). Maximal inhibition, to 11% of control, was at 1 hr, with significant inhibition still evident at 24 hr. The similarity between this time-course of inhibition of apparent HD inhibition at the whole-body level, and that for individual tissues measured *ex vivo* [8], lends

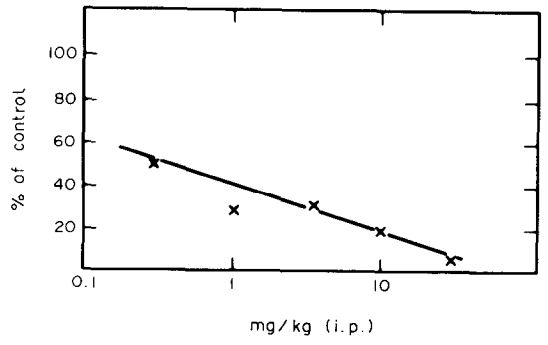

Fig. 3. Inhibition of apparent whole-body histidine decarboxylase in mice by α-FMH. Treatment was at 20 min prior to i.v. injection of α-methylhistidine-^{14}COOH at 1.0 mg (100,000 dpm total).

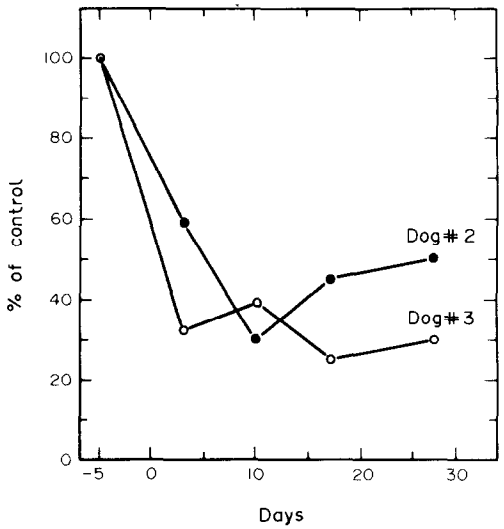

Fig. 4. Inhibition of apparent whole-body histidine decarboxylase in dogs by α-FMH, at 5 mg/kg b.i.d. orally. α-Methylhistidine-^{14}COOH was given intravenously at 0.05 mg/kg (total dpm = 1×10^{-6}).

additional credibility to the α-methylhistidine-^{14}COOH test protocol.

The effects of subacute treatment with α-FMH upon apparent whole-body HD in dogs is summarized in Fig. 4. In each of two animals receiving α-FMH at 10 mg/kg-day for 21 days, α-methylhistidine-^{14}COOH decarboxylation was depressed to <50% of control, and remained so on day 4 after the last treatment.

As a test of specificity of the decarboxylase inhibition by α-FMH *in vivo*, $^{14}CO_2$ was collected at intervals after an i.v. bolus of DOPA[1-^{14}C]. Respective rate constants for recovery of $^{14}CO_2$ were 0.0135 and 0.0129 min^{-1} for controls and animals pretreated with α-FMH at 100 mg/kg.

Histidine decarboxylase in vivo*; indirect method.* As an indirect index of HD inhibition, tissue histamine levels were examined after single and multiple doses of α-FMH. In normal rats and mice, acute reductions in histamine concentration could be demonstrated only in stomach and brain. In rat stomach, reduction in histamine was maximal at 6 hr post-treatment and gradually rose thereafter (Table 5). At doses 7-fold higher, two other HD inhibitors, bromcresine and McNeil A-1293, failed to achieve significant reductions at any time through 24 hr (Table 5).

In pregnant rats, α-FMH admixed with diet from day 11 of gestation onwards effected dose-related reductions in gastric histamine and in the abnormally high levels of urinary histamine characteristic of pregnancy [9]. There were also large, concomitant reductions in HD activity and histamine content of the fetuses (Table 6). In the animals killed incrementally, and in paired ones allowed to proceed to term, there were no discernible changes in litter size or in fetal weights.

