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α-Fluoromethylhistidine

Kinetics of Uptake and Inhibition of Histamine Synthesis in Basophil (2H3) Cell Cultures

ELIZABETH WOLDEMUSSIE AND MICHAEL A. BEAVEN

Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

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SUMMARY

Labeled histidine was taken up into rat leukemic basophil 2H3 cells by a system with high affinity for histidine and then decarboxylated to form histamine. Uptake was partially inhibited and decarboxylation was completely blocked by α-fluoromethylhistidine (α-FMH) at concentrations of 10–100 μM. α-FMH appeared to be co-transported by a histidine uptake system but the affinity of the system for α-FMH was lower than that for histidine (K_m 130 and 24 μM, respectively). The drug rapidly penetrated into and became highly localized within the cells. By 60 min the apparent IC_{50} for inhibition of histamine synthesis in intact cell suspensions was 0.2 μM compared to an IC_{50} of 1–2 μM α-FMH for inhibition of soluble histidine decarboxylase preparations. Turnover of histidine decarboxylase activity in 2H3 cells was rapid ($t_{1/2}$, 37 min), and biphasic effects were noted after 24-h exposure of 2H3 cells to drug. At low concentrations (<0.1 μM), decarboxylase activity was increased (up to 134 ± 9% of control values). Higher concentrations of the drug (0.1–10 μM) were inhibitory, and inhibition was related to drug concentration. No detectable decarboxylase activity was observed with 10 μM α-FMH after 4 days. Histamine levels increased (up to 232 ± 2% of control values) or decreased in parallel with decarboxylase activity. Even in cultures devoid of histamine or decarboxylase activity (with 10 μM α-FMH) cell division and growth were not affected. Thus the drug appeared to inhibit specifically histamine synthesis without impairing essential cellular metabolic processes. However, kinetics of drug uptake and perturbation of enzyme turnover are additional factors to be considered in the action of α-FMH in intact cell systems.

INTRODUCTION

Release of histamine and other inflammatory mediators from mast cells and blood basophils occurs in a variety of allergic and inflammatory conditions. Histamine release from gastric mucosa is also the underlying factor in the pathogenesis of duodenal ulcers (3). Selective histamine antagonists are widely used to treat these conditions (4). Another potential therapeutic approach is the use of drugs that selectively inhibit histidine decarboxylase (EC 4.1.1.22) (Ref. 5 and see Refs. 6 and 7), the enzyme responsible for histamine synthesis (6, 8). Of these inhibitors, α-FMH[1] appears to be the most selective and promising drug tested to date (9). This drug covalently binds to histidine decarboxylase and irreversibly inhibits enzyme activity (9). The drug has been shown to inhibit histidine decarboxylase activity in various tissues of rats and mice *in vivo* (10, 11) and in rat peritoneal mast cells *in vitro* (12). However, because of its amino acid structure it is unlikely to readily penetrate cells unless it is taken up by one of the amino acid transport systems. Rate of uptake and accumulation of drug within the cell thus should be important determinants of its potency *in vivo*.

In the present studies we have used rat leukemic basophil (2H3) cell cultures to observe and quantitate uptake of α-FMH and to determine whether or not uptake occurs by the same system that transports histidine into 2H3 cells (13) and rat peritoneal mast cells (14). The histidine transport system has a high affinity for histidine (apparent K_m, 44 μM in rat peritoneal mast cells and 24 μM in 2H3 cells) (13, 14) and is inhibited by

[1]The abbreviations used are: α-FMH, (*S*)-α-fluoromethylhistidine; MEM, Eagle's minimum essential medium; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; AIB, 2-aminoisobutyric acid; MeAIB, methylaminoisobutyric acid; BCH, (±)-B-2 aminobicyclo-[2,2,1]-heptane-2-carboxylic acid. The designations of amino acid transport systems as A, ASC, L, Ly^+, and N are as defined by Christensen and associates (1, 2).

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histidine analogs, glutamine (15) and aromatic amino acids (14). The 2H3 cells, however, have characteristics that were useful for the present studies. The numbers of histidine transport carriers decrease markedly as 2H3 cells approach cell division (13), and similar changes should be evident in α-FMH uptake if both amino acids share the same transport system. The cells possess appreciable histidine decarboxylase activity (13), and the effect of uptake and intracellular localization of α-FMH on histamine synthesis could be assessed. Advantage also was taken of the ability to grow these cells as monolayer cultures in cluster plates (16, 17) to conduct detailed kinetic studies.

EXPERIMENTAL PROCEDURES

Soluble preparations of histidine decarboxylase activities from rat stomach and fetal liver. Rats, fasted overnight, were injected s.c. with pentagastrin, 1 mg/kg, to induce gastric histidine decarboxylase activity and killed 2 hr later. The fundic portion of the stomach was removed, briefly washed with physiological saline, and a 1:10 homogenate prepared in 0.01 M sodium phosphate buffer, pH 7.4. The homogenate was centrifuged (100,000 × *g* for 60 min), and the supernatant fraction was concentrated by lyophilization. One ml of final preparation contained enzyme activity recovered from 0.2 g of tissue. Enzyme activity was also extracted from livers of rat fetuses (16-day term) and purified through steps I and II as described by Watanabe and associates (18). The final preparation contained enzyme activity recovered from 0.5 g tissue in 1 ml. All procedures were carried out at 4°.

Preparation and maintenance of 2H3 cells. The 2H3 cell line was supplied to us by Dr. Reuben P. Siraganian (Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health). The cells were maintained and subcultured in MEM with Earle's balanced salt solution supplemented with 15% heat-inactivated fetal calf serum, penicillin, streptomycin, and fungizone (supplemented MEM) by procedures described by Barsumian *et al.* (16). Cells were detached from the surface of the flasks by treatment with trypsin (37°, 5 min), recovered by centrifugation (200 × *g* for 10 min), and resuspended in the indicated medium. For most experiments, the cells were grown as monolayer cultures by plating 1×10^5 cells in 0.5 ml of supplemented MEM in cluster plates (Costar, 24 × 1-cm wells). Plates were incubated for 16 hr at 37° in a humidified atmosphere of 5% CO_2/95% air. The cultures were washed once and medium replaced with a modified Hanks' medium (Hanks' medium supplemented with 10 mM HEPES and 0.1% bovine serum albumin).

For comparison of uptake of α-FMH at different stages of development, 2H3 cells were separated into fractions of different size by elutriation before transfer to cluster plates in modified Hanks' medium (13). The plates were kept at 37° for 2 hr before determination of uptake as described below.

