MOLECULAR PHARMACOLOGY, 12, 720-730

2-Fluoro-L-histidine, an Inhibitor of Enzyme Induction

DAVID C. KLEIN,[1] KENNETH L. KIRK,[2] JOAN L. WELLER,[1] TAKAMI OKA,[3] ANDREW PARFITT,[1] AND IDA S. OWENS[4]

Section on Physiological Controls, Laboratory of Biomedical Sciences, and Section on Developmental Pharmacology, Neonatal and Pediatric Medicine Branch, National Institute of Child Health and Human Development; and Section on Biochemical Mechanisms, Laboratory of Chemistry, and Section on Intermediary Metabolism, Laboratory of Biochemistry and Metabolism, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

(Received April 1, 1975)

SUMMARY

KLEIN, DAVID C., KIRK, KENNETH L., WELLER, JOAN L., OKA, TAKAMI, PARFITT, ANDREW & OWENS, IDA S. (1976) 2-Fluoro-L-histidine, an inhibitor of enzyme induction. *Mol. Pharmacol.*, 12, 720-730.

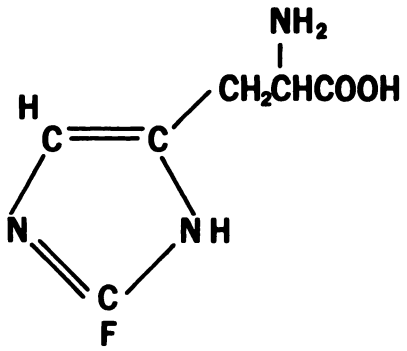
The effect of a new amino acid analogue, 2-fluoro-L-histidine, on the stimulation of certain enzyme activities in the rat pineal gland, mouse mammary gland, a hepatoma cell line, and rat liver explants was investigated. This compound inhibited the isoproterenol stimulation of pineal *N*-acetyltransferase activity *in vivo*, and in organ culture it blocked the isoproterenol, norepinephrine, and N^6,2′-*O*-dibutyryladenosine 3′,5′-monophosphate stimulation of this enzyme. Inhibitory effects of 2-fluorohistidine on the steroid induction of tyrosine aminotransferase in liver explants and on benz[*a*]anthracene induction of aryl hydrocarbon hydroxylase in established hepatoma cell line cultures were also observed. 2-Fluorohistidine did not block the spontaneous increase in ornithine decarboxylase activity in explants of mouse mammary gland, but did partially block the spontaneous increase of this enzyme in cultured pineal glands. In cultured pineal glands, no evidence was found for direct competitive or noncompetitive inhibition of *N*-acetyltransferase by 2-fluorohistidine, for the existence of any inhibitory effect on *N*-acetyltransferase activity in 2-fluorohistidine-treated glands, or for rapid irreversible toxic effects of this analogue. Unlike cycloheximide, which blocks the stimulation of pineal *N*-acetyltransferase activity by blocking protein synthesis, 2-fluorohistidine did not substantially inhibit the incorporation of [^{3}H]leucine into protein. However, the incorporation of [^{14}C]histidine is reduced by 50-90% in pineal glands treated with 2-fluorohistidine. This, together with the observation that the inhibitory effect of the analogue on enzyme induction and on [^{14}C]histidine incorporation was reversed by an equal concentration of histidine, suggests to us that 2-fluorohistidine may act by competing with histidine in a metabolic pathway, perhaps protein synthesis.

[1] Section on Physiological Controls, Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development.

[2] Section on Biochemical Mechanisms, Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases.

[3] Section on Intermediary Metabolism, Laboratory of Biochemistry and Metabolism, National Institute of Arthritis, Metabolism, and Digestive Diseases.

[4] Section on Developmental Pharmacology, Neonatal and Pediatric Medicine Branch, National Institute of Child Health and Human Development.



2-FLUORO-L-HISTIDINE

INTRODUCTION

Fluorine-containing amino acid analogues have proven to be valuable biochemical and pharmacological tools (1, 2). Recently a fluorine-containing analogue of histidine, 2-fluoro-L-histidine, has been synthesized (3). 2-Fluorohistidine is of particular interest because, while fluorine substitution has only minor steric consequences (2), the characteristic basicity and nucleophilicity of the imidazole ring nitrogens are greatly reduced by the strong electron-withdrawing effect of fluorine. 2-Fluorohistidine attracted our attention initially because of our long-standing studies of pineal *N*-acetyltransferase[5] (4, 5) and the suggestion that histidine may be located at the active site of many types of acetyltransferase molecules (6), participating in enzyme action via the imidazole group. In the present study we have examined the effects of 2-fluorohistidine on the induction[6] of pineal *N*-acetyltransferase and other enzymes. The results indicate that 2-fluorohistidine acts to inhibit the induction of most of the enzymes examined, and, based on our more detailed studies with the pineal gland, it appears that the analogue acts acutely without producing a severe blockade of cellular metabolism or protein synthesis.

[5] The abbreviations used are: *N*-acetyltransferase, acetyl-CoA:arylamine *N*-acetyltransferase (EC 2.3.1.5); tryrosine aminotransferase, L-tyrosine:2-oxoglutaric aminotransferase (EC 2.6.1.5); phosphodiesterase, 3',5'-cyclic AMP 5'-nucleotide hydrolase (EC 3.1.4.17); hydroxyindole *O*-methyltransferase, *S*-adenosylmethionine:*N*-acetylserotonin *O*-methyltransferase (EC 2.1.1.4); ornithine decarboxylase, L-ornithine carboxylase (EC 4.1.1.7).

