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Effect of Hydrocortisone on Histidine Decarboxylase Activity in Rat Stomach

NORIAKI IMANISHI, EIJI OHMORI, KIMIO YATSUNAMI and ATSUSHI ICHIKAWA*

Department of Health Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606, Japan

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Hydrocortisone-stimulated synthesis of histidine decarboxylase (EC 4.1.1.22) was studied in rat stomach. Single or daily administration of hydrocortisone increased the histidine decarboxylase activity but not the histamine content in the fundic glands of stomachs from fed and fasted rats with or without adrenalectomy, and also in those from adrenalectomized fasted rats with concomitant treatment with pirenzepine, an inhibitor of gastrin secretion. The increase in histidine decarboxylase activity was maximal at 2 h after the administration of hydrocortisone, and the activity had returned almost to the pretreatment level by 4 h after the administration. Furthermore, the increase was not augmented by daily administration of hydrocortisone for 3 d. Cycloheximide prevented the increase in gastric histidine decarboxylase due to hydrocortisone. Rat fundic gland tissue was found to possess binding sites for [^{3}H]glucocorticoids in the cytosol. These results suggest that hydrocortisone stimulates the *de novo* synthesis of histidine decarboxylase in the fundic gland of rat stomach, though the response is not mediated by changes in the gastrin level.

Keywords——hydrocortisone; histidine decarboxylase; histamine; rat stomach; induction

Histamine synthesis in the stomach is controlled by various secretogogues, such as gastrin,[1] acetylcholine,[2] insulin[3] and single feeding.[1] Gastrin stimulates histamine synthesis by increasing the *de novo* synthesis of histidine decarbxylase in the stomach,[4] while other secretogogues such as acetylcholine, insulin and single feeding are believed to act through the change of gastrin level, thereby increasing histamine synthesis.[5]

In addition, administration of hydrocortisone to mice[6] and rats[7,8] is known to stimulate gastric histamine synthesis, while inconsistent results of adrenalectomy in rats have been reported.[7,8] However, little is known about the involvement of any other secretogogues in the corticoid-stimulated increase of histamine synthesis. Therefore, we have studied the effects of adrenalectomy, feeding and the gastrin level on hydrocortisone-induced histidine decarboxylase activity in rat stomach to gain more insight into the roles of corticoids in gastric histamine metabolism.

Experimental

Materials——The chemicals used were obtained from the following sources: hydrocortisone sodium phosphate, L-histidine, histamine, pyridoxal-5′ phosphate, *o*-phthalaldehyde and cycloheximide, from Nakarai Chemical Co. (Kyoto, Japan); polyethylene glycol and aminoguanidine, from Wako Chemical Co. (Osaka, Japan); Amberlite CG-50 from Organo Co. (Tokyo, Japan); phenylmethylsulfonyl fluoride and pentagastrin (*N-tert*-Boc-alanyl-L-tryptophanyl-L-methionyl-L-aspartyl-L-phenylalanine amide) from Sigma Chemical Co. (St. Louis, MO., U.S.A.); pirenzepine from Boehringer Ingelheim.

Animals——Male Wistar rats (180—200 g), from the Shizuoka Experimental Animals and Agricultural Cooperative, Hamamatsu, Japan, were used. Bilateral adrenalectomy was carried out under ether anesthesia *via* the translumbar route. The adrenalectomized rats were maintained on a 0.9% NaCl solution, as a drinking fluid, and a laboratory chow. Six days after the operation, some animals were, if necessary, deprived of food but allowed free

access to the 0.9% NaCl solution for a further 24 h. Sham-operated controls were included in the above. For steroid treatment, hydrocortisone sodium phosphate (10 or 50 mg/kg body weight) was given intraperitoneally (i.p.) once or once daily for 3 d. The third dose of the steroid was given at 2 h before sacrifice.

Preparation of Tissue Homogenates——After decapitation, the stomachs and other tissues of the rats were quickly removed. The fundic gland was separated from the forestomach. To determine the enzyme distribution in response to hydrocortisone, the stomach was carefully separated into four anatomical parts; cardia, forestomach, fundic gland and pylorus. Each tissue was carefully sliced and then homogenized with 7 vol. of ice-cold 0.1 M potassium phosphate buffer (pH 6.8) containing 10 μM pyridoxal 5'-phosphate, 0.2 mM dithiothreitol and 1% polyethylene glycol (average molecular weight, 300) with a Teflon homogenizer in an ice both. Homogenates were centrifuged at 30000 × *g* for 20 min. The supernatants were dialyzed three times against 50 vol. of the above-mentioned buffer.