Cell count and size analysis were performed using a Celloscope (Particle Data Inc.) or hemocytometer. Viability of cells was assessed by ethidium bromide/fluorescein diacetate-staining procedure (19).

Effect of drugs on histidine decarboxylase activity and histamine synthesis. Histidine decarboxylase activity of soluble enzyme preparations and intact cell suspensions was assayed by measurement of release of $^{14}CO_2$ from L-[*carboxyl*-^{14}C]histidine (52 mCi/mmol, Amersham Corp.) exactly as described previously (13, 20). The final reaction mixture contained 10 μl of enzyme preparation or 5×10^4 cells, 20 nCi of labeled histidine (10 μM), and 10 μM pyridoxal phosphate in 40 μl of modified Hanks' medium. When samples were incubated with α-FMH at 37° before assay of decarboxylase activity, sufficient drug was added to the final assay reaction mixture to maintain the indicated drug concentration. Release of $^{14}CO_2$ was measured over a 60-min period, unless noted otherwise (5 min in short term experiments or 15 min in studies with cylcoheximide).

Histamine synthesis was determined by incubation (37°) of 2H3 monolayer cultures in cluster plates with L-[2-*ring carbon*, ^{14}C]histidine (50 nCi, 59 mCi/mmol, Amersham Corp.) and the indicated concentration of α-FMH in 500 μl of modified Hanks' medium for 5 or 60 min. The reactions were terminated and labeled histamine and histidine assayed in cell extracts by procedures reported elsewhere (13).

To study long term effects of α-FMH, 2H3 cultures were grown in supplemented MEM in culture flasks (75 cm^2 surface area) with α-FMH for 1–4 days. Cells were recovered by treatment with trypsin (16), resuspended in modified Hanks' medium and, after determination of cell count, diluted as indicated for determination of histamine (5×10^3 cells/10 μl) by radioenzymatic assay (20) and histidine decarboxylase activity by the procedure described above. α-FMH, however, was not added back to the reaction mixture for the decarboxylase assay.

Turnover of histidine decarboxylase activity was assessed by addition of cycloheximide (10 μM) to spinner cultures of 2H3 cells (6×10^5 cells/ml in 50 ml). At various times, 5-ml samples were removed, cells were collected by centrifugation (200 × *g* for 10 min) and resuspended in modified Hanks' medium for assay of decarboxylase activity.

Measurement of α-FMH and histidine uptake in monolayer cultures. Modified Hanks' medium (500 μl) that contained either (*S*)[*ring*-4-^{3}H] α-FMH ([^{3}H]α-FMH, 9 Ci/mmol), 180 nCi, or L-[2-*ring carbon*, ^{14}C] histidine, 50 nCi, and unlabeled amino acids in the concentrations indicated were added to each well of washed cultures in cluster plates. In some experiments medium contained both labeled amino acids. Medium was prewarmed to 37°. The plates were incubated (37°) for 1 min or the stated time. The reactions were terminated by placing trays on ice. Medium was removed by aspiration, and the cultures were washed twice with 500 μl of ice-cold modified Hanks' medium. Cells were lysed in 200 μl of 0.01 N HCl. Radiolabel was assayed in 100-μl samples of lysed cells. Intracellular levels were calculated on an assumed cell volume of 1100 fl/cell (13).

A variety of metabolizable and nonmetabolizable amino acids were examined for their effects on uptake of α-FMH or histidine. Selection was based on their ability to be preferentially taken up by amino acid transport systems as defined by Christensen and associates (1, 2). The model substrates used to discriminate between the various transport systems included MeAIB (system A), AIB (systems A and ASC), BCH and L-phenylalanine (system L), and L-glutamine (system N) (see "Discussion").

Materials. Medium, reagents, enzymes, and Hanks' medium for cell culture work were purchased from GIBCO (Grand Island, NY) and bovine serum albumin (fraction V) from Miles Laboratories (Elkhart, IN). BCH was obtained from New England Nuclear, and other amino acids from Sigma. Labeled and unlabeled (*S*)-α-FMH were gifts from Dr. J. Kollonitsch, Merck Sharp and Dohme. Sodium-free Hanks' medium was prepared in the laboratory according to the manufacturer's formula, but choline chloride and choline bicarbonate were substituted for their respective sodium salts. The medium was supplemented with HEPES and bovine serum albumin as described above. To prepare media of different pH values, the pH was adjusted by addition of 1 N NaOH or HCl.

Expression of results. All results were expressed as the mean ± S.E. of 6 culture wells in studies of amino acid uptake or of 3–6 samples for measurement of histidine decarboxylase activity. Each experiment was repeated at least twice. Where values are presented from several experiments the mean or mean ± S.E. of mean values from individual experiments is given for the number (*n*) of experiments indicated. In most experiments with cluster plates standard errors were ± 1–5% of mean values, and error bars have been omitted where these fall within the data point. In the studies of amino acid uptake, the data were plotted as velocity of uptake (*v*) *versus* substrate concentration ([*s*]) or *v versus v/s* (21) and analyzed by computer. Computations were done through the MLAB program (Division of Computer Research and Technology, National Institutes of Health) and were based on least squares analysis of theoretical equations of the sum of two or more hyperbolas from *v* against *s* plots. For the data shown in Fig. 6, scatter of data did not allow discrimination between the sum of 2 or 3

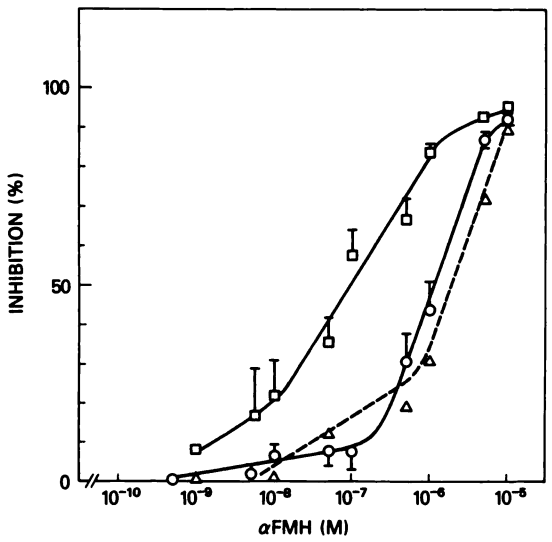

FIG. 1. *Inhibition of histidine decarboxylase activity of soluble enzyme preparations from rat stomach (△) and fetal liver (○, □) in the presence of various concentrations of α-FMH*

α-FMH was added to enzyme preparation together with L-[*carboxyl*-^{14}C]histidine and $^{14}CO_2$ release determined over a 60-min period (△, ○) or was incubated with fetal liver enzyme preparation for 24 hr before addition of labeled histidine (□) as described under "Experimental Procedures." Values are from 2 (△) or 4 (○, □) experiments and indicate decrease in activity compared to activity of samples assayed in the absence of α-FMH (2.4 ± 0.3 and 1.1 ± 0.1 nmol of $^{14}CO_2$ released/hr/ml of liver and stomach enzyme preparation, respectively).

