Regulation of Histidine Decarboxylase in Rat Stomach by Gastrin: the Effect of Inhibitors of Protein Synthesis

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SUMMARY

Histidine decarboxylase activities in the stomachs of freely feeding rats or fasted rats treated with gastrin are 3 and 2 times, respectively, greater than enzyme activity of fasted rats. Administration of gastrin produces a marked increase of gastric histidine decarboxylase in 30 min with maximal effects between 2 and 3 hr, and a decline of enzyme activity to fasting levels after 8 hr. Puromycin and cycloheximide completely prevent the rise of gastric histidine decarboxylase activity induced by gastrin, whereas actinomycin D tends to stimulate this rise in activity. When cycloheximide is administered to freely feeding rats there is an exponential fall in gastric histidine decarboxylase activity with a half-life of 2.1 hr. Cycloheximide treatment accelerates the decline of gastric histidine decarboxylase activity after enhancement by gastrin.

INTRODUCTION

Large concentrations of histamine are present in the gastric mucosa of many mammalian species. Histamine has been thought to be the final common mediator of gastric acid secretion (2). Histamine is formed in the stomach by a specific histidine decarboxylating enzyme (3, 4). Both histamine and histidine decarboxylase activity are localized to the acid secreting regions of the stomach (4–6).

Kahlson *et al.* (6) reported that either feeding fasted rats or gastrin injection markedly increased the ability of stomach tissue minces to form histamine from histidine-^{14}C. They proposed that this effect was secondary to release of histamine, which freed histidine decarboxylase from product inhibition by histamine. Levine and Watts (7, 8) suggested the presence of a soluble inhibitor other than histamine in the stomachs of fasted rats.

This report describes studies of the enhancement of histidine decarboxylase in rat stomach by gastrin. The effect of drugs whose primary effect has been ascribed to interference with protein and nucleic acid synthesis has been examined.[1]

METHODS AND MATERIALS

Sprague-Dawley female rats, body weight 150–200 g (Huntingdon Farms), were deprived of food but received water ad libitum for 24 hr prior to all experiments unless otherwise indicated. Animals were killed by neck fractures; their stomachs were removed rapidly and opened and the mucosal surface was rinsed with saline. Only the thick-walled, glandular portion of the stomach was used for enzyme assay. Stomachs (about 1 g wet weight) were homogenized in 5 volumes of 0.05 M sodium-potassium phosphate buffer (pH 7.2) in a conical glass homogenizer with a loosely fitting pestle. Homogenates were centrifuged for 20 min at 10,000 *g*, and the super-

[1] A preliminary report of this work has been published (1).

natant fluid was used for enzyme assay unless otherwise indicated.

Assay for activity of histidine decarboxylase: Incubations were carried out in a 50-ml Erlenmyer flask equipped with a rubber stopper supporting a polyethylene center well (Kontes Glass Company No. 88230) containing 0.3 ml of a 1:2 mixture of ethanolamine and 2-methoxyethanol. Incubation mixtures consisted of 0.1 μmole of pyridoxal phosphate, 0.1 μmole of streptomycin, 0.1–0.6 ml of enzyme preparation, 0.1 μC of L-histidine-carboxyl-^{14}C (7 mμmoles), 0.05 μmole of nonradioactive histidine, and 0.05 M sodium-potassium phosphate buffer pH 7.2 to make a final volume of 2.0 ml. All components of the system except for substrate were incubated for 10 min at 37° in an agitating metabolic shaker prior to the addition of the radioactive and nonradioactive histidine preparations, and the incubation was continued for 2 hr at 37°. The reaction was stopped by injecting 1.0 ml of 1.0 M citric acid into the reaction mixture through the rubber stopper. The mixture was agitated for a further 45 min at 25° to allow complete absorption of the evolved $^{14}CO_2$. The center well was carefully removed, placed in a counting vial containing 4 ml of ethanol and 10 ml of a phosphor [0.4% 2,4-diphenyloxazole and 0.01% β-bis-(2-phenyloxazole) benzene in toluene] and assayed for radioactivity in a liquid scintillation spectrometer. All values were corrected for a heated enzyme blank.