Assays for Histidine Decarboxylase Activity and Histamine Level——Histidine decarboxylase activity was measured according to the method of Watanabe *et al.*[9] Briefly, aliquots of the supernatants obtained from the tissue homogenates were incubated at 37°C for various times in an incubation mixture (1.0 ml) containing 0.25 mM histidine, 100 μg/ml phenylmethylsulfonyl fluoride and 0.2 mM aminoguanidine. The reactions were stopped by adding 2.1 mM $HClO_4$ and then clear supernatants were obtained from the mixture by centrifugation. Histamine was purified on a column of Amberlite CG-50, type I, equilibrated with 0.2 M sodium phosphate buffer, pH 6.5, and it was measured fluorometrically using *o*-phthalaldehyde.[10] Assays were carried out in parallel with a mixture containing 1 nmol of histamine as an internal standard. The recovery of histamine through these steps was more than 90% in all cases. Protein was measured by the method of Lowry *et al.*[11] with bovine serum albumin as a standard.

Assay for Steroid Binding in the Cytosol Fraction of Rat Fundic Gland and Serum Cortisol Level——The minced fundic glands from fed rats were suspended in 3 vol. of 10 mM Tris–HCl buffer (pH 7.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 12 mM thioglycerol and 0.5 mM antipain, homogenized with a Polytron homogenizer in an ice bath and then centrifuged at 100000 × *g* for 60 min to obtain the supernatant (cytosol) fraction. Cytosol binding of [^{3}H]dexamethasone was assayed essentially according to the method described previously.[12]

Serum cortisol (unconjugated cortisol and corticosterone) was measured by the fluorometric method of Nielsen and Asfeldt.[13]

Student's *t* Test——Student's *t* test was used to determine the significance of differences between groups.[14]

Results

Effects of Hydrocortisone on the Histidine Decarboxylase Activity and Histamine Content in the Stomachs of Fed and Fasted Rats with or without Adrenalectomy

With adrenalectomy, the cortisol level in the serum decreased as follows: adrenalectomized rats 26.9 ± 4.1 ng/ml ($n=7$) *versus* sham-operated rats 279 ± 38 ng/ml ($n=7$), $p<0.01$. The effects of adrenalectomy and hydrocortisone treatment on the histidine decarboxylase activity and histamine content are shown in Table I. A slight change in the gastric histidine decarboxylase activity was seen in the stomachs from adrenalectomized rats compared with in those from sham-operated animals. On the other hand, hydrocortisone markedly increased the histidine decarboxylase activity but not the histamine content in the stomachs from both fed and fasted rats with adrenalectomy treatment and in those from sham-operated, fed rats. However, the increase in histidine decarboxylase activity was no greater after daily treatment for 3 d with hydrocortisone at a dose of 10 mg/kg body weight or 50 mg/kg body weight, than after a single administration of each dose.

The increase in gastric histidine decarboxylase activity, but not that in histamine content, was time-dependent after administration of hydrocortisone; it increased to reach a peak by 2 h but had returned almost to the pre-administration level by 4 h after the administration (Fig. 1). No tissue other than the stomach, such as the liver, small intestine, spleen or skin showed a response to hydrocortisone treatment, as was the case with histidine decarboxylase activity (data not shown).

The effect of hydrocortisone on histidine decarboxylase activity was compared in four anatomically different sections of the stomachs from adrenalectomized, fasted rats (Table II). More than 80% of the histidine decarboxylase activity in the stomach was found to be localized in the fundic gland. In addition, hydrocortisone specifically stimulated the activity by 2.5-fold in this section.