Table 7 summarizes the effects of α-FMH in normal mice and in the mast-cell deficient strain

Table 5. Comparison of effects of histidine decarboxylase inhibitors upon gastric histamine in normal rats*

	Histamine (μg/g tissue)					
Hours post = treatment	N	α-FMH 14.4 mg/kg, i.p.	N	Bromcresine 100 mg/kg, i.p.	N	McNeil A-1293 100 mg/kg, i.p.
0 (control	12	71.8 ± 13.7	5	57.2 ± 15.5	5	58.2 ± 13.9
2	6	39.2 ± 15.4†	5	62.3 ± 11.4	5	48.5 ± 11.9
4	6	37.4 ± 11.2†	5	57.9 ± 15.4	5	60.3 ± 29.4
6	6	28.9 ± 10.6†	5	64.8 ± 24.7	5	68.4 ± 21.1
8	8	43.3 ± 17.9†	5	101.5 ± 26.1	5	61.3 ± 17.6
16	6	57.1 ± 15.5				
24	6	65.2 ± 13.2	5	65.6 ± 10.5	5	71.1 ± 13.5

* Male SD rats (150–160 g) were allowed free access to food and water until time of dosing and then deprived throughout the indicated intervals until sacrifice.
† $P < 0.05$.

Table 6. Effects of α-FMH upon histamine synthesis in pregnant rat and fetus*

		Maternal histamine		Fetal liver	
Gestation day	Rx (mg/kg/day)	Stomach (μg/tissue)	Urine (μg/6 hr)	HD activity (μg HA/g tissue/hr)	Histamine (μg/tissue)
14	0	44.5 ± 4.4	6.9 ± 1.1	21.6 ± 8.2	52.3 ± 22.0
	10	15.8 ± 2.7	8.4 ± 4.7	15.2 ± 4.3	38.0 ± 11.8
	50	7.3 ± 1.6	6.2 ± 4.2	6.8 ± 0.8	20.2 ± 0.9
18	0	32.2 ± 1.9	33.7 ± 16.1	15.0 ± 5.5	34.3 ± 13.2
	10	11.7 ± 4.2	34.5 ± 32.5	11.2 ± 3.3	30.2 ± 11.8
	50	6.6 ± 0.7	20.9 ± 19.2	5.0 ± 0.8	14.1 ± 0.7
22	0	34.3 ± 17.8	37.5 ± 30.0	19.2 ± 6.4	48.4 ± 15.7
	10	18.6 ± 2.5	79.9 ± 53.6	4.2 ± 2.5	12.3 ± 9.3
	50	13.5 ± 2.1	7.5 ± 5.7	1.6 ± 0.3	4.3 ± 2.9

* Drug was admixed with powdered diet at a concentration calculated to deliver the daily levels indicated. The animals had constant access to the diet commencing with day 11 of gestation. All values are means ± S.D. for two animals or for pooled fetuses from each of two animals. HA = histamine.

Table 7. Effects of acute and subacute treatment with α-FMH upon tissue hisuamine in normal and mast-cell deficient mice*

				Histamine (μg/g tissue)		
Expt. no.	Mouse	N	α-FMH	Stomach	Ear	Tongue
I	+/+	5	None	13.0 ± 2.2	60.8 ± 4.8	16.7 ± 2.2
	+/+	5	14.4 mg/kg, i.p.	10.9 ± 2.3	65.6 ± 12.5	17.2 ± 4.1
	W/W^v	5	None	10.3 ± 3.2	0.48 ± 0.17	0.28 ± 0.17
	W/W^v	5	14.4 mg/kg, i.p.	6.5 ± 1.2	0.31 ± 0.06	0.18 ± 0.02
II	W/W^v	4	None	10.1 ± 2.7	0.77 ± 0.01	0.30 ± 0.02
	W/W^v	4	33 mg/kg/day†	5.0 ± 1.1	0.44 ± 0.14	0.16 ± 0.03

† Drug was admixed with diet at a level calculated to deliver daily dosage indicated for 31 days.
* Values are means ± S.D.

