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# α-FLUOROMETHYL HISTIDINE

## INHIBITION OF HISTIDINE DECARBOXYLASE IN PYLORUS LIGATED RAT

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**Abstract**—α-Fluoromethyl histidine is an irreversible inhibitor of histidine decarboxylase. The injection of a single dose to pyloric-ligated rats inhibits gastric mucosal histidine decarboxylase in a dose-dependent manner but does not modify histamine content and gastric acid secretion even at the highest dose used. Administration of cimetidine increases histidine decarboxylase activity, decreases histamine level in gastric mucosa and inhibits gastric acid secretion. The co-administration of α-fluoromethyl histidine blocks the augmentation in enzyme activity, maintains lowered histamine level and prolongs the antisecretory action of cimetidine.

Studies with $H_2$ receptor antagonists leave little doubt that histamine has an important role in gastric acid secretion [1] although acetylcholine and gastrin are equally known to be involved (for reviews, see ref. [2]). The question of whether it is the stored or the newly formed histamine which is important for regulation of gastric secretion may be answered by blocking *de novo* synthesis of histamine. Most histamine found in mammalian tissues arises from decarboxylation of histidine by a specific histidine decarboxylase (EC 4.1.1.22 [3]). Although potent inhibitors of the specific decarboxylase are available [4], until recently none were unequivocally adequate in terms of both specificity and *in vivo* effectiveness. Some years ago an irreversible histidine decarboxylase inhibitor, α-chloromethyl histidine, was developed in this centre [5]. However, this compound was relatively unstable at neutral and basic pH. More recently, α-fluoromethyl histidine has been reported to be a potent suicide inhibitor of histidine decarboxylase found in foetal rat liver [6] and mouse brain [7]. Here we report the effects of this compound on histamine biosynthesis in gastric mucosa and on gastric acid secretion in pylorus ligated rat.

### METHODS

*Materials.* α-Fluoromethyl histidine was synthesized in our laboratory. Histamine, histidine and *S*-adenosyl methionine were purchased from Merck, Darmstadt, West Germany. Cimetidine was obtained from SK & F, U.K. L-[1-$^{14}$C]Histidine (50 mCi/mmole) came from New England Nuclear Corporation, Boston, U.S.A. *S*-Adenosyl-L [methyl-$^{3}$H]methionine (80 Ci/mmole) was supplied by the Radiochemical Center, Amersham, U.K.

*Purification of histidine decarboxylase from rat gastric mucosa.* The enzyme was purified from frozen mucosa by a described procedure [8], through ammonium sulphate fractionation, chromatography on DEAE-Sephadex and gel filtration on Sephadex G-200.

The specific activity of the final enzyme preparation was 40 nmoles of $CO_2$/hr·mg protein. The purified histidine decarboxylase preparation was free of aromatic amino acid decarboxylase activity.

*Animal and tissue preparations.* Male Wistar rats, body weight 100–125 g, were used. For the *in vivo* studies, rats were fasted for 48 hr before the expt and the pylorus was ligated under light ether anaesthesia according to the method of Shay *et al.* [9] 90 min before decapitation. The pyloric ligation has two advantages: it allows measurement of gastric acid secretion and produces a slight induction of histidine decarboxylase activity in the gastric mucosa.

For determination of gastric acid secretion, the stomach was removed, and the contents and saline washings collected and titrated to pH 7 with $2 \times 10^{-2}$ N NaOH.

Gastric mucosa was prepared as follows: the stomach was opened along the minor curvature, the gastric mucosa was scraped off with a scalpel and homogenized in five vols. of phosphate buffer 0.1 M, pH 7, containing $10^{-5}$ M pyridoxal phosphate. The crude homogenates were used for determination of histidine decarboxylase activity. The supernatants obtained after centrifugation were used for measuring histamine.

*Determination of histamine content.* The method used was a modification of the enzymatic isotopic assay described by Taylor and Snyder [10].