[6] The term induction is used to describe an increase in enzyme activity which may be due to the new synthesis of enzyme protein or to activation of previously existing enzyme molecules via a mechanism dependent upon protein synthesis.

MATERIALS AND METHODS

Culture techniques. Published methods for the culture of rat pineal glands (5, 7, 8), fetal liver explants (9), a hepatoma cell line (10, 11), and mouse mammary gland explants (12) were used. Except where indicated, BGJ[b] (Fitton-Jackson modification) culture medium (5, 8), containing 0.1 mM histidine and supplemented with serum albumin, fraction V (1 mg/ml), ascorbic acid (0.1 mg/ml), and glutamine (2 mM), was used.

Assays. Published methods for measurement of the activities of *N*-acetyltransferase (5, 8), hydroxyindole *O*-methyltransferase (5), tyrosine aminotransferase (13), aryl hydrocarbon hydroxylase (10), and ornithine decarboxylase (14) were used. A filter disc method (15) was used to recover radiolabeled macromolecules from tissue homogenates. Protein was measured by the method of Lowry *et al.* (16).

Materials. 2-Fluorohistidine and 2-fluorohistamine were synthesized (3). The sources of drugs, culture media, animals, and chemicals have been given (8–15). Histidine-free BGJ[b] culture medium was produced by the NIH Media Unit; histidine was added as required.

Statistical analysis. Statistical analyses were performed using Student's *t*-test.

RESULTS

Effect of 2-fluorohistidine on adrenergic stimulation of pineal N-acetyltransferase in vivo. Pineal *N*-acetyltransferase is regulated by an adrenergic mechanism (5). A single injection of isoproterenol causes a 60-fold increase in pineal *N*-acetyltransferase activity within 2 hr (17). In animals treated with 2-fluorohistidine the isoproterenol-induced increase in *N*-acetyltransferase activity was inhibited 75% (Table 1). A similar treatment with histidine was

TABLE 1

Inhibition by 2-fluorohistidine of isoproterenol-induced pineal N-acetyltransferase activity in vivo

Animals (100-g male Sprague-Dawley rats) were deprived of food overnight. Group B rats were injected subcutaneously in the lower back region with a suspension of 2-fluorohistidine (250 mg/kg in 0.25 ml of NaCl) at 9:00 a.m. This injection was repeated at 10:30 a.m. Group B and C rats were injected subcutaneously in the nape of the neck with isoproterenol (20 mg/kg in 0.1 ml of NaCl) at 10:00 a.m. All animals were killed at noon, and their pineal glands were removed rapidly. Values are based on four glands; data are means ± standard errors.

Group and treatment	*N*-Acetyltransferase activity
	nmoles/gland/h
A. Control	0.2 ± 0.011
B. Isoproterenol	12.6 ± 1.42
C. Isoproterenol, 2-fluorohistidine	3.0 ± 0.58

ineffective. Superficial examination of the animals indicated that 2-fluorohistidine treatment had no apparent toxic or behavioral effect. This study indicated that the analogue can act in the whole animal; the limited amounts of it available precluded further studies *in vivo*.

Effect of 2-fluorohistidine on adrenergic–cyclic AMP stimulation of pineal N-acetyltransferase activity in organ culture. Isoproterenol treatment of pineal glands cultured in histidine-free or 0.1 mM histidine-containing medium caused an increase in *N*-acetyltransferase activity (Table 2). This increase was inhibited if glands were cultured in 2-fluorohistidine-containing medium. The same inhibitory effect was observed using chronically denervated pineal glands or glands which had been cultured for 48 hr to allow nerve

TABLE 2

Inhibition by 2-fluorohistidine of drug-induced increase in pineal N-acetyltransferase activity in organ culture

Superior cervical ganglionectomy (SCGX) was performed 14 days prior to organ culture. Pineal glands were removed between 10:00 and 12:00 a.m. and placed in culture. The concentration of 2-fluorohistidine was 3 mM, and, when present, the concentration of histidine was 0.1 mM. Isoproterenol or norepinephrine was added in 5 μl of 0.01 M HCl to a final concentration of 10 μM. Dibutyryl cyclic AMP was added in 5 μl of culture medium to a final concentration of 1 mM. Each value is based on four glands. Data are means ± standard errors.

Expt.	Surgical treatment	Treatment in organ culture	*N*-Acetyltransferase activity
			nmoles/gland/hr
1	None	Histidine (0–11 hr)	0.2 ± 0.03
	None	Histidine (0–11 hr); isoproterenol (3–11 hr)	10.5 ± 1.5
	None	Histidine, 2-fluorohistidine (0–11 hr); isoproterenol (3–11 hr)	4.9 ± 0.31[a]
	None	Isoproterenol (3–11 hr)	16.4 ± 2.74
	None	2-Fluorohistidine (0–11 hr); isoproterenol (3–11 hr)	3.2 ± 0.56[a]
2	None	Isoproterenol (3–11 hr)	10.4 ± 2.11
	None	2-Fluorohistidine (0–11 hr); isoproterenol (3–11 hr)	1.6 ± 0.09[a]
	SCGX	Isoproterenol (3–11 hr)	18.8 ± 3.00
	SCGX	2-Fluorohistidine (0–11 hr); isoproterenol (3–11 hr)	2.5 ± 0.52[a]
3	None	Histidine (0–55 hr); norepinephrine (51–55 hr)	9.8 ± 0.42
	None	Histidine (0–55 hr); 2-fluorohistidine (48–55 hr); norepinephrine (51–55 hr)	3.1 ± 0.14[a]
	None	Histidine (0–55 hr)	0.08 ± 0.020
4	None	Dibutyryl cyclic AMP (3–11 hr)	22.7 ± 3.43
	None	2-Fluorohistidine (0–11 hr); dibutyryl cyclic AMP (3–11 hr)	2.6 ± 0.64[a]