Michaelis-Menten components. Calculations for the high affinity component were based on an assumed 2-component system. No assumption was made that such a component represented a single homogenous system. In experiments where single components appeared to be involved curve fitting was by least squares analysis by a computer. Tests for statistical significance were done by the two tailed Student's *t* test.

RESULTS

Inhibition of histidine decarboxylase activity by α-FMH in soluble enzyme preparations and intact 2H3 cells. Histamine synthetic activity of 2H3 cells was largely destroyed (by 90–98%) upon cell disruption even in the presence of inhibitors of proteolytic enzymes (see Ref. 15), and insufficient enzyme activity could be recovered to test the effects of α-FMH directly on cell extracts. With soluble preparations of enzyme from rat stomach and fetal liver, inhibtion of decarboxylase activity increased progressively with time and was dependent on concentration of α-FMH. In the presence of 1 μM α-FMH, for example, inactivation of the stomach enzyme activity followed first order kinetics with a half-life of 22 min (data not shown). Assay of decarboxylase activity (60-min incubation) in the presence of various concentrations of drug indicated almost complete inhibition of enzyme activity with 10 μM α-FMH and 50% inhibition (IC_{50}) with 1–2 μM drug. The apparent IC_{50} shifted to lower values (~0.1 μM with fetal liver preparations) after 24-hr exposure to drug (Fig. 1). Although these data confirmed previously published work (9, 10), they provided the basis for comparison of the effects of α-FMH in intact cells.

Inhibition of histamine synthesis, as indicated by formation of labeled histamine from ring-labeled histidine or release of $^{14}CO_2$ from carboxyl-labeled histidine, was also apparent in intact 2H3 cells within 5 min of addition of α-FMH (Fig. 2*B*). By 60 min, inhibition of histidine decarboxylase activity and histamine synthesis in the intact cells was greater than that observed with soluble enzyme preparations. The observed values for IC_{50} were

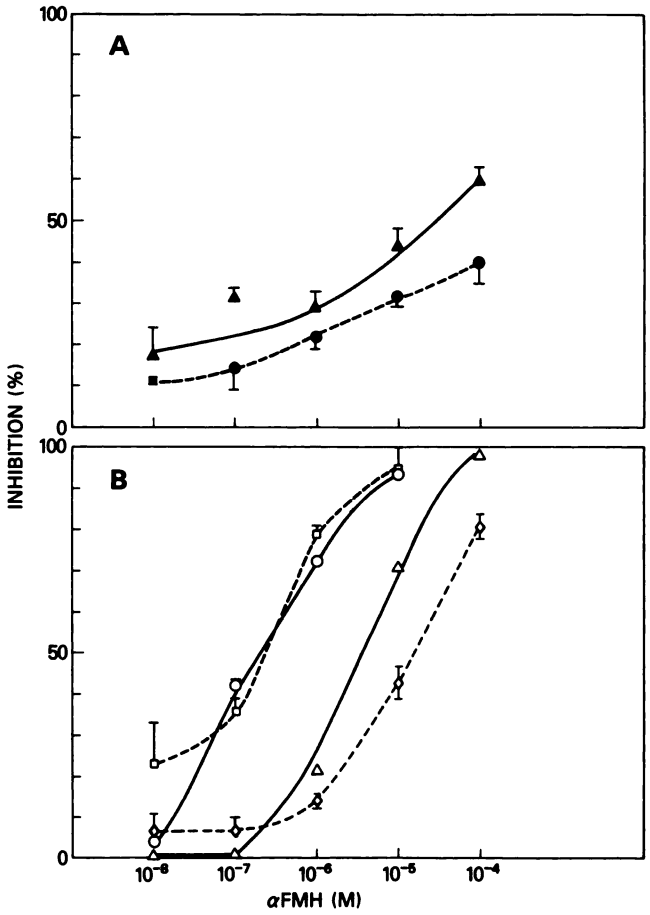

FIG. 2. *Effect of 5- or 60-min exposure to α-FMH on histidine uptake (Panel A) and histamine synthesis (Panel B) in intact 2H3 cells*

For panel *A*, 2H3 cell cultures in cluster plates were incubated with the indicated concentration of α-FMH for 5 (▲) or 60 (●) min, and L-[*2-ring carbon*, ^{14}C]histidine (2 μM) was added 5 min before termination of the incubation to allow determination of intracellular concentration of label. Values are from one of two similar experiments and indicate inhibition of uptake compared to uptake measured in the absence of α-FMH (for all experiments, per cent label taken up in the absence of drug ranged from 2.6 ± 0.1 to 4.0 ± 0.1, equivalent to 260–400 pmol of histidine/10^6 cells/5 min). For panel *B*, the cultures were incubated with L-[*2-ring carbon*, ^{14}C]histidine and α-FMH for 5 (△) and 60 (○) min at which times intracellular levels of labeled histidine and histamine were determined by thin layer chromatography of extracts prepared from pooled samples from 4 cultures (see "Experimental Procedures"). Data are shown from an additional series of 3 experiments in which 2H3 cells in suspension were incubated for 5 (◇) or 60 (□) min with L-[*carboxyl*-^{14}C]histidine (10 μM) and α-FMH and $^{14}CO_2$ release measured over the 5- or 60-min period as described under "Experimental Procedures." Values indicate per cent decrease in labeled histamine formed or $^{14}CO_2$ released compared to samples assayed in the absence of drug. (In control cultures 0.20 and 1.86% of the added label was converted to [^{14}C]histamine by 5 and 60 min, respectively, and 0.1 and 1.4% of carboxyl-labeled histidine was converted to $^{14}CO_2$, respectively).