Supernatant (20 μl) from the tissue extract was incubated in a tube containing 30 μl of the following reagent mixture: 10 μg of histamine-*N*-methyl transferase prepared as described [11], 0.5 μCi of *S*-adenosyl-L-[methyl-$^{3}$H]methionine (final concentration $2.5 \times 10^{-5}$ M) and 0.1 M phosphate buffer, pH 7.9.

After 1 hr incubation at 37°, 100 μl of 1 N NaOH, saturated with NaCl was added and the ethyl histamine formed was extracted into 1 ml chloroform. The chloroform layer was washed with 100 μl 0.1 N NaOH saturated with NaCl. Chloroform was evap-

orated in an air-current and, subsequently, the residue was re-dissolved in a scintillation cocktail and radioactivity measured.

*Determination of histidine decarboxylase activities in gastric mucosa.* The method is based on measuring $^{14}CO_2$ liberated from L-[1-$^{14}$C]histidine The closed reaction vessel with the hyamine hydroxide soaked filter paper described previously [12] contained, in 1 ml of assay medium: $5 \times 10^{-5}$ M L-histidine, 0.1 $\mu$Ci L-[1-$^{14}$C]histidine, $10^{-5}$ M pyridoxal phosphate, $10^{-1}$ M phosphate buffer (pH 7) and gastric mucosa homogenate which was added to start the reaction.

After incubation at 37° for 1 hr, the reaction was stopped by the addition of 500 $\mu$l of 40% trichloroacetic acid. The $^{14}CO_2$ trapped on the filter paper was counted in a standard scintillation cocktail.

## RESULTS

### In vitro *studies*

Incubation of purified rat gastric mucosa histidine decarboxylase with $\alpha$-fluoromethyl histidine led to a time-dependent inhibition of the enzyme (Fig. 1). The inhibition was not reversed by dialysis. By plotting the half-life of enzyme activity as a function of the reciprocal of initial inhibitor concentration, as represented in the insert of Fig. 1 [13], it can be shown that the inhibition follows saturation kinetics. The apparent dissociation constant and minimal half-life at infinite inhibitor concentration are 13 $\mu$M and 5 min, respectively. Given at 1 mM (i.e. approximately 80 times the $K_I$ for histidine decarboxylase), $\alpha$-fluoromethyl histidine had no inhibitory effect on aromatic amino acid decarboxylase nor on glutamic acid decarboxylase.

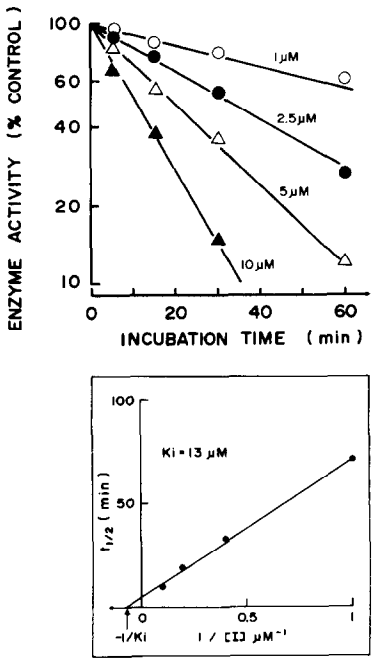


Fig. 1. Time-dependent inhibition of purified rat gastric mucosa histidine decarboxylase by $\alpha$-fluoromethyl histidine. A purified enzyme preparation corresponding to 50 $\mu$g of protein was incubated at 37° in 50 mM phosphate buffer, pH 7, containing 10 $\mu$M pyroxidal phosphate with inhibitor at the following concentrations: 1 $\mu$M (○); 2.5 $\mu$M (●); 5 $\mu$M (△); 10 $\mu$M (▲). At various times, aliquots of the solution were removed and assayed for enzyme activity.