[a] Significantly less than glands treated with isoproterenol, dibutyryl cyclic AMP, or norepinephrine alone ($p < 0.01$; *t*-test).

processes to disintegrate. It would appear from these findings that 2-fluorohistidine acts directly on postsynaptic cells, probably pinealocytes, in the pineal gland, and that neuronal elements do not mediate its effects.

2-Fluorohistidine also inhibited the increase in *N*-acetyltransferase activity caused by dibutyryl cyclic AMP (Table 2), a compound which appears to act either by mimicking cyclic AMP or by preventing the destruction of cyclic AMP by phosphodiesterase (5). We conclude from this that 2-fluorohistidine acts at a "post-cyclic AMP" step in the adrenergically initiated sequence of events which leads to an increase in pineal *N*-acetyltransferase activity.

Effects of 2-fluorohistidine on induction of tyrosine aminotransferase by dexamethasone. To determine whether the effects of 2-fluorohistidine are unique to pineal *N*-acetyltransferase, we examined the effects of this compound on the dexamethasone induction of tyrosine aminotransferase in fetal liver explants (18–20). The culture method used was essentially the same as that used for the pineal gland. Treatment with 5 μM dexamethasone for 8 hr caused a 9-fold increase in tyrosine aminotransferase activity; this effect was blocked by 3 mM 2-fluorohistidine (Table 3). The activity of tyrosine aminotransferase in tissues treated with 2-fluorohistidine but not dexamethasone was unchanged. Tyrosine aminotransferase, like pineal *N*-acetyltransferase, is regulated via a cyclic AMP mechanism (21). Thus it is possible that 2-fluorohistidine may act by blocking the effects of cyclic AMP in both enzyme systems, or by blocking a process necessary for the effects of cyclic AMP.

Effects of 2-fluorohistidine on induction of aryl hydrocarbon hydroxylase by benz[a]anthracene. We examined the effects of 2-fluorohistidine on aryl hydrocarbon hydroxylase induction by benz[*a*]anthracene, which might also be mediated by cyclic AMP (22). It was determined that the analogue could block induction of this enzyme in Hepa-1 cells, an established hepatoma cell line (Table 4). Treatment with 13 μM benz[*a*]anthracene caused an

TABLE 3

Effect of 2-fluorohistidine on dexamethasone induction of tyrosine aminotransferase activity in fetal rat liver explants in culture

After a 24-h incubation period with 1 mM histidine, the tissue was transferred to fresh medium containing 0.1 mM histidine for the indicated treatments. The concentration of 2-fluorohistidine was 3 mM. Dexamethasone treatment was initiated by adding 5 μl of culture medium containing dexamethasone, resulting in a final medium concentration of 5 μM. Each value is the average of the means of duplicate determinations (13) performed on duplicate cultures. The duplicate means did not differ by more than 3 nmoles/mg of protein per minute.

Treatment in culture		Tyrosine aminotransferase activity
0–3 hr	3–11 hr	
		nmoles/mg protein/min
Control	Control	6.5
Control	Dexamethasone	54.2
2-Fluorohistidine	2-Fluorohistidine	6.6
2-Fluorohistidine	2-Fluorohistidine + dexamethasone	10.4

8-fold increase in aryl hydrocarbon hydroxylase activity. This increase was blocked by 3 mM 2-fluorohistidine, which also reduced aryl hydrocarbon hydroxylase activity in cells not treated with inducer.

Effects of 2-fluorohistidine on spontaneous increase in ornithine decarboxylase in midpregnant mouse mammary explants. Ornithine decarboxylase in explants of midpregnant mouse mammary tissue increases spontaneously at the start of culture, without the addition of an inducer, by a process which appears to involve cyclic AMP (23). Enzyme activity rapidly reaches a peak and returns to control levels within 6 hr. After 3 hr of incubation it was found that ornithine decarboxylase activity increased 8-fold (Table 5). This increase was blocked only slightly by 2-fluorohistidine. Identical inhibition resulted from treatment with an equal concentration of histidine, indicating that this minor inhibition was not specific to the fluorinated analogue.

Effect of 2-fluorohistidine on spontaneous increase in ornithine decarboxylase in pineal glands. We have recently ob-

TABLE 4

Effect of 2-fluorohistidine on benz[a]anthracene induction of aryl hydrocarbon hydroxylase in Hepa-1 cells

Hepa-1 cells were obtained from a stock maintained in culture and transferred to 15 × 60 mm Falcon dishes containing 3 ml of Waymouth MAB medium with 10% fetal calf serum for 72 hr, until 75–80% confluency was achieved (11). The medium was then replaced with 2 ml of BGJ[b] containing 0.1 mM histidine for the treatments detailed below. The concentration of 2-fluorohistidine was 3 mM. Benz[a]anthracene was added in a concentrated solution resulting in a final concentration of 13 μM. Data are present as the average of the means of duplicate determinations (11) performed on duplicate cultures. The individual values did not differ by more than 5%.