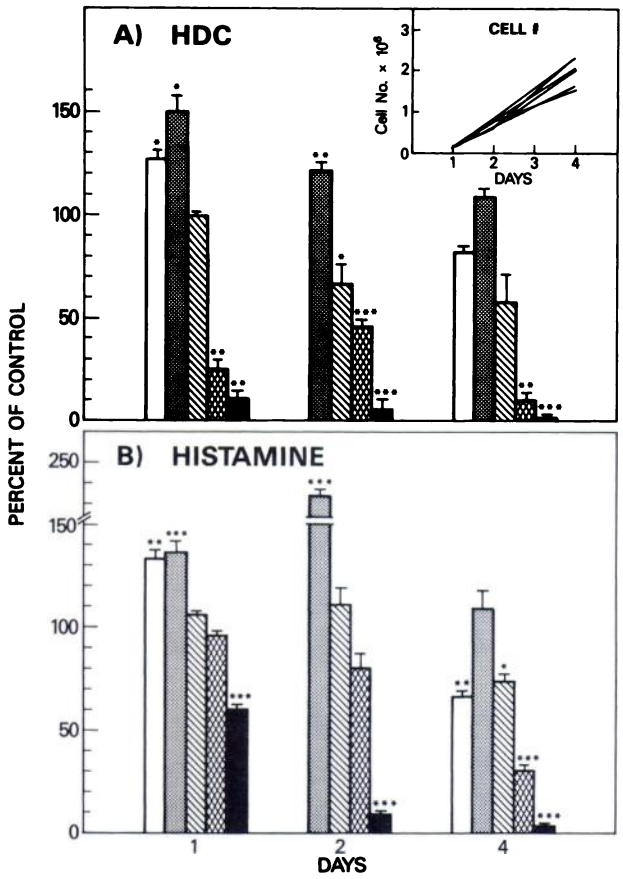

FIG. 3. *Histidine decarboxylase (HDC) activity (Panel A), histamine content (Panel B), and cell numbers (inset) in 2H3 cell cultures exposed to various concentrations of α-FMH for 1, 2, or 4 days*

Cultures were prepared in supplemented MEM in flasks (see "Experimental Procedures") that contained 0 (controls), 0.001 (□), 0.01 (□), 0.1 (■), 1 (■), and 10 (■) μM α-FMH. Cells were harvested by trypsinization. Histidine decarboxylase activity, as measured by rates of $^{14}CO_2$ release from L-[*carboxyl*-^{14}C]histidine in cell suspensions, intracellular histamine content, and cell numbers were determined (in triplicate). Decarboxylase activity (pmol/10^6 cells/hr) of control cultures was 217 ± 24 (day 1), 95 ± 3 (day 2), and 51 ± 10 (day 3), and histamine content (nmol/10^6 cells) of the same cultures was 1.7 ± 0.05, 1.3 ± 0.2, and 1.8 ± 0.5, respectively. Values for drug-treated cultures were expressed as a per cent of control values. Values are mean ± S.E. of 3 culture flasks and significant difference from control values is indicated by: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. No significant difference was observed in cell numbers between controls and drug-treated cultures nor was there any trend to decreased growth rates with high drug concentrations.

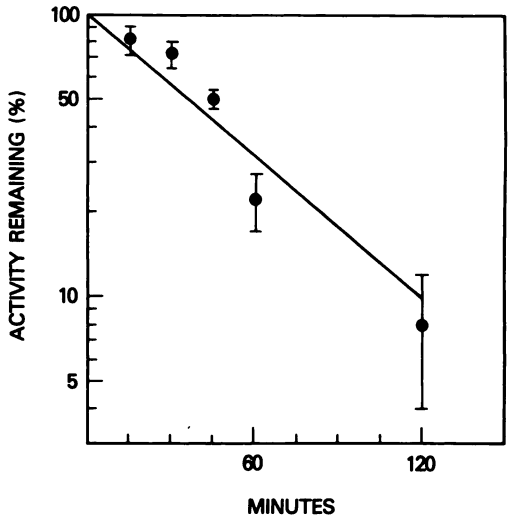

FIG. 4. *Decline in histidine decarboxylase activity in 2H3 cells in the presence of 10 μM cycloheximide*

Samples (in triplicate) were removed from spinner culture at various times after the addition of cycloheximide and assayed for decarboxylase activity by measurement of $^{14}CO_2$ release from L-[*carboxyl*-^{14}C]histidine as described under "Experimental Procedures." Values are from 4 experiments. Preliminary studies indicated that with low concentrations (0.1 μM) of cyloheximide enzyme activity was increased by 51% within 60 min, and at higher concentrations (1–10 μM) enzyme activity was reduced. Maximal rate of decline in enzyme activity was observed with 10 μM cycloheximide. The calculated half time ($t_{1/2}$) for decay in enzyme activity was 37 min.

approximately 0.2 μM. Although α-FMH inhibited histidine uptake, the effects were much less than those observed on decarboxylase activity. Furthermore, inhibition of uptake was not increased upon longer exposure of cells to drug (Fig. 2A).

Long term effects of α-FMH on 2H3 cultures. Cells maintained in supplemented MEM medium in the presence of low concentrations of α-FMH did not show inactivation of decarboxylase activity. Rather, there was an increase in enzyme activity. Increase in activity was apparent in cultures grown for 1, 2, and 4 days in the presence of the 0.01 μM α-FMH. At concentrations of 0.1 μM or greater, the drug inhibited activity in a dose-dependent manner (Fig. 3A). Histamine content of the cells was similarly elevated or decreased in the presence of low or high concentrations of drug, respectively (Fig. 3B). By 2–4 days in the presence of 10 μM drug cells were almost devoid of decarboxylase activity and histamine. Cell growth and division as indicated by increase in cell numbers, however, was not affected at any drug concentration tested (Fig. 3, *inset*). It should be noted that decarboxylase activity was measured in the absence of drug after washing of cell cultures and that decreases in activity were due to irreversible inactivation of enzyme activity.

The increase in enzyme activity noted above could indicate stimulation of enzyme synthesis in the presence of low α-FMH concentrations. Studies with cycloheximide suggested that turnover of the enzyme was rapid ($t_{1/2}$, 37 min) in 2H3 cells (Fig. 4).