The efficiency of $^{14}CO_2$ trapping by this system was tested by incubating stomach homogenates with 0.1 μC $Na_2{}^{14}CO_3$, pyridoxal phosphate, streptomycin, nonradioactive histidine, and sodium-potassium buffer for 2 hr at 37°. After the addition of citric acid, the mixture was agitated at 25° for 45 min and the center well contents were assayed for radioactivity. Between 95 and 100% of the radioactivity of the added $Na_2{}^{14}CO_3$ was recovered at $^{14}CO_2$.

Enzyme activity was linear with time for at least 2 hr and proportional to enzyme concentration between 0.1 and 0.6 ml of the enzyme preparation described above (Fig. 1). No activity was obtained when D-histidine–carboxyl-^{14}C was used as substrate.

L-Histidine–carboxyl-^{14}C (14 mC/m-

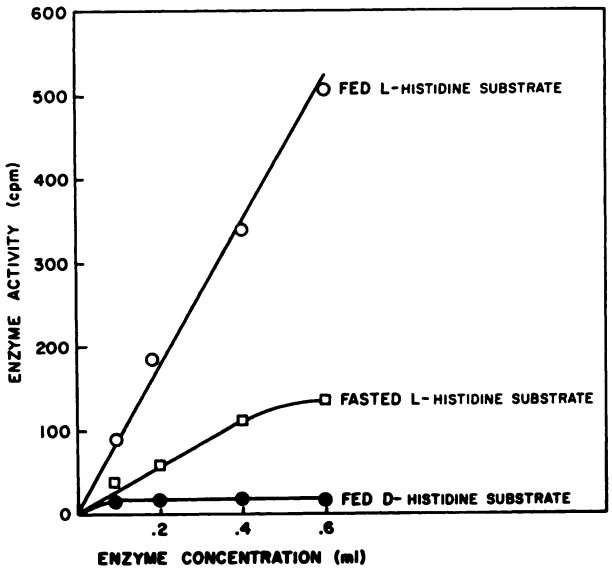

FIG. 1. *Effect of varying tissue concentration on gastric histidine decarboxylase*

Enzyme activity was measured as described in Methods and Materials and expressed as counts per minute of $^{14}CO_2$ evolved during the 2-hr incubation. Freely feeding rats and rats fasted for 24 hours were used. Values are the means of duplicate determinations. The inability of enzyme preparations to decarboxylate D-histidine is also shown.

mole), D-histidine–carboxyl-^{14}C (14 mC/mmole), and $Na_2{}^{14}CO_3$ (4 mC/mmole) were obtained from the New England Nuclear Corporation. Puromycin hydrochloride was purchased from the Nutritional Biochemical Corporation. Actinomycin D was generously supplied by Dr. C. Porter of the Merck Institute for Therapeutic Research and was dissolved in saline immediately prior to use. Purified gastrin was purchased from the Leo Pharmaceutical Company (Cogenhagen). It is a mixture of gastrin I and II. Maximal acid stimulation is produced in adult humans injected subcutaneously with 3 Leo units/kg. Gastrin was dissolved in saline and injected intraperitoneally in a volume of 0.4 ml. Cycloheximide was obtained from the Aldrich Chemical Company. Sephadex G-25 and G-10 gels were obtained from the Pharmacia Corporation.

RESULTS

Histidine Decarboxylase Activity of Stomachs from Fasted, Fed, and Gastrin-Treated Rats

Enzyme activity of freely feeding animals was compared with that of fasted rats killed 90 min after receiving 10 units of gastrin and fasted rats injected with the same volume of saline. Enzyme activity of fed and gastrin-treated rats were about 3 and 2 times greater, respectively, than activity in fasted controls (Fig. 2). Similar differences in enzyme activity were obtained using 10,000 *g* supernatants of gastric homogenate or crude homogenates. The same differences also were obtained when enzyme was prepared by centrifuging gastric homogenates at 100,000 *g* for 1 hr and when minces of stomach tissue were employed.