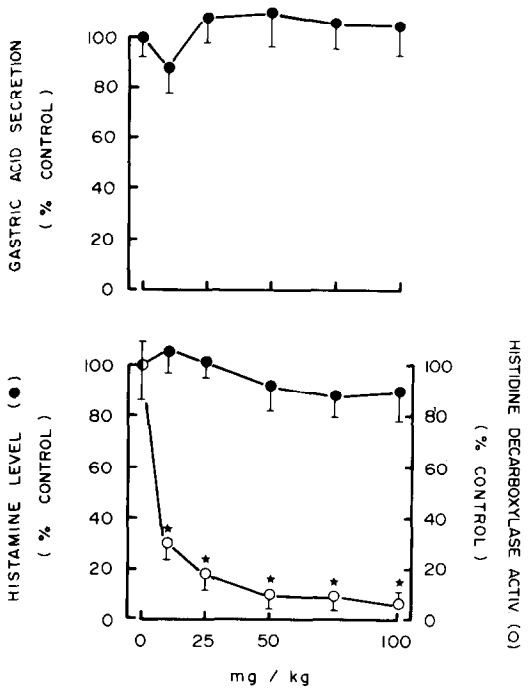


Fig. 2. Dose-dependent effects of $\alpha$-fluoromethyl histidine. Rats were injected i.p. with indicated doses of $\alpha$-fluoromethyl histidine. Pylorus ligation, measurement of acid secretion and biochemical determinations were performed as described in the Methods. The control values were 220 + 20 $\mu$Eq $H^+$/hr for gastric secretion, 2.2 ± 0.1 nmoles/g tissue/hr for histidine decarboxylase activity and 39 ± 3 ng/mg tissue for the histamine concentration. Each point is the mean ± S.E.M. of five animals. * $P < 0.001$ (Student's $t$ test).

### In vivo *studies*

*Dose-dependent effect of $\alpha$-fluoromethyl histidine (Fig. 2).* $\alpha$-Fluoromethyl histidine given i.p. 2 hr before killing the animal (30 min before pyloric ligation) inhibits histidine decarboxylase activity in the gastric mucosa in a dose-dependent manner. A dose of 50 mg/kg produces 90% inhibition. No effects were found on gastric acid secretion, measured 90 min after pyloric ligation, or on gastric mucosa histamine content.

*Time-course of the effect of a single dose of $\alpha$-fluoromethyl histidine, 50 mg/kg i.p. (Fig. 3).* The drug was given at various times before pyloric ligation. Inhibition of histidine decarboxylase activity was maintained between 60–80% for 12 hr. At 24 hr after injection the enzyme activity was the same as in control animals. Gastric acid secretion and mucosa histamine level were not different from the control, at any time point.

*Effect of $\alpha$-fluoromethyl histidine on the cimetidine induced changes of gastric mucosal histamine metabolism and acid secretion (Fig. 4).* Rats were divided

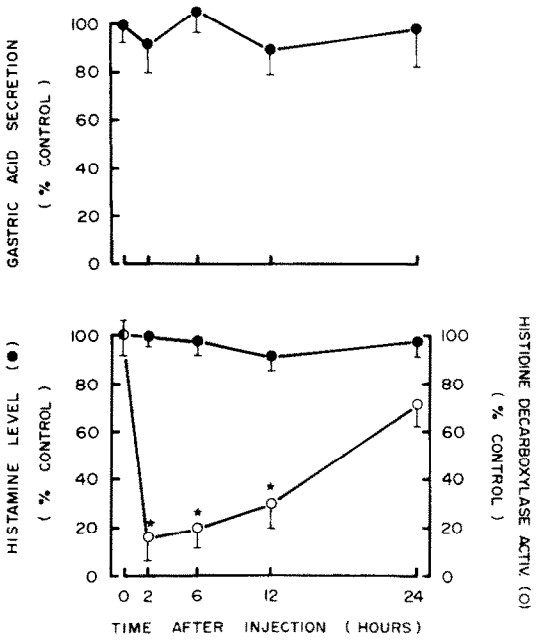


Fig. 3. Time-course of effects of single dose α-fluoromethyl histidine (50 mg/kg, i.p.). Animals were prepared as in the previous expt except for the interval between α-fluoromethyl histidine administration and pylorus ligation. The control values are 190 ± 30 μEq $H^+$ secreted/hr for gastric acid secretion, 2.6 ± 0.2 nmoles/g tissue/hr for histidine decarboxylase activity and 40 ± 2 ng/mg tissue for histamine level. Each point is the mean ± S.E.M. of five animals. * $P < 0.001$ (Student's $t$ test).