TABLE I. Effects of Hydrocortisone on the Histidine Decarboxylase Activity and Histamine Level in Stomachs from Fed and Fasted Rats with or without Adrenalectomy

Treatment (dose × times)	Histidine decarboxylase activity (pmol/min/g tissue)		Histamine level (nmol/g tissue)	
	Fed	Fasted	Fed	Fasted
Sham-operated				
Control	544±113	72±22	388±58	342±98
Hydrocortisone 10 mg/kg B.W. × 3	840± 29[a)]	168±46[a)]	313±67	344±88
Adrenalectomized				
Control	480±191	71±10	247± 9	304±33
Hydrocortisone				
10 mg/kg B.W. × 3	992±112[a)]	133±29[a)]	335±43	271±57
50 mg/kg B.W. × 3	—	135±10[c)]	—	252±18
10 mg/kg B.W. × 1	—	153±29[a)]	—	218±14[a)]
50 mg/kg B.W. × 1	—	149±51[c)]	—	228±32

Adrenalectomized and sham-operated rats were injected intraperitoneally with hydrocortisone sodium phosphate (10 mg/kg, once or once daily for 3 d) and were then fed freely or fasted for 24 h before sacrifice. At 2 h after the third steroid injection each rat was decapitated and plasma was collected, and the stomach was removed, then homogenized with a Teflon homogenizer. Aliquots of the samples obtained were subjected to both histamine level and histidine decarboxylase activity determinations. Each value represents the mean±S.E. for twelve samples. Statistical significance: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$. B.W.: body weight.

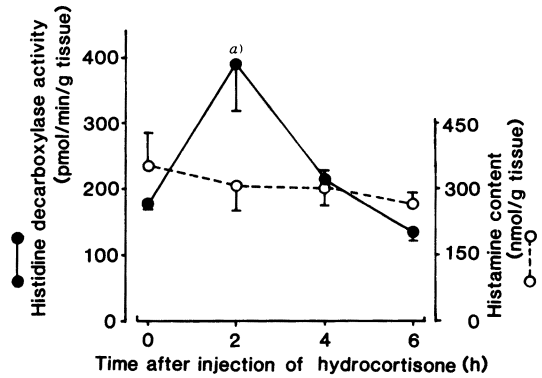

Fig. 1. Time Courses of Gastric Histidine Decarboxylate Activity and Histamine Content in the Fundic Glands of Stomachs from Fasted Rats for 6 h after Addition of 10 mg/kg Body Weight of Hydrocortisone

Each value represents the mean±S.E. for four samples. Statistical significance: *a*) $p<0.05$.

Effects of Fasting, Pentagastrin and Pirenzepine on Hydrocortisone-Stimulated Activity of Histidine Decarboxylase

As shown in Table III, gastric histidine decarboxylase activity decreased drastically on deprivation of food for 24 h. The residual enzyme activity was not further affected by treatment with pirenzepine, an inhibitor of gastrin secretion.[15)] On the other hand, the level of gastric histidine decarboxylase activity in fasted rats considerably recovered with a single administration of pentagastrin at 1.5 h before sacrifice. Hydrocortisone, even in pirenzepine-treated, fasted rats, showed a stimulatory effect on histidine decarboxylase activity. Pentagastrin caused additional stimulation of histidine decarboxylase activity in the presence of hydrocortisone.

Effect of Cycloheximide on Gastric Histidine Decarboxylase Activity in Rats Treated with or without Hydrocortisone

The administration of cycloheximide alone significantly reduced the gastric histidine

TABLE II. Effects of Hydrocortisone on the Histidine Decarboxylase Activity and Histamine Level in Rat Stomach

Tissues	Treatment	Histidine decarboxylase activity (pmol/min/g tissue)	(pmol/min/stomach)	(% of total)	
Cardia	Control	33.7 ± 0.1	1.57±0.12	3.63[b]	
	Hydrocortisone	29.1 ±11.8	1.01±0.38		0.95[c]
Forestomach	Control	8.84± 1.82	2.77±0.70	6.40	
	Hydrocortisone	8.51± 3.10	2.92±1.06		2.77
Fundic gland	Control	59.2 ±14.8	37.7 ±9.4	87.1	
	Hydrocortisone	165 ± 4.6[a]	101 ±2.8[a]		95.5
Pylorus	Control	7.26± 1.71	1.22±0.44	2.82	
	Hydrocortisone	5.24± 1.77	0.88±0.15		0.83

Adrenalectomized and fasted rats were used. Other experimental procedures were the same as in Table I. After being opened along the greater curvature, each stomach was dissected into four anatomically different sections: cardia, forestomach, fundic gland and pylorus. Each value represents the mean±S.E. for five samples. Statistical significance; *a*) $p<0.05$: *b*, *c*) Represents percent of total activity in the stomach for control animals *b*) or for hydrocortisone-treated animals *c*) (100%=43.3 *b*) or 106 *c*) pmol/min/stomach, respectively).