Uptake of α-FMH by 2H3 cells. The ability of α-FMH to inhibit enzyme activity in intact cells within 5 min, and more so by 60 min, suggested that drug readily entered into the cell cytosol. Uptake of drug was confirmed in studies with radiolabeled α-FMH. Maximum uptake of drug was observed within 60 min with low but not high concentrations of α-FMH (Fig. 5). At this time the calculated intracellular concentration of drug was 60 times that in the medium. A plot of drug concentration ([*s*]) versus rates of uptake (*v*) was curvilinear but did not follow simple Michaelis-Menten kinetics (Fig. 6, *inset*). When *v* was plotted against *v*/[*s*] according to Hofstee (21), uptake at concentrations below 50 μM appeared to be through a component with an apparent V_{max} of 390 ± 50 pmol/10^6 cells/min and K_m of 130 ± 9

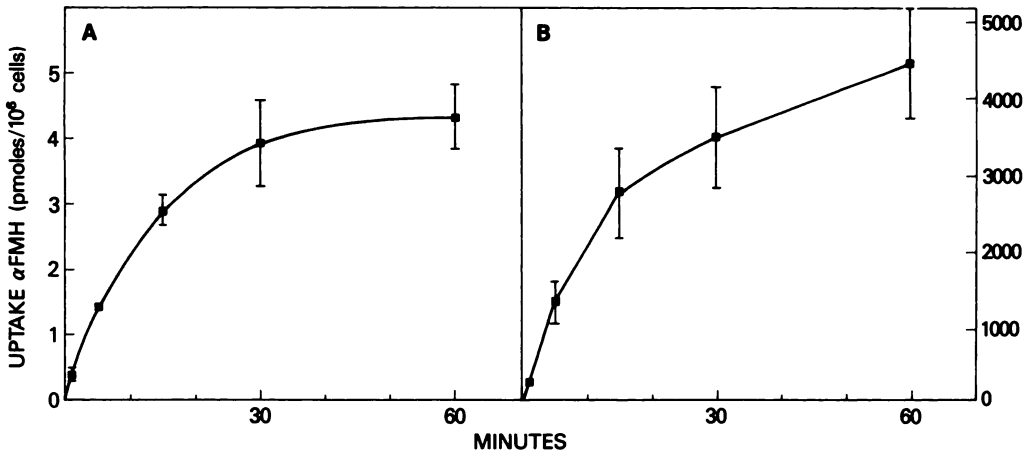

FIG. 5. *Time course of [³H]α-FMH uptake by 2H3 cultures in cluster plates*

Cultures were incubated (37°) for the indicated times in Hanks' medium (0.5 ml) that contained [³H]α-FMH (185 nCi) and unlabeled drug to make final concentrations of 0.08 (Panel *A*) and 50 (Panel *B*) μM α-FMH. Intracellular levels of radiolabel were determined as described under "Experimental Procedures." Data points are from 3 experiments all of which showed similar time course of uptake.

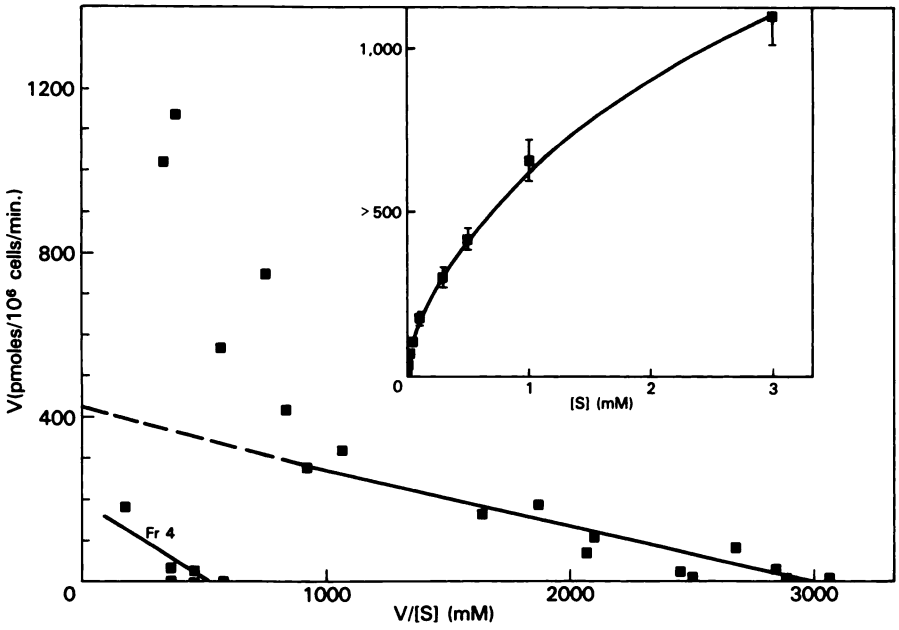

FIG. 6. *Relationship between rate of uptake and α-FMH concentration plotted according to Hofstee (21)*

2H3 cultures in cluster plates were prepared from confluent cultures or from fraction 4 (*Fr 4*) of cells separated by elutriation (see "Experimental Procedures"). Cultures were washed and uptake determined with different α-FMH concentrations. Uptake, pmol/10⁶ cells/min (*V*), was plotted as a function of ratio of uptake to α-FMH concentration (*V*/[*S*]). Values are mean of 6 cultures and are from 2 of 4 similar experiments. The *inset* shows the data from 3 experiments (mean ± S.E.) plotted as *V* against [*S*]. Computer simulation indicated that the data at least fit the sum of two hyperbolic functions; based on this assumption calculated values for the high affinity component (main panel, *solid line*) were 145 ± 108 μM for K_m and 421 ± 265 pmol/10⁶ cells/min for V_{max} (see text for calculated kinetic parameters for all experiments). Note: fraction 4 cells represent small cells in early G_1 phase of growth (see Ref. 13).

μM (values for 4 experiments). At higher concentrations additional component(s) with low affinity for drug were observed (Fig. 6).

At all concentrations of drug tested, the drug had become highly localized within cells by 20 min with ratios of intra- to extracellular drug concentrations ranging from 55 (with 10 μM α-FMH) to 6.5 (with 3 mM α-FMH) (Fig. 7). On the assumption that intracellular drug concentrations of 10 μM should be sufficient to inhibit enzyme activity by >90% (see Fig. 1), the data in Fig. 7 suggested that complete inhibition could be achieved with external drug concentrations of 0.2 μM. These calculations were not consistent with the data in Fig. 2 which indicated that drug concentrations >1 μM were required for substantial inhibition of enzyme activity in intact cells. In view of the rapid turnover and induction of enzyme activity noted in the previous section, increased rates of enzyme synthesis might account for this discrepancy.