Treatment in culture		Aryl hydrocarbon hydroxylase activity
0–3 hr	3–11 hr	
		pmoles/mg protein/min
Control	Control	19
Control	Benz[a]anthracene	159
2-Fluorohistidine	2-Fluorohistidine	6.2
2-Fluorohistidine	2-Fluorohistidine + benz[a]anthracene	21

TABLE 5

Effect of 2-fluorohistidine on spontaneous increase of ornithine decarboxylase activity in midpregnant mouse mammary explants

Midpregnant mouse (CH_3/Hen) mammary explants were prepared by mincing mammary tissue as described (11). Tissue which was not incubated was prepared for enzyme assay immediately. The culture medium used was M199 (histidine concentration, 0.175 mM). Data are presented as the mean enzyme activity in two cultures. Enzyme activity in each culture is based on duplicate determinations, which were within 1% of the mean.

Treatment	Ornithine decarboxylase activity
	pmoles $^{14}CO_2$ produced/mg tissue/hr
Not incubated	5.0
Incubated (0–3 hr)	
Control	41.2
+ Histidine (3 mM)	33.6
+ 2-Fluorohistidine	33.2

served that the activity of ornithine decarboxylase in cultured pineal glands increases after glands are placed in culture.[7] This increase occurs in chronically denervated pineal glands and, as is true of the effect in the mammary tissue explants, occurs without the addition of an inducer. This increase is first detected after about 6 hr of culture and reaches a maximum at about 11 hr. At 24 hr of culture, enzyme activity has returned to baseline values.

Pineal glands incubated for 12 hr in 0.1 M histidine-containing medium exhibited a 10-fold increase in ornithine decarboxylase activity as compared to glands which were not cultured (Table 6). In the presence of 3 mM 2-fluorohistidine the increase in ornithine decarboxylase activity was reduced about 50%. In the same experiment, isoproterenol-dependent induction of *N*-acetyltransferase activity was blocked about 70% by 2-fluorohistidine.

Effects of 2-fluorohistidine on gross protein and RNA syntheses in cultured pineal gland. We determined whether 2-fluorohistidine would nonspecifically inhibit cellular metabolism by incubating pineal glands with this compound for 3, 27, or 51 hr and adding [^{14}C]leucine and [^{3}H]uridine during a 3-hr test to estimate the synthesis of macromolecules (Table 7). After 3 or 27 hr of 2-fluorohistidine treatment no more than a 20–25% reduction in the incorporation of [^{14}C]leucine into protein was detected, and slight inhibition of [^{3}H]uridine incorporation into RNA occurred. Only after 51 hr of 2-fluorohistidine treatment were substantial inhibitory effects of the analogue on both [^{14}C]leucine and [^{3}H]-uridine incorporation apparent. In short-term experiments (3 hr), [^{3}H]leucine uptake was not decreased by 3 mM 2-fluorohistidine treatment, suggesting that the small inhibitory effects observed at 3 and 27 hr were not due to inhibition of uptake.[8]

We also measured the long-term effects of 2-fluorohistidine on the activity of pineal hydroxyindole *O*-methyltransferase (Table 8). No inhibitory effect of the ana-

[7] D. C. Klein and T. Oka, unpublished observations.

[8] D. C. Klein and J. L. Weller, unpublished observations.

TABLE 6

Effect of 2-fluorohistidine on spontaneous increase of ornithine decarboxylase activity in pineal glands

Pineal organ cultures were prepared as described in Table 2. Each value is the mean ± standard error of four to six determinations. Glands which were not incubated were prepared for enzyme assay immediately prior to the start of culture. For the determination of ornithine decarboxylase activity, individual glands were sonicated in 100 μl of the assay buffer (23) immediately after being removed from culture. The homogenate was stored at −20° for 24 hr, thawed, and transferred to a reaction vial for enzyme assay. The final volume of the reaction was 125 μl and contained pyridoxal phosphate (40 μM), dithiothreitol (5 mM), EDTA (4 mM), Tris-HCl (50 mM), and DL-[1-^{14}C]ornithine (20 μM; specific activity, 43 Ci/mole). Other details of the assay have been described (23).

Treatment	*N*-Acetyltransferase activity	Ornithine decarboxylase activity
	nmoles/gland/hr	*pmoles $^{14}CO_2$ produced/gland/hr*
Not incubated	0.15 ± 0.02	6.38 ± 0.86
Incubated		
Control (0–11 hr)		67.45 ± 6.47
2-fluorohistidine		34.52 ± 9.5″
Control (0–3 hr) + isoproterenol (3–11 hr)	13.24 ± 4.17	
2-Fluorohistidine (0–11 hr) + isoproterenol (3–11 hr)	4.37 ± 0.84	

logue on this enzyme was detectable after 24 or 48 hr of treatment.

Effects of 2-fluorohistidine on incorporation of [^{14}C]histidine into macromolecules. Pineal glands were incubated with [^{3}H]leucine and [^{14}C]histidine to determine the relative effects of 2-fluorohistidine on the incorporation of these amino acids into proteins (Table 9). Again a 20–25% inhizition of [^{3}H]leucine incorporation was detected in 2-fluorohistidine-treated glands, confirming the previous finding of only a small inhibitory effect on the incorporation of [^{3}H]leucine. The inhibition of [^{14}C]histidine incorporation was more dramatic; in the presence of [^{14}C]-histidine (7 μM) this parameter was decreased 80% by 3 mM 2-fluorohistidine.