To examine the effect of varying gastrin doses on histidine decarboxylase, fasted rats received several different doses of gastrin and were killed 90 min later; their stomachs were assayed for histidine decarboxylase activity (Fig. 3). There was a steep increase in response to the administration of between 1 and 5 units of gastrin and a lesser increase (not statistically sig-

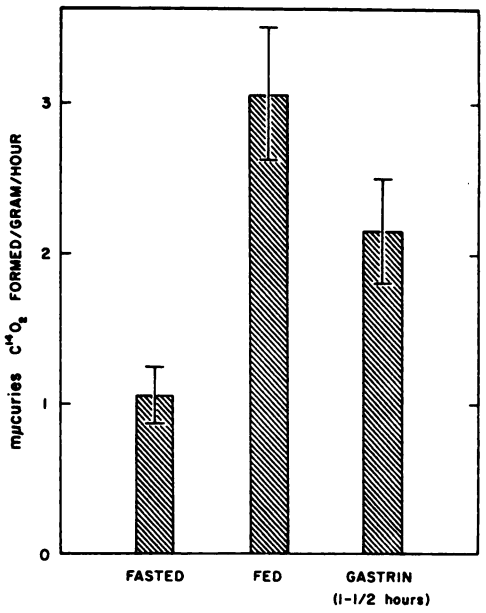

FIG. 2. *Gastric histidine decarboxylase activity after feeding and gastrin*

Animals were prepared as described in the text, and their stomachs were assayed for histidine decarboxylase activity. Values are expressed as the means for groups of 8 animals. Vertical bars indicate the standard error of the mean.

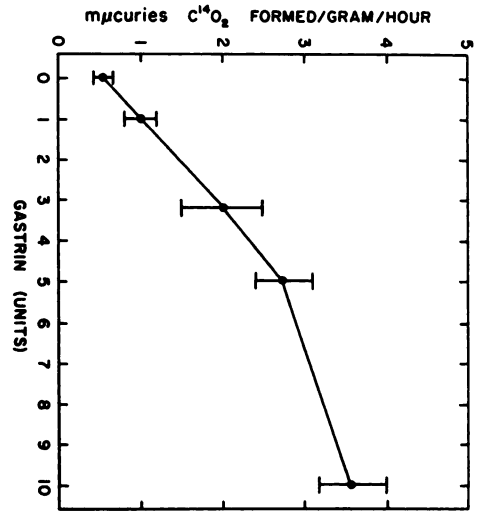

FIG. 3. *Effect of varying doses of gastrin on gastric histidine decarboxylase*

Data are presented as the mean ± standard error of the mean for groups of 6 rats.

nificant) between 5 and 10 units. In preliminary experiments, 15 and 20 units of gastrin produced the same effect as 10 units.

In several experiments gastrin was added directly to gastric enzyme preparations *in vitro*. Gastric minces, crude homogenates, or supernatants of 10,000 *g* centrifugations from fasted or fed rats were incubated with 0.01, 0.1, 1.0, and 10 units of gastrin in sodium-potassium phosphate buffer pH 7.5 (total volume 2 ml) for 30–60 min prior to enzyme assay. No changes in histidine decarboxylase activity were observed under any of these conditions.

Levine and Watts (7, 8) have suggested that histidine decarboxylase activity of fasted stomachs may be low because of the presence of soluble inhibitor in the stomachs of fasted rats, since they observed an increase in enzyme activity after Sephadex G-25 chromatography of stomach homogenates from fasted rats. Using a Sephadex chromatography procedure similar to that described by Levine and Watts (7), we found no change in enzyme activity.

A soluble inhibitor might be removed by dialysis. We observed no alteration in histidine decarboxylase activity of stomachs from fasted or fed rats after dialysis of enzyme preparations for 12, 24, or 48 hr against 200 volumes of 0.001 sodium-potassium phosphate buffer pH 7.2.

In another experiment designed to detect the presence of an inhibitor in the stomachs of fasted rats, enzyme preparations from fasted and either fed or gastrin-treated rats were mixed together and assayed for histidine decarboxylase activity. Enzyme activity was additive in all cases, an observation suggesting the absence of either inhibitors or activators in stomachs of fasted or fed rats.

Time Course of Histidine Decarboxylase Activation by Gastrin

Groups of fasted rats received 10 units of gastrin and were killed at varying time intervals; their stomachs were assayed for histidine decarboxylase activity (Fig. 4). There was a rapid initial rise in enzyme activity which was marked even after 30 min. Peak enzyme activity occurred between 2 and 3 hr after gastrin injection. Thereafter enzyme activity declined rapidly and attained fasting levels 8 hr after gastrin injection.