Further characterization of the α-FMH uptake system: similarities with histidine uptake. Our earlier studies had indicated that uptake of histidine was much reduced in

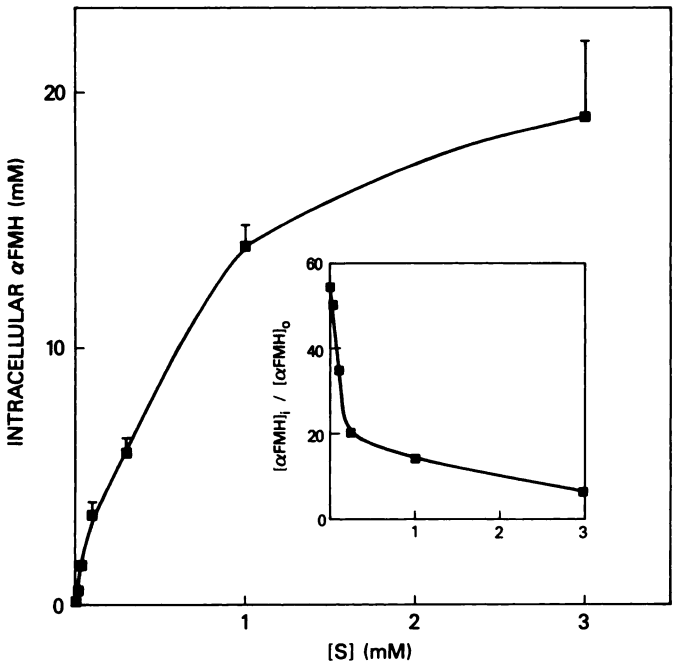

FIG. 7. *Relationship between intracellular and extracellular concentration of α-FMH in 2H3 cells*

Intracellular levels of [^{3}H]α-FMH were determined after 20 min incubation (37°) of 2H3 cell cultures in cluster plates in the presence of various concentrations of α-FMH as described in the legend to Fig. 6. Intracellular concentrations of α-FMH were calculated on an assumed cell volume of 1100 fl/cell (see "Experimental Procedures"). The ratio of intra- and extracellular drug concentrations plotted for each drug concentration tested is shown in the *inset*. Values (6 cultures) are from one of 2 similar experiments.

fractions of small 2H3 cells (*i.e.* Fractions 3 and 4 of elutriated cells) compared to fractions of large cells or confluent cultures (13). Similarly rates of α-FMH uptake were much lower in fraction 4 of elutriated cells than those observed in cultures of confluent cells (Fig. 6).

Christensen and associates have characterized a variety of amino acid transport systems in mammalian cells. These included systems with broad specificity for neutral amino acids (systems A, ASC, and L), one specific for cationic amino acids (system Ly$^+$), and one for amino acid amides and histidine (system N) (1, 2). Uptake of α-FMH and histidine was not inhibited by MeAIB, the model substrate of system A. Uptake of both was weakly inhibited by AIB, a substrate for system A and ASC, and strongly inhibited by BCH and phenylalanine which are substrates of system L. Glutamine, a substrate of system N, also inhibited uptake. The pattern and extent of inhibition of uptake for both α-FMH and histidine were similar (Fig. 8).

Transport of amino acids by L is independent of sodium ion concentration whereas transport by systems A, ASC, and N is substantially reduced (typically by more than 80% at low substrate concentrations) when choline salts were substituted for sodium salts in the medium (1, 2). Partial reduction (by 52%) in rates of histidine uptake has been noted with mast cells suspended in sodium-depleted medium (14). With 2H3 cells the data were equivocal; uptake of histidine and α-FMH by 2H3 cells was only moderately impaired (31 ± 6% reduction, 4 experiments) by sodium deprivation, nor was uptake substantially reduced in the absence of a Na$^+$ and presence of 5 mM L-homoarginine (to inhibit uptake by system Ly$^+$) (Table 1). In all experiments the data did indicate that the uptakes of α-FMH and histidine were reduced by the same extent (Table 1). Also uptake of both amino acids was not highly sensitive to changes in pH when uptake was measured between pH 5.0–8.0 (Fig. 9). These pH values were chosen because Kilberg and associates (2) have shown that uptake of glutamine into hepatocytes was increased 3-fold when pH of the medium was increased from 7.0–8.0 whereas uptake of BCH was little affected by these changes in pH.

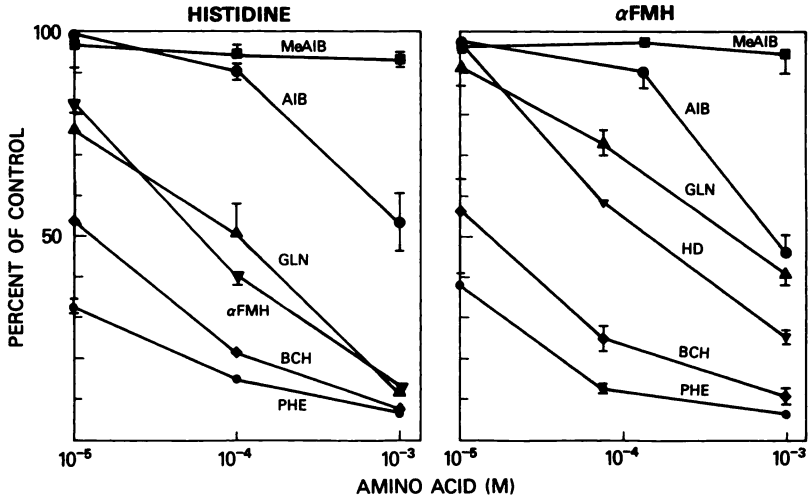

FIG. 8. *Inhibition of uptake of labeled histidine and α-FMH by 2H3 cells in the presence of different concentrations of various amino acids*

Uptake of labeled histidine (2 μM) and α-FMH (25 nM) was determined in 2H3 cultures in cluster plates as described in previous figure legends. Values are from 3 experiments and show uptake as the per cent of that in cultures without added amino acid (*i.e.*, 120 ± 10 pmol of histidine and 0.11 ± 0.01 pmol of α-FMH/10^6 cells/min). Unlabeled amino acids tested were MeAIB, AIB, L-glutamine (*GLN*), L-histidine (*HD*), α-FMH, BCH, and L-phenylalanine (*PHE*). Similar data were obtained in a second series of experiments with sodium-deficient medium (choline salts were substituted for sodium salts).

TABLE 1

Uptake of [^{3}H]α-FMH in the absence of Na^+ ions and presence of homoarginine (5 mM)

2H3 cultures in cluster plates were incubated (37°) in Hanks' medium (control) or medium in which choline salts were substituted for sodium salts and L-homoarginine (5 mM) was added. [^{3}H]α-FMH (180 nCi) and unlabeled α-FMH were added to give the indicated concentration of α-FMH. Intracellular levels of label were determined at 5 min. In a separate experiment uptake of [^{14}C]histidine (2 μM) was slightly impaired in the absence of Na^+ and presence of L-homoarginine (1.27 ± 0.10 *versus* 1.52 ± 0.07% for controls) as was the uptake of [^{3}H] α-FMH (2.5 μM) (0.38 ± 0.05 *versus* 0.46 ± 0.02%). In both cases (i.e., controls and presence of homoarginine) the ratio of ^{3}H to ^{14}C in the cells was the same (3.34 *versus* 3.30). Uptake in the presence of excess amino acid (10 mM) was less than 0.04%, and this value has been subtracted from above values.