The finding of a much greater 2-fluorohistidine inhibition of [^{14}C]histidine incorporation than of [^{3}H]leucine incorporation led us to consider the possibility that the analogue might compete with histidine in protein synthesis. If this were so, the inhibitory effects of 2-fluorohistidine might be overcome with high concentrations of histidine (Fig. 1). In a series of studies both [^{14}C]histidine incorporation into total protein and *N*-acetyltransferase activity in glands treated with 2-fluorohistidine and isoproterenol increased as the concentration of histidine was increased from 7 μM to 3 mM. In the presence of 3 mM histidine no effect of 2-fluorohistidine was seen on either the stimulation of *N*-acetyltransferase activity or [^{14}C]histidine incorporation into protein. We also found that the small inhibitory effect of 3 mM 2-fluorohistidine on [^{3}H]leucine incorporation did not occur in the presence of 3 mM histidine.[8]

Studies on mechanism of action of 2-fluorohistidine. 2-Fluorohistamine, a possible physiological metabolite of 2-fluorohistidine via decarboxylation, was found not to inhibit the isoproterenol induction of *N*-acetyltransferase activity (Table 10). In addition, no evidence emerged which indicated that 2-fluorohistidine inhibited *N*-acetyltransferase during enzyme assay (Table 11), in that homogenates of glands treated with 2-fluorohistidine and isoproterenol did not contain an inhibitor of *N*-acetyltransferase activity, or that 2-fluorohistidine rapidly and abruptly inactivated molecules of *N*-acetyltransferase present in glands which had been treated for 3 hr with isoproterenol (Fig. 2).

Reversibility of inhibitory effects of 2-fluorohistidine. We determined whether tissue could recover from treatment with 2-fluorohistidine by treating pineal glands with the analogue for 3 hr, incubating the tissue for 24 hr in the absence of 2-fluorohistidine, and then testing for isoproterenol responsiveness (Table 12). A complete response occurred, indicating that the effects of a 3-hr treatment with 2-fluorohistidine are reversible.

DISCUSSION

It is clear from these results that 2-fluorohistidine can decrease the adrenergic-

TABLE 7

Effect of long-term 2-fluorohistidine treatment in organ culture on incorporation of radioactivity into macromolecules in pineal glands incubated with [^{14}C]leucine and [^{3}H]uridine for 3 hr

Pineal glands were removed between 10:00 and 12:00 a.m. and placed in organ culture. The culture medium contained no histidine. The concentration of 2-fluorohistidine was 3 mM. [^{3}H]Uridine (3 μM; specific activity, 21 Ci/mole) and [^{14}C]leucine (0.38 mM; 8.62 Ci/mole) were present only for the final 3 hr of the incubation period. Isoproterenol was added in 5 μl of 0.01 mM HCl to a final concentration of 10 μM. At the end of the experiment glands were sonicated in 100 μl of buffer, pH 6.9, at 4°. A 25-μl sample was used for precipitation of trichloracetic acid-insoluble material, using a filter disc method as previously described (15). Each precipitate was washed several times with trichloracetic acid and with ether. Values, which are based on four or five glands, were computed from the specific activity of the radioactive amino acid and the radioactivity per gland. Data are means ± standard errors.

Treatment in organ culture	Total incubation time	Radioactivity incorporated	
		[^{14}C]Leucine	[^{3}H]Uridine
	hr	*nmole/gland*	*pmoles/gland*
Control	3	0.34 ± 0.041	1.04 ± 0.098
2-Fluorohistidine	3	0.27 ± 0.031	0.87 ± 0.167
Control	27	0.37 ± 0.018	1.14 ± 0.177
2-Fluorohistidine	27	0.28 ± 0.018[a]	1.30 ± 0.122
Control	51	0.43 ± 0.035	1.47 ± 0.095
2-Fluorohistidine	51	0.10 ± 0.014[b]	0.81 ± 0.081[b]

[a] Significantly less ($p < 0.01$) than the value for control glands incubated for 27 hr, but not significantly less than [^{14}C]leucine incorporation into glands treated for 3 hr 2-fluorohistidine.

[b] Significantly lower than the value for control glands incubated for 4 hr ($p < 0.01$).

TABLE 8

Lack of effect of long-term treatment with 2-fluorohistidine on hydroxyindole O-methyltransferase activity

Pineal glands were removed between 10:00 and 12:00 a.m. and placed in organ culture in medium containing 0.1 mM histidine, or frozen on solid CO_2. Each value is based on four glands, and is presented as the mean ± standard error. The concentration of 2-fluorohistidine was 3 mM.

Treatment	Total incubation time	Hydroxyindole O-methyltransferase activity
	hr	*pmoles/gland/hr*
Not incubated	0	69.2 ± 11.1
Incubated		
Control	24	109.2 ± 19.1
2-Fluorohistidine	24	132.8 ± 8.4
Control	48	88.8 ± 6.0
2-Fluorohistidine	48	92.8 ± 8.1

cyclic AMP stimulation of pineal *N*-acetyltransferase activity, the spontaneous increase of pineal ornithine decarboxylase, the steroid induction of fetal liver tyrosine aminotransferase activity, and the benz[*a*]anthracene induction of Hepa-1 cell aryl hydrocarbon hydroxylase activity. Consideration of how this decrease is brought about presents interesting possibilities.