TABLE III. Effect of Hydrocortisone on Histidine Decarboxylase Activity of Fundic Glands from Adrenalectomized Rats Treated with Pentagastrin or Pirenzepine

Treatment	Hydrocortisone	Histidine decarboxylase activity (pmol/min/g tissue)	*p*
Fed rats			
(1) None	−	434±121	
(2) None	+	891±195	(1)−(2)[a]
Fasted rats			
(3) None	−	127± 14	(1)−(3)[b]
(4) None	+	235± 43	(3)−(4)[a]
(5) Pentagastrin	−	301± 26	(3)−(5)[d]
(6) Pentagastrin	+	415± 19	(5)−(6)[c]
			(4)−(6)[a]
(7) Pirenzepine	−	158± 45	(3)−(7) N.S.
(8) Pirenzepine	+	343± 41	(7)−(8)[b]
			(4)−(8) N.S.

Adrenalectomized and fasted rats were injected intraperitoneally with hydrocortisone sodium phosphate (10 mg/kg, once daily for 3 d). At 24 h before sacrifice, all rats were deprived of food. Some rats were given pirenzepine (27 mg/kg, three times orally at 8 h intervals, the third administration being made at 2 h before sacrifice), and the others pentagastrin intraperitoneally as a single injection at 1.5 h before sacrifice. Each value represents the mean±S.E. for seven rats. Statistical significance: *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.001$. N.S.: not significant.

decarboxylase activity, to 18% of the control level, in adrenalectomized, fasted rats. When cycloheximide was added along with the third dose of hydrocortisone at 1.5 h before sacrifice, the steroid-induced increase in histidine decarboxylase activity was completely inhibited (Table IV).

Glucocorticoid Binding to the Cytosol of the Fundic Gland of Rat Stomach

Scatchard plot analysis of the total [^{3}H]dexamethasone binding to the cytosol fraction of fundic gland tissue indicated the existence of one type of binding site with a K_d value of 6.0×10^{-9} M and a binding capacity of 57 fmol/mg of protein (Fig. 2a). Among various steroids tested, glucocorticoids effectively replaced [^{3}H]dexamethasone (Fig. 2b).

TABLE IV. Effect of Cycloheximide on Histidine Decarboxylase Activity Stimulated by Hydrocortisone

Treatment	Histidine decarboxylase activity (pmol/min/g tissue)	p
(1) Control	322 ±59	
(2) Cycloheximide	57.2±13.9	(1)−(2)[b]
(3) Hydrocortisone	442 ±47	(1)−(3)[a]
(4) Hydrocortisone+cycloheximide	46.3±20.5	(1)−(4)[b]
		(3)−(4)[c]
		(2)−(4) N.S.

Adrenalectomized and fasted rats were used. The experimental procedures were the same as in Table I except for the concomitant administration of cycloheximide (50 mg/kg, intraperitoneally) with the third dose of hydrocortisone at 2 h before sacrifice. Each value represents the mean±S.E. for four samples. Statistical significance: *a*) $p<0.01$, *b*) $p<0.001$. N.S.: not significant.

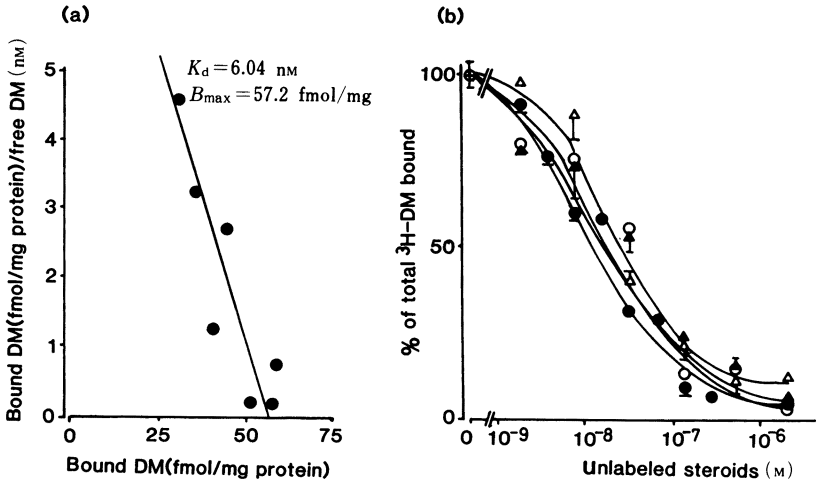

Fig. 2. Analysis of [^{3}H]Dexamethasone Binding to the Cytosol Fraction of the Fundic Gland of Rat Stomach