α-FMH	Label taken up	
	Control	$-Na^+$, +homoarginine
μM	%	
5	0.53 ± 0.04	0.45 ± 0.02
10	0.42 ± 0.004	0.39 ± 0.01
25	0.42 ± 0.05	0.39 ± 0.01
50	0.46 ± 0.04	0.36 ± 0.05
100	0.37 ± 0.005	0.38 ± 0.003
500	0.23 ± 0.02	0.25 ± 0.03
1000	0.21 ± 0.02	0.15 ± 0.02

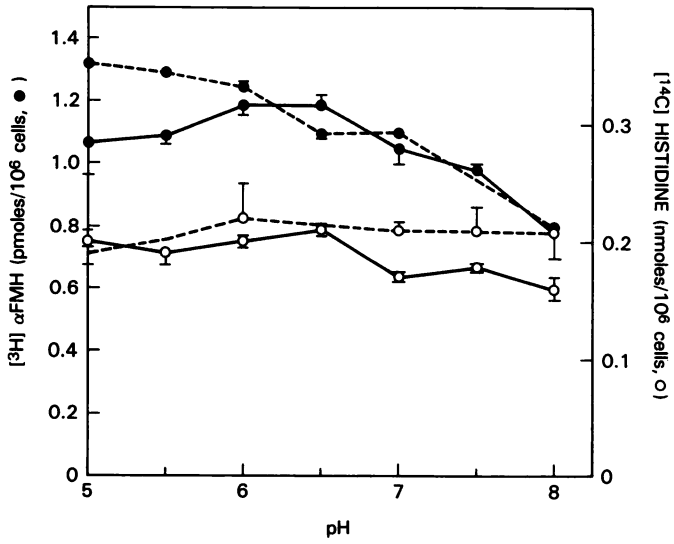

FIG. 9. *Effect of pH on uptake of [^{3}H]α-FMH (●) and [^{14}C]histidine (○)*

In one experiment 2H3 cultures in cluster plates were incubated (37°) with [^{3}H]α-FMH (30 nM) and [^{14}C]histidine (1.0 μM) for 5 min before measurement of intracellular content of radiolabel (*solid lines*). In another experiment uptake of each amino acid was determined in separate culture plates (*dashed lines*). The pH of the medium was adjusted with HCl or NaOH as described under "Experimental Procedures." Values are means ± S.E. of 6 cultures.

Although the above data do not permit definite assignment of α-FMH uptake to one or more amino acid transport systems, the results implied that histidine and α-FMH were taken up by the same mechanism(s). Competition studies at low amino acid concentrations provided additional evidence for this view. With increasing concentrations of histidine, the kinetics of α-FMH uptake showed progressive increases in values for K_p (Fig. 10) where $K_p = K_m (1 + i/K_i)$, the apparent Michaelis-Menten constant for α-FMH in the presence of the histidine concentration i. Extrapolation of the lines in the right-hand panel to Fig. 10 indicated competitive inhibition but such analyses mask other kinetic parameters. At low histidine concentrations (10 μM), for example, uptake of α-FMH was enhanced (Fig. 10) with an increase in V_{max} but apparent decrease in affinity for α-FMH (*i.e.* increased K_p). Although the data suggested an interaction of the amino acids at common sites, the kinetics did not fit a simple model of uptake through a single carrier system.[2]

DISCUSSION

The uptake and concentration of the histidine decarboxylase inhibitor, α-FMH, in 2H3 cells had several important consequences. The drug penetrated the cells at a sufficiently rapid rate to reach inhibitory concentrations within 5 min (Fig. 2). By 20 min intracellular concentrations were 5–55 times that in the medium, and this resulted in an apparent enhancement in drug potency. In the presence of 0.1 μM drug, a concentration that produced no significant inhibition of soluble histidine decarboxylase activity (Fig. 1), significant inhibition of histidine decarboxylation was observed in intact cells (Fig. 2).

The ability of the drug to rapidly penetrate cells is also apparent from other studies. The half-life for inhibition of histidine decarboxylase activity in mouse stomach and brain after a single i.p. injection of α-FMH has been estimated to be 3 and 15 min, respectively (10). Similarly, a 10-min period of preincubation with α-FMH has been employed in studies of inhibition of histidine decarboxylation in rat peritoneal mast cells (12). There are factors, however, that are likely to reduce efficacy of α-FMH *in vivo*. If turnover of enzyme is rapid, as appears to be the case in 2H3 cells (Fig. 4), the drug must inhibit newly synthesized enzyme molecules as well as existing enzyme. Indeed, the results obtained with 2H3 cells suggest that synthesis of enzyme is increased at a sufficient rate to compensate for rates of enzyme inactivation at low drug concentrations (Fig. 3). Another factor is that inactivation of histidine decarboxylase by α-FMH is retarded by the presence of L-histidine as both inhib-

[2] In some amino acid transport systems, analysis is complicated by the phenomenon of countertransport or accelerative exchange diffusion whereby influx is accelerated by outward flux of substrate (or vice versa) once intracellular substrate concentration is increased (22). Addition of histidine (25 μM) to 2H3 cells does result in subsequent acceleration of α-FMH uptake (data not shown), and countertransport (*i.e.*, histidine out, α-FMH in) might explain the increase in V_{max} noted in Fig. 10. If this is the explanation the effect is paradoxical because 2H3 cells which have high intracellular histidine concentrations demonstrate histidine efflux in the presence or absence of external histidine (13). Presumably countertransport might still operate without external histidine. A further difficulty in defining the exact kinetic model is the uncertainty of the contribution of additional low affinity components of uptake both for α-FMH (this paper) and histidine (13) in 2H3 cells. Analysis of data indicates an apparent K_i for histidine of 140 μM, but this value may have little meaning both from kinetic considerations (see Ref. 22) and the uncertainties noted above.