First, it is apparent that 2-fluorohistidine does not have a rapid toxic effect, since neither protein synthesis nor RNA synthesis in the pineal gland was significantly inhibited after 24 hr of 2-fluorohistidine treatment. Thus, in contrast to the effects of cycloheximide, which blocks enzyme induction by blocking all protein synthesis (5, 10, 15, 21, 23), 2-fluorohistidine does not act by nonspecifically blocking the synthesis of protein. The explanation of the inhibitory effect is, according to our data on the pineal gland, more complex than classical forms of competitive or noncompetitive enzyme inhibition. In addition, it was demonstrated that 2-fluorohistidine need not be decarboxylated to be active.

Second, one mechanism of inhibition consistent with our observations assumes that 2-fluorohistidine is incorporated into newly synthesized protein, as a replacement for histidine. Thus we found that 2-fluorohistidine inhibited the incorporation of [^{14}C]histidine into protein and that the effects of the analogue on [^{14}C]histidine

TABLE 9

Effect of 2-fluorohistidine on incorporation of [^{3}H]leucine and [^{14}C]histidine into protein of pineal glands

Pineal glands were removed between 10:00 and 12:00 a.m. and placed in organ culture. The culture medium contained 0.38 mM [^{3}H]leucine (specific activity, 51.2 Ci/mole) and 1 mM [^{14}C]histidine (24 Ci/mole). The concentration of 2-fluorohistidine was 3 mM. Glands were sonicated in 100 μl of 2 mM penicillamine in 0.01 M sodium phosphate buffer, pH 6.9, at 4°, to preserve *N*-acetyltransferase activity during handling. A 50-μl sample was used for enzyme assay, and a 25-μl sample was used for trichloracetic acid precipitation. For further details, see the legend to Table 7.

Treatment in organ culture	Radioactivity incorporated		*N*-Acetyltransferase activity
	[^{3}H]Leucine	[^{14}C]Histidine	
	nmoles/gland		*nmoles/gland/hr*
Control (0–11 hr)	1.25 ± 0.071	0.30 ± 0.019	0.34 ± 0.068
2-Fluorohistidine (0–11 hr)	1.17 ± 0.158	0.12 ± 0.018[a]	0.18 ± 0.034
Control (0–3 hr); isoproterenol (3–11 hr)	1.65 ± 0.076	0.41 ± 0.022	14.3 ± 0.034
2-Fluorohistidine (0–11 hr); isoproterenol (3–11 hr)	1.43 ± 0.075	0.12 ± 0.005[b]	3.68 ± 0.548[b]

[a] Statistically significantly less than control value ($p < 0.01$).

[b] Statistically significantly less than the group treated with isoproterenol alone ($p < 0.01$).

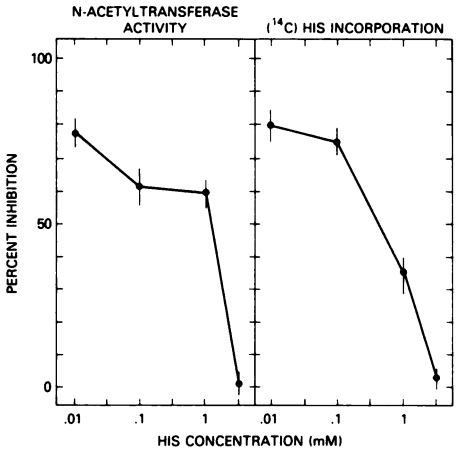


FIG. 1. *Effect of histidine (HIS) on N-acetyltransferase activity and [^{14}C]histidine incorporation into proteins in pineal glands treated with isoproterenol and 2-fluorohistidine*

Data were collected from four experiments; a different concentration of histidine was used in each. Percentage inhibition was calculated as follows: 100 × [isoproterenol value − (isoproterenol + 2-fluorohistidine value)] ÷ isoproterenol value. In each experiment four glands were treated with 10 μM isoproterenol, and four other glands were treated with 10 μM isoproterenol and 3 mM 2-fluorohistidine.

incorporation and enzyme activity could be reversed by treatment with an equal concentration of histidine. It seems quite possible that replacement of histidine by 2-fluorohistidine in newly synthesized protein would result in faulty enzyme proteins. If 2-fluorohistidine were incorporated into protein it could substitute for histidine at catalytic sites, thus producing inactive enzyme molecules. While this might explain the effects of 2-fluorohistidine on pineal *N*-acetyltransferase, other consequences of 2-fluorohistidine incorporation such as changes in enzyme conformation, must be considered. The substitution of 2-fluorohistidine for histidine at a regulatory site of an enzyme could also result in loss of enzyme activity. For example, it appears that specific protein kinases phosphorylate histidine moieties in histones (24, 25), and probably other proteins. Fluorine substitution on the imidaz-

TABLE 10

Effect of 2-fluorohistamine on isoproterenol-induced pineal N-acetyltransferase activity

Pineal glands were removed between 10:00 and 12:00 a.m. and placed in organ culture for 48 hr before the indicated drug treatment. Each value is based on four glands. Data are means ± standard errors. The concentrations of 2-fluorohistamine and isoproterenol were 3 mM and 10 μM, respectively