The cytosol fraction (1 mg protein/ml) was incubated for 2 h at 4 C with 7 nM [^{3}H]dexamethasone in the presence of various concentrations of unlabeled dexamethasone or other steroids. (a) Scatchard plots of [^{3}H]dexamethasone binding to the cytosol fraction. (b) Specificity of [^{3}H]dexamethasone binding. Symbols: ●, dexamethasone; ○, hydrocortisone; ▲, triamcinolone; △, prednisolone. Each value represents the mean±S.E. for four samples.

Discussion

The present results showed that hydrocortisone stimulated tissue-specifically the *de novo* synthesis of histidine decarboxylase in rat stomach. This observation is basically consistent with those of previously reported studies,[6-8] but extended them in the following respects.

Firstly, the effect of hydrocortisone on histidine decarboxylase activity in rat stomach is probably not mediated by changes in the serum gastrin level, because hydrocortisone stimulated histidine decarboxylase activity in the stomachs from fed rats (Table I) and in those from pirenzepine-treated, fasted rats (Table III) as well. It is known that fasting in rats abolishes most of the effect of the endogenous gastrin on the histidine decarboxylase activity in rat stomach,[5] and that pirenzepine inhibits the gastrin secretion in the rat stomach.[15]

On the other hand, adrenalectomy was found not to affect the gastric histidine

decarboxylase activity or histamine content (Table I), although it clearly depressed the serum cortisol level to as low as one-tenth of the control value. This means that such a low concentration of serum cortisol, formed probably in extra-adrenal tissues, might be sufficient to maintain the basal level of histidine decarboxylase activity in the stomachs of adrenalectomized, fasted rats.

On hydrocortisone treatment, the histamine content in the stomach did not change, while the histidine decarboxylase level apparently increased (Table I). A similar phenomenon was also reported in the case of gastrin administration to rats.[1] However, we previously reported that the increase in histidine decarboxylase activity of mastocytoma P-815 cells by glucocorticoids was accompanied with an increment of cellular histamine content.[12] One possible explanation for the discrepancy between the stomach and mastocytoma P-815 cells is that the newly synthesized gastric histamine was rapidly transported into the blood stream, and then removed by urinary secretion, since the administration of glucocorticoid to rats was shown to stimulate the urinary secretion of histamine.[16]

In addition, we showed that the increase in gastric histidine decarboxylase activity caused by hydrocortisone was a result of accelerated synthesis of the enzyme protein, since cycloheximide inhibited the steroid-inducible enzyme activity (Table IV), similar to the case of gastrin.[17] The induction of histidine decarboxylase by glucocorticoids has been demonstrated in histamine-forming neoplastic mast cells.[12] The histidine decarboxylase activities in human, canine and pig stomachs are known to be entirely contained in mast cell-like cells.[18] However, the enzyme activity in rat stomach was reported to be localized in enterochromaffin-like (ECL) cells.[19] This point certainly requires further investigation, using isolated ECL cells stimulated by glucocorticoids in an *in vitro* incubation system.

Our results indicate that rat fundic glands possess considerable amounts of cytosolic [^{3}H]dexamethasone-binding sites (Fig. 2b), which are specific for glucocorticoids. Glucocorticoids are generally known to bind first, with high affinity, to specific cytosolic receptors on the target cells, and then to acceptor sites in the chromatin of the nucleus as a complex with the activated receptors. The resultant increase in ribonucleic acid polymerase activity and in the rate of transcription of a specific gene lead to the enhanced synthesis of a spcific protein. Studies of possible involvement of glucocorticoid receptors in the regulation of histidne decarboxylase activity in rat fundic glands are now required.

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