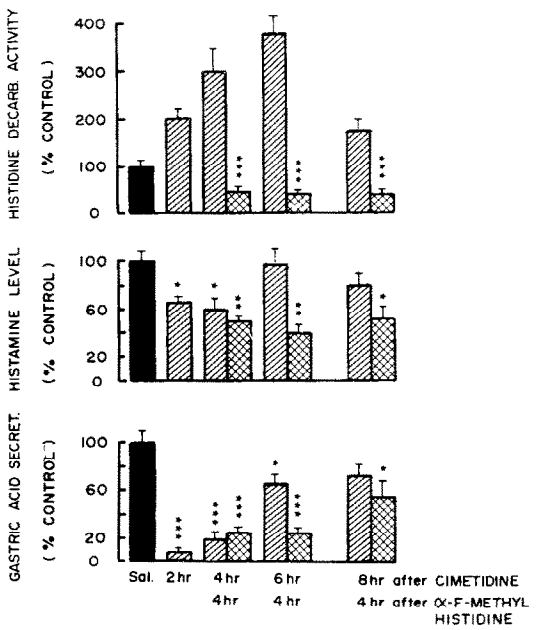


Fig. 4. Effects of combined treatment with cimetidine, (100 mg/kg i.p.) and α-fluoromethyl histidine (25 mg/kg, i.p.) on gastric mucosal histamine metabolism and acid secretion. Fasted rats received the different treatments indicated (saline, black columns; cimetidine alone, hatched columns; cimetidine plus α-fluoromethyl histidine, cross hatched columns). The indicated times refer to times between drug administration and death. The interval between pylorus ligation and death was 90 min. The control values are 2.1 and 0.3 nmoles/g tissue/hr for histidine decarboxylase activity, 42 ± 3 ng/mg tissue for histamine content and 250 ± 40 μEq $H^-$ secreted/hr for gastric acid secretion. Each point is the mean ± S.E.M. of five animals, except for controls, where $n = 20$. (Control rats were killed 2, 4, 6 and 8 hr following saline injection. Results obtained at each time were not significantly different and were pooled.) * $P < 0.01$; ** $P < 0.05$; *** $P < 0.001$. (Comparison of mean to control levels by the Student's $t$ test.)

into three groups: one group received only saline; one group received cimetidine (100 mg/kg, i.p.) at time, $t = 0$; and the third group received cimetidine at $t = 0$ plus α-fluoromethyl histidine 4 hr before sacrifice. All animals were submitted to pyloric ligation 90 min before decapitation. Acid secretion, mucosal histidine decarboxylase activity and histamine content were measured as described in the Methods.

(a) In this experiment it was confirmed that cimetidine causes an increase of histidine decarboxylase activity. The maximal effect was seen 6 hr after cimetidine administration and corresponded to a 3.5–4-fold elevation of enzyme activity. At longer time points the enzyme activity returned to the control activity. In animals treated with cimetidine plus α-fluoromethyl histidine, the supra-inductive effect of cimetidine was totally blocked and the residual enzyme activity reduced to about 50% of values found in control pylorus ligated animals.

(b) Cimetidine treatment caused a 40% depletion of gastric histamine stores at the short time intervals (2 and 4 hr). At 6 hr, the time of maximal induction of histidine decarboxylase, the histamine levels had returned to normal values. The administration of α-fluoromethyl histidine, which prevented the increase of histidine decarboxylase activity also prevented the replenishment of histamine pools depleted by cimetidine. At 8 hr after cimetidine, the histamine level in α-fluoromethyl histidine treated rats was still 55% of the control ($P < 0.01$).