Treatment in organ culture (48–54 hr)	*N*-Acetyltransferase activity
	nmoles/gland/hr
Control	0.10 ± 0.012
2-Fluorohistamine	0.25 ± 0.056
Isoproterenol	4.5 ± 1.18
2-Fluorohistamine + isoproterenol	3.1 ± 0.67

TABLE 11

Studies with broken cell preparations

Isoproterenol-treated and isoproterenol- and 2-fluorohistidine-treated glands were obtained as described in Table 2. Histidine-free culture medium was used. Sonicates (four glands per 225 μl) and 2-fluorohistidine were prepared in 0.1 M sodium phosphate buffer, pH 6.9, at 40°. Tubes containing 1 mM [^{14}C]acetyl-CoA (1 Ci/mole) and 20 mM tryptamine HCl in 50 μl of buffer received the additions indicated below in 25-μl volumes. Buffer was added to bring the final volume to 100 μl when necessary. The final concentration of 2-fluorohistidine was 3 mM. The *N*-acetyltransferase activity of the isoproterenol-treated gland sonicate was 7.24 nmoles/gland/hr. The results of duplicate determinations are presented.

Additions to assay				*N*-[^{14}C]Acetyltryptamine formed
None	Isoproterenol-treated gland homogenate	Isoproterenol + 2-fluorohistidine-treated gland homogenate	2-Fluorohistidine	
				nmoles/20 min
+				0.033, 0.025
	+			1.10, 1.10
		+		0.246, 0.245
	+	+		1.29, 1.22
	+		+	1.20, 1.15
			+	0.041, 0.041

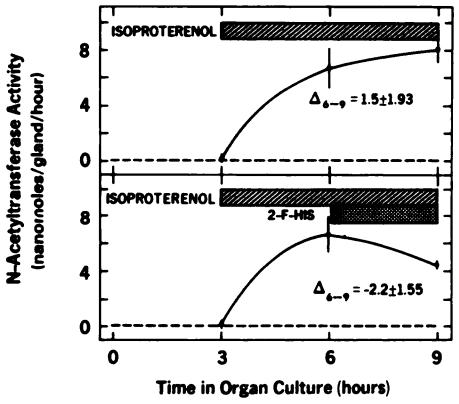


FIG. 2. *Effect of addition of 2-fluorohistidine (2-F-HIS) on induced N-acetyltransferase activity in organ culture*

Glands were incubated for a total of 9 hr. The horizontal bars indicate the duration of treatment periods. The concentration of isoproterenol was 10 μM, and the concentration of 2-fluorohistidine was 3 mM. Δ_{6-9} represents the change in enzyme activity between the 6th and 9th hours of culture. Each point is based on four glands. The vertical bars represent standard errors.- - -, untreated control values. Drug treatment was initiated by transferring glands to culture medium containing the indicated drug or drug combination.

ole of histidine should retard phosphorylation of the imidazole ring. Although we view this particular mode of action as a highly speculative one, it gains some interest upon consideration of two points. First, we found that 2-fluorohistidine inhibited the induction of three enzymes which clearly are regulated by cyclic AMP (5, 19, 22). Second, cyclic AMP may control the activities of these enzymes via a phosphorylation mechanism similar to other well-documented enzyme regulatory mechanisms involving phosphorylation (26). It is possible that induction involves both the synthesis of new enzyme molecules and the subsequent cyclic AMP-regulated activation of these newly formed enzyme molecules, in part via phosphorylation of histidine.

It was striking that the spontaneous increase in ornithine decarboxylase activity in the mouse mammary gland and the steady-state activity of hydroxyindole *O*-methyltransferase activity were not altered substantially by 2-fluorohistidine. In the case of mouse mammary gland ornithine decarboxylase, the increase in enzyme activity depends upon protein synthesis and appears to involve cyclic AMP (23). The lack of an effect of 2-fluorohistidine in the mouse mammary gland might be explained on the basis of a higher intracellular concentration of histidine, slower entry into cells, or a more rapid intracellular degradation of 2-fluorohistidine as compared to the pineal gland. It is also

TABLE 12

Recovery from 2-fluorohistidine treatment in organ culture

Pineal glands were removed between 10:00 and 12:00 a.m. and placed in organ culture for a 48-h incubation under control conditions. At 48 hr glands were transferred to fresh medium as indicated below. The concentration of isoproterenol was 10 μM, and the concentration of 2-fluorohistidine was 3 mM. The culture medium contained 0.1 mM histidine.

Treatment in organ culture			*N*-Acetyltransferase activity
48–51.5 hr	51.5–72 hr	72–75.5 hr	
			nmoles/gland/hr
Control			0.06 ± 0.004
Isoproterenol			4.9 ± 1.04
2-Fluorohistidine			1.2 ± 0.318
Isoproterenol + 2-fluorohistidine			0.92 ± 0.115
Control	Control		0.05 ± 0.013
2-Fluorohistidine	Control		0.06 ± 0.028
2-Fluorohistidine	Control	Isoproterenol	3.95 ± 0.592
Control	Control	Isoproterenol	3.53 ± 0.89

possible that the different culture medium used in this study explains the lack of an effect of 2-fluorohistidine, because the histidine concentration was almost twice as high (0.175 mM) as that used in most of the studies presented. In the case of pineal hydroxyindole *O*-methyltransferase activity, 2-fluorohistidine, even though incorporated into the polypeptide chain, may have been ineffective because it replaced a histidine which is not involved in enzyme action. Alternatively, the turnover of this enzyme may have been so low as to have precluded the demonstration of an effect on enzyme activity of the incorporation of any amino acid analogue into enzyme protein. It is not clear whether the regulation of hydroxyindole *O*-methyltransferase involves cyclic AMP. This prevents meaningful discussion of the effectiveness of 2-fluorohistidine relative to the role of cyclic AMP in the regulation of the activity of this enzyme.