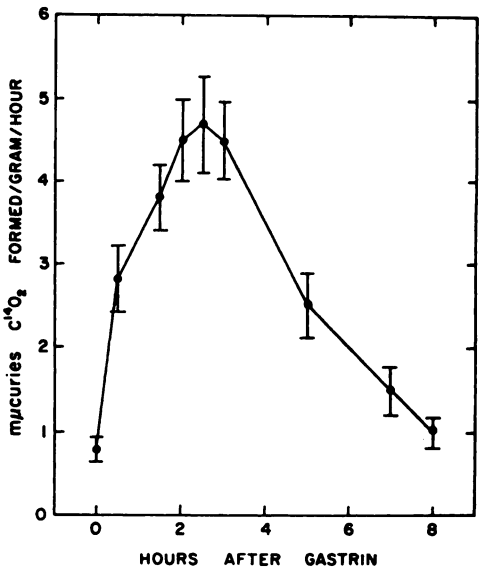

FIG. 4. *Time course of the effect of gastrin on gastric histidine decarboxylase*

Fasted rats received intraperitoneal injections of 10 units of gastrin and were killed at varying times. Data are expressed as the mean ± standard error of the mean for groups of 6 rats.

Effects of Puromycin and Cycloheximide on Gastric Histidine Decarboxylase

Puromycin and cycloheximide are both potent inhibitors of protein synthesis in mammals. Their interaction with the effect of gastrin on histidine decarboxylase was examined in the following experiments. Fasted rats were injected intraperitoneally with puromycin (100 mg/kg) or saline. After 10 min some of the rats received 10 units of gastrin and the remainder received an equal volume of saline. Animals were killed 90 min later, and their stomachs were assayed for histidine decarboxylase activity (Fig. 5). Puromycin did not alter the enzyme activity of rats who were not injected with gastrin but completely prevented any enhancement of enzyme activity by gastrin.

In other experiments, the effect of cyclo-

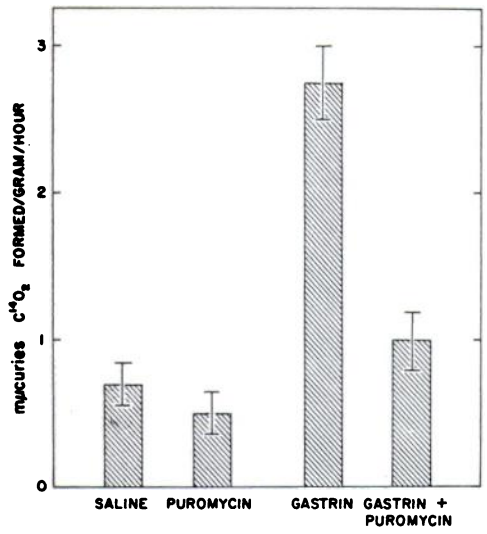

FIG. 5. *Effect of puromycin on the induction by gastrin of gastric histidine decarboxylase*

Groups of rats received puromycin and gastrin as described in the text. Vertical bars indicate the magnitude of the standard error of the mean.

heximide was studied on the gastrin induced increase of histidine decarboxylase. Fasted rats received cycloheximide (50 mg/kg) or saline injections. Fifteen minutes later rats received 10 units of gastrin or an equivalent volume of saline and were killed after 90 min (Fig. 6). Cycloheximide

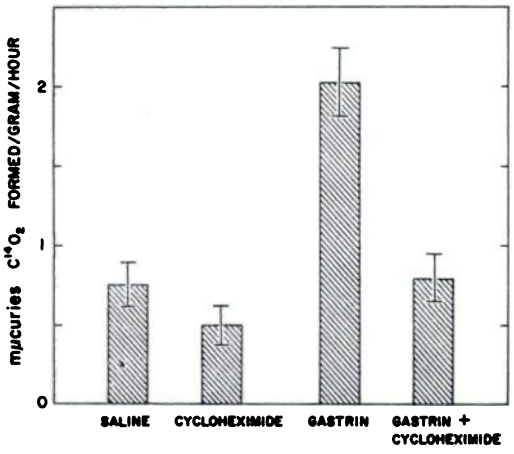

FIG. 6. *Effect of cycloheximide on the induction by gastrin of gastric histidine decarboxylase*