(c) Cimetidine blocked $H^+$ secretion, as expected. Maximal effect was at 2 hr (90% inhibition). When the time interval between cimetidine injection and pyloric ligation was increased, the anti-secretory effect became weaker and was no longer significant when this interval was greater than 6.5 hr (8 hr time point on the graph). Presumably, this reflects cimetidine clearance from the body. It should also be noted that the maximal anti-secretory effect occurred earlier than the maximal histidine decarboxylation induction.

As mentioned earlier, α-fluoromethyl histidine alone had no effect on gastric acid secretion in this model. However, when it was given together with cimetidine, the acid secretion was inhibited for longer time periods: inhibition at 6 hr was the same as at 4 hr, and it was still significantly reduced at 8 hr (55% of control as compared to 25% (N.S.) in the absence of α-fluoromethyl histidine).

## DISCUSSION

The data presented here show that α-fluoromethyl histidine is a potent, irreversible inhibitor of gastric mucosal histidine decarboxylase *in vitro* and *in vivo*.

Gastric histidine decarboxylase is an adaptive enzyme in the rat. Thus, prolonged fasting reduces the enzyme activity to very low values whereas feeding, gastrin and vagal stimulation increase the enzyme activity [14, 3]. In these expts, enzyme activity was measured in fasted rats, 90 min after pyloric ligation. The latter manipulation produces a small induction of the enzyme. α-Fluoromethyl histidine leads to an almost complete loss of histidine decarboxylase activity in gastric mucosa (Figs. 2 and 3). In these short term treatments, histamine content of the gastric mucosa was not decreased. This can be explained by the slow turnover of histamine in the gastric mucosa of fasted rats [15]. The lack of effect on gastric secretion leads us to conclude that new histamine synthesis is not necessary for the maintenance of basal gastric secretion in the Shay-rat model. Other histidine decarboxylase inhibitors, such as 4-imidazolyl-3-amino-2-butanone and brocresine, have been found to inhibit gastric acid secretion in the rat following adminstration of a single dose [4]. Our preliminary data with these inhibitors did not show any changes in gastric mucosal histamine levels after a single injection. The observed decrease in gastric acid secretion could be the result of an interaction of the drug with the $H_2$ histamine receptor or at another site.

Histamine $H_2$ receptor antagonists are potent inhibitors of gastric acid secretion [1]. As confirmed in this study, cimetidine produces an increase of histidine decarboxylase activity and a decrease of histamine content of the rat gastric mucosa [16, 17]. The effect is probably a consequence of inhibiting gastric acid secretion, leading to gastrin release [18] which in turn causes a release of histamine. Administration of α-fluoromethyl histidine to cimetidine treated rats not only suppressed the increased histidine decarboxylase activity but also reduced the residual activity of the enzyme. The depletion of histamine which lasted less than 6 hr if cimetidine was administered alone could be maintained for at least 8 hr by co-administration of α-fluoromethyl histidine. Interestingly, the antisecretory effect was prolonged to a similar extent. It is tempting to suggest that the newly synthesized histamine, which is induced by cimetidine, displaces the $H_2$ blocker from its receptor. This would explain the prolonged anti-secretory effect of the combination of $H_2$-antagonist and synthesis inhibitor.

Using another histidine decarboxylase inhibitor (2 - hydroxy - 5 - carboxymethoxy - benzyloxyamine) Huszti *et al.* [19] observed an increase in the potency of the anti-secretory activity of $H_2$-receptor antagonists. They suggested that the increase of histamine biosynthesis following administration of an histamine $H_2$-receptor antagonist would lead to a weaker potency of the drug after prolonged administration. Thus, the simultaneous application of a potent and specific histidine decarboxylase inhibitor, such as α-fluoromethyl histidine, could potentiate the anti-secretory effect of $H_2$-antagonists, although there have been no reports on loss of potency of cimetidine on long-term administration to humans.

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