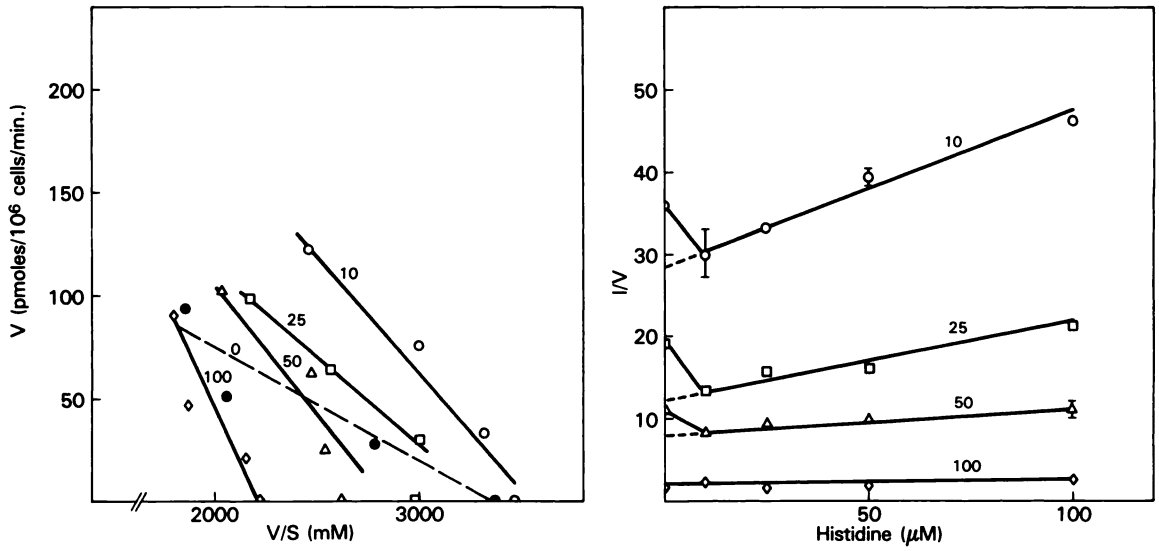

FIG. 10. *Inhibition of α-FMH uptake in the presence of different concentrations of L-histidine*

The design of the experiment was exactly as described in Fig. 6 except that uptake was determined in the presence of 0 (●), 10 (○), 25 (□), 50 (△), 100 (◇) μM L-histidine. Note the apparent increase in V_{max} (i.e., the intersects with the *y* axis) for α-FMH uptake in the presence of histidine and the progressive decrease in affinity for α-FMH with increasing histidine concentrations as indicated by the change in slopes of the curves. A plot of the data by the method of Dixon (right-hand panel) indicated a K_i for histidine of 140 μM. The data were from 1 of 2 similar experiments.

itor and substrate compete for the active site on the enzyme (9, 10). The half-life for inactivation of enzyme by 1 μM α-FMH is reported to be 20 min in the absence of histidine and 57 min in the presence of 50 μM histidine (10). High intracellular histidine levels (1.8 mM) are maintained in 2H3 cells against extracellular concentrations of 0.25 mM (13). Nevertheless, despite high intracellular histidine levels substantial inhibition of enzyme activity is achieved in 2H3 cells with 1 μM concentrations of drug. Complete suppression of histamine synthetic activity and depletion of histamine stores is observed with 10 μM drug.

Uptake of α-FMH through system(s) that transport histidine in mast cells[3] was considered likely because of our previous finding that uptake of histidine into rat peritoneal mast cells was inhibited by α-methyl-L-histidine and other histidine analogs (15). The histidine transport systems in rat peritoneal mast cells (12) and basophilic leukemia 2H3 cells (13) appear to be similar if not identical. The uptake systems in both mast cells (12, 14, 23) and 2H3 cells (this paper) have high affinity for histidine and are inhibited by the same amino acids, namely phenylalanine, BCH, and glutamine, but not by MeAIB. The similar patterns of uptake for histidine and α-FMH when studied under a variety of experimental conditions and the competition studies now provide suggestive evidence that α-FMH and histidine are taken up by the same transport system(s).

The histidine transport system in mast cells and 2H3 basophils has an affinity for substrate an order of magnitude greater than those described for histidine transport in other cells where calculated K_m values range from 0.4–4.0 mM for different components of uptake (23, 24).

[3] Our comments in the remaining paragraphs refer specifically to the high affinity components of uptake for histidine (13) and α-FMH.

The transport in 2H3 cells also differs from that in Ehrlich cells which, at neutral pH, take up histidine primarily through Na^+-independent system L and to a lesser extent through Na^+-dependent system A (23). When pH is decreased below pH 7 net rates of histidine uptake are decreased markedly but uptake through Na^+-dependent system Ly^+ becomes of increasing importance (23, 24). In 2H3 cells, the insensitivity of uptake of α-FMH and histidine to changes in pH suggests that protonation of the imidazole ring (pK_2 of histidine is 5.97) does not impede net uptake of these amino acids. The profile obtained with pH (*i.e.* Fig. 9) is similar to that for uptake of BCH through system L (24) but not that for uptake of glutamine through system N.

The above data do not rule out the involvement of multiple components of uptake, which may vary in importance with change in pH. The data do indicate, however, that at neutral pH a large proportion of uptake of α-FMH and histidine into 2H3 cells is independent of Na^+ and is not suppressed by inhibitors of systems A and ASC (*i.e.*, AIB and MeAIB) or Ly^+ (*i.e.*, homoarginine). Uptake is suppressed by inhibitors of system L (*i.e.*, aromatic amino acids and BCH) and of system N (*i.e.*, glutamine). These characteristics are most consistent with uptake by system L, which has been proposed as a possible mediator of histidine uptake in mast cells (14, 25), but the inhibition of uptake by glutamine is paradoxical. An alternative explanation is that histidine uptake in mast cells and 2H3 cells represents a specialized high affinity transport system that allows histidine to be maintained at sufficient concentration within the cell to saturate intracellular histidine decarboxylase (K_m, 0.2–0.4 mM) (15). Plasma histidine concentrations (90 μM) would be insufficient to do so (26). There are indications that histidine uptake is restricted to basophils in

guinea pig bone marrow cells (27) and mast cells in rat peritoneal cells (12, 15), but more studies are required to determine whether or not the presence of a high affinity uptake system is a unique feature of histamine-containing cells. The possibility of using α-FMH as a model amino acid for the histidine transport system may also warrant further study.

Previous studies have demonstrated the exquisitely selective action of α-FMH. The drug inhibits histidine decarboxylase, but not other pyridoxal-dependent decarboxylase enzymes (9, 10), and is nontoxic in animals (10, 28, 29) at doses that produce substantial reduction in tissue histamine and decarboxylase activity. That growth and cell division of 2H3 cells was not affected by the presence of inhibitory concentrations (*i.e.*, 10 μM) of drug indicated that essential metabolic processes were not impaired and histamine synthesis was not essential for the cell division.

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Send reprint requests to: Dr. Michael A. Beaven, Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205.