Groups of 7 rats received cycloheximide and gastrin as described in the text. Vertical bars indicate the magnitude of the standard error of the mean.

alone did not alter fasting levels of histidine decarboxylase activity. However, like puromycin, cycloheximide completely prevented any enhancement of enzyme activity by gastrin. After both puromycin and cycloheximide treatment, stomachs were mildly distended, but there was no gross evidence of ulceration.

Effect of Actinomycin D on Gastric Histidine Decarboxylase

It is well known that actinomycin D interferes with the biosynthesis of some forms of ribonucleic acid. The effect of actinomycin D on the stimulation of histidine decarboxylase activity induced by gastrin was studied in the following experiment. Gastrin (10 units) or saline was administered to fasted rats. Animals received an intraperitoneal injection of actinomycin D (2 mg/kg) or saline 15 min prior to gastrin treatment. All animals were killed 90 min after gastrin (Fig. 7). Acti-

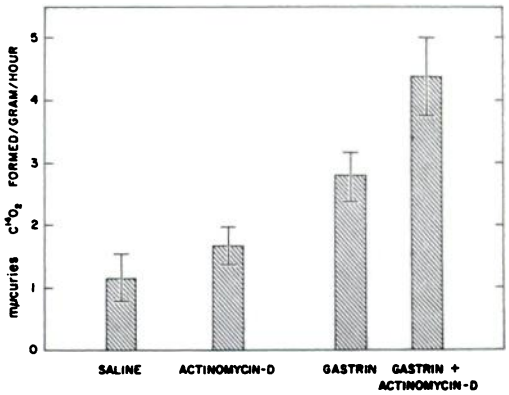

FIG. 7. *Effect of actinomycin D on the induction by gastrin of gastric histidine decarboxylase*

Groups of 7 rats received actinomycin D and gastrin as described in the text. Vertical bars indicate the standard error of the mean.

nomycin did not significantly affect enzyme activity of fasted rats. In contrast to the actions of puromycin and cycloheximide, this drug tended to enhance ($p < 0.05$) the gastrin-induced increase of enzyme activity. The stomachs of actinomycin D-treated rats were similar to those treated with puromycin and cycloheximide, being mod-

erately distended but with no macroscopic evidence of ulceration.

Actinomycin D (2 mg/kg) was administered to freely feeding rats who were then killed at several time intervals up to 5 hr (Table 1). The drug did not alter the high

TABLE 1
Effect of actinomycin D on gastric histidine decarboxylase

Freely feeding rats received intraperitoneal injections of actinomycin D (2 mg/kg) and were killed at varying time intervals. Data are expressed as the mean ± the standard error of the mean for groups of 6 rats. Enzyme activity is expressed as millimicrocuries of $^{14}CO_2$ formed per hour per gram of stomach.

Time (hr)	Histidine decarboxylase activity
0	5.6 ± 0.43
0.5	5.2 ± 0.56
1.5	6.0 ± 0.40
3.0	6.0 ± 0.64
5.0	5.0 ± 0.56

"fed" histidine decarboxylase activity in the stomachs of the rats at any time interval studied.

Regulation of the Rate of Decline of Gastric Histidine Decarboxylase

Freely feeding rats were injected with cycloheximide (50 mg/kg) and killed at varying time intervals up to 5 hr; their stomachs were assayed for histidine decarboxylase activity. There was a marked decrease in enzyme activity in the stomachs of cycloheximide-treated rats (Fig. 8). The decrease was statistically significant ($p < 0.01$) after 1 hr and reached fasting levels after 5 hr. The half-life for the decline was about 1.8 hr. In contrast, during the 5-hr period there was no decline in gastric histidine decarboxylase of rats that received saline in place of cycloheximide.

The decline of gastric histidine decarboxylase after enhancement of activity by gastrin is rapid (Fig. 4), with enzyme activity falling from peak to fasting levels in about 5 hr. To examine the decline phase of