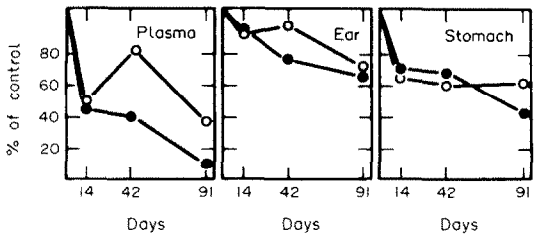

Fig. 5. Effects of chronic treatment with α-fluoromethylhistidine at 10 (○) and 50 (●) mg/kg daily upon histamine levels in plasma, ear and stomach.

W/W^v. In two representative mast-cell tissues, ear and tongue, α-FMH effected no changes in histamine levels in the normal animals. In the mast-cell deficient mutant, however, pretreatment concentrations of histamine in the ear and tongue, which were only 0.8 and 1.6% of those in normals, were depressed by a single dose of α-FMH, but showed no further reduction upon subacute treatment.

In Fig. 5 are summarized the effects of chronic α-FMH treatment in rats upon the histamine content of one mast-cell tissue (ear), and those of plasma and stomach. For stomach, chronic treatment did not, on average, reduce histamine to a greater extent than did a single treatment. In ear, on the other hand, depletion of histamine was clearly time dependent. Qualitatively, the time-course for plasma histamine appeared to share characteristics with both of the others, achieving significant reduction through the first sampling interval and, in the case of the higher dose, a subsequent decline. For larger numbers of animals of both sexes examined at 13 weeks, histamine levels for these and other tissues were decreased (Table 8). Only in stomachs of both sexes, and in brain and diaphragm of female rats, was histamine depletion clearly dose-related. In brain there was an absolute sex difference, no significant changes occurring in males at any dosage level.

DISCUSSION

A K_{cat} or "suicide-substrate" inhibitor is one possessing a latentiated grouping which is activated only by the target enzyme and, at its active site, leading to a covalent adduct between enzyme and inhibitor product (or among enzyme, co-factor, and inhibitor product) [2]. In addition to this irreversible aspect, K_{cat} inhibition is characterized by time-dependency and a high degree of selectivity. The present results with partially purified enzyme, with isolated mast cells, and *in vivo* are consistent with such a definition for α-FMH.

Irreversibility was evidenced by the inability of dialysis to restore activity and by the persistent association of α-FMH[^{3}H] label with protein upon repeated Sephadex chromatography. Similar results have been reported for more stringently purified histidine decarboxylase separated from unbound inhibitor by chromatography and by immuno-

Table 8. Effects of 13 weeks treatment with α-FMH upon tissue histamine levels in male and female rats*

Tissue	Dose (mg/kg/day)	Histamine (μg/g tissue) Female	Male
Plasma	0	0.032 ± 0.025	0.015 ± 0.001
	10	0.012 ± 0.005	0.016 ± 0.007
	50	0.003 ± 0.001†	0.003 ± 0.002†
	250	0.003 ± 0.002‡	0.014 ± 0.015
Brain	0	0.104 ± 0.035	0.058 ± 0.029
	10	0.073 ± 0.023	0.025 ± 0.032
	50	0.038 ± 0.024‡	0.040 ± 0.060
	250	0.002 ± 0.004‡	0.048 ± 0.052
Diaphragm	0	14.9 ± 2.4	15.7 ± 4.3
	10	15.1 ± 2.5	6.8 ± 1.5
	50	10.1 ± 5.6	4.5 ± 0.73‡
	250	4.3 ± 1.1	8.3 ± 8.5
Ear	0	16.0 ± 3.3	14.5 ± 0.91
	10	11.6 ± 3.2	10.9 ± 2.6‡
	50	10.8 ± 3.0†	11.8 ± 5.0
	250	7.0 ± 1.3‡	6.4 ± 2.5‡
Stomach	0	37.7 ± 6.0	62.4 ± 25.4
	10	23.8 ± 6.2‡	34.5 ± 4.8
	50	15.9 ± 5.0‡	28.0 ± 2.2‡
	250	10.9 ± 6.3‡	19.8 ± 8.5‡

* All values are mean ± S.D. for four animals of each sex, fasted for 24 hr prior to sacrifice.
† $P < 0.1$.
‡ $P < 0.05$.

precipitation [10], and analogous ones for aromatic amino acid decarboxylase incubation with α-fluoromethyl DOPA[6]. In the latter instance, both ring-labeled (^3H) and carboxyl-labeled inhibitors were available, enabling direct demonstration that inactivation involved loss of the carboxyl group and almost stoichiometric formation of ^3H-labeled enzyme–inhibitor covalent adduct, i.e. little or no free amine product was liberated. The fact that both enantiomers of α-fluoromethylhistamine, the decarboxylation product of α-FMH, are substrates for histamine *N*-methyltransferase provided the basis for their direct measurement in the α-FMH[^3H]/histidine decarboxylase reaction mixture. The virtual absence of labeled free amine indicates a highly efficient inactivation process, involving no turnover of the inhibitor–substrate to product other than the adduct with enzyme, identical with that demonstrated for α-fluoromethyl DOPA. Further evidence for irreversible adduct formation between α-FMH and enzyme is seen in autoradiograms of fresh stomach sections incubated with α-FMH[^3H] [11].

The selectivity of α-FMH is emphasized by its inability at 1 mM to inhibit other amino acid decarboxylases *in vitro*. *In vivo* it has no effect, as does α-hydrazinohistidine, for example, upon aromatic amino acid decarboxylase, as measured by DOPA[1-^{14}C] → $^{14}CO_2$. The histamine-catabolizing enzymes, diamine oxidase and histamine-*N*-methyltransferase, were not affected by α-FMH at a concentration >100-fold in excess of its I_{50} for histamine formation. A final and compelling demonstration of selectivity is its absolute stereospecificity with respect to the one enzyme which it does inhibit. Of the other known chiral inhibitors of histidine decarboxylase, the enantiomers of α-hydroazinohistidine differ only 7-fold in inhibitory activity [7], and α-methylhistidine has not been resolved.

The time-dependent nature of HD inhibition by α-FMH was evident in each of the several systems examined, both *in vitro* and *in vivo*. The interval required for maximal inhibition *in vivo*, as measured for apparent whole-body HD activity (present $^{14}CO_2$ data), or as measured in individual tissues *ex vivo* [8], is probably largely a reflection of the kinetics of the enzyme inhibition process *per se*, rather than of disposition kinetics of the drug, whose absorption, distribution and elimination rates are quite high (unpublished observations). On the other hand, the onset and duration of the desired effect (depletion of tissue histamine) are governed by other kinetic considerations viz. the turnover rates for histamine pools and the rate of resynthesis of histidine decarboxylase. Thus, the maximum decrement in HD activity in any given tissue precedes that of the corresponding histamine pool, and decrements in both parameters persist through at least 24 hr, whereas the average $T_{1/2}$ for circulating inhibitor in three species is only 2 hr[11].

While there is no consensus regarding exact values for the half-lives of various histamine pools, it is clear that there are two kinetically distinct types [12]. Mast-cell storage pools are relatively inert [13] and would be expected to deplete quite slowly, even under conditions of sustained inhibition of new synthesis. The "nascent" pools composing the major fraction of total histamine of gastric and intestinal mucosa and brain have putative regulatory functions, e.g. in gastric secretion [14] and neurotransmission [15], and turn over rapidly [16, 17]. A substantial inhibition of enzyme involved in the maintenance of these pools would be expected to result in the rapid depletion observed.

The respective effects of acute and chronic treatment with α-FMH upon histamine concentrations in the various tissues examined are consistent with this traditional distinction between mast cell and non-mast cell tissues, based upon their turnover kinetics.

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