Our observation that after 51 hr of 2-fluorohistidine treatment both protein and RNA synthesis were decreased indicates that although 2-fluorohistidine is not acutely toxic, it may have toxic effects after prolonged treatment. It seems possible that with such extended treatment, more and more cellular processes might be affected as the effects of 2-fluorohistidine F-HIS became more generalized. Assuming that 2-fluorohistidine acts primarily by substituting for histidine in proteins, any protein (e.g., hemoglobin) or enzyme (e.g., ribonuclease, trypsin) in which a histidine moiety plays an important functional or structural role could be influenced. Specificity would then be determined in part by the importance of histidine in the activity or stability of the enzyme and in enzyme turnover. Rapidly turning over protein would probably be influenced the most, whereas slowly turning over proteins would be the least affected. Assuming that 2-fluorohistidine is incorporated into protein by cells, it appears that this analogue may be a valuable tool in determining the role of histidine in enzyme action, in the action of histones, and in the function of proteins in general.

ACKNOWLEDGMENTS

We thank Drs. C. R. Creveling and Louis A. Cohen for their valuable discussions and advice during the course of this investigation. Special acknowledgment is made of the generous contributions of Dr. Howard Eisen, who conducted the liver explant culture experiment.

REFERENCES

1. Shive, W. & Skinner, C. G. (1963) in *Metabolic Inhibitors, a Comprehensive Treastise* (Hochster, R. M. & Quastel, J. H., eds.) Vol. 1, pp. 1–60, Academic Press, New York.
2. Weygand, F. & Oettmeir, W. (1970) *Russ. Chem. Rev.*, **39**, 290–300.
3. Kirk, K. L., Nagai, W. & Cohen, L. A. (1973) *J. Am. Chem. Soc.*, **95**, 8389–8392.
4. Klein, D. C. (1974) in *The Neurosciences, Third*

Study Program (Schmitt, F. O. & Worden, F. G., eds.), pp. 509–516, MIT Press, Cambridge, Mass.
5. Klein, D. C. & Weller, J. L. (1973) *J. Pharmacol. Exp. Ther.*, **186**, 516–527.
6. Jencks, W. P. (1969) *Catalysis in Chemistry and Enzymology*, pp. 163–168, McGraw-Hill, New York.
7. Klein, D. C. & Weller, J. (1970) *In Vitro*, **6**, 197–204.
8. Parfitt, A. P., Weller, J. L. & Klein, D. C. (1975) *Neuropharmacology*, **15**, in press.
9. Eisen, H. J., Glinsmann, W. H., & Sherline, P. (1973) *Endocrinology*, **92**, 584–588.
10. Gielen, J. E. & Nebert, D. W. (1972) *J. Biol. Chem.*, **247**, 7591–7598.
11. Benedict, W. F., Gielen, J. E., Owens, I. S., Niwa, A. & Nebert, D. W. (1973) *Biochem. Pharmacol.*, **22**, 2766–2769.
12. Jurgens, W. G., Stockdale, F. E., Topper, Y. J. & Elias, J. J. (1965) *Proc. Natl. Acad. Sci. U. S. A.*, **54**, 629–634.
13. Diamondstone, T. I. (1966) *Anal. Biochem.*, **16**, 395–399.
14. Russel, D. H. & McVicker, T. A. (1972) *Biochem. J.*, **130**, 71–77.
15. Klein, D. C., Yuwiler, A., Weller, J. L. & Plotkins, S. (1973) *J. Neurochem.*, **21**, 1261–1272.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265–275.
17. Deguchi, T. & Axelrod, J. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, **69**, 2547–2550.
18. Kenny, F. T. (1962) *J. Biol. Chem.*, **237**, 1610–1614.
19. Tomkins, G. M., Gelehrter, T. D., Granner, D., Martin, D., Jr., Samuels, H. H. & Thompson, F. B. (1969) *Science*, **166**, 1474–1476.
20. Holton, D. & Kenny, F. T. (1967) *J. Biol. Chem.*, **242**, 4372–4377 (1967).
21. Kenny, F. T. (1970) in *Mammalian Protein Metabolism* (Munro, H. N., ed.), Vol. 4, pp. 131–145, Academic Press, New York.
22. Yamasaki, H., Huberman, E. & Sachs, L. (1975) *J. Biol. Chem.*, **250**, 7766–7770.
23. Oka, T. & Perry, J. (1976) *J. Biol. Chem.*, **251**, in press.
24. Chen, C. C., Smith, D. L., Bruegger, B. B., Halprin, R. M. & Smith, R. A. (1974) *Biochemistry*, **13**, 3785–3789.
25. Smith, D. L., Chen, C.-C., Bruegger, B. B., Holtz, S. L., Halpern, R. M. & Smith, R. A. (1974) *Biochemistry*, **13**, 3780–3785.
26. Segal, H. L. (1973) *Science*, **180**, 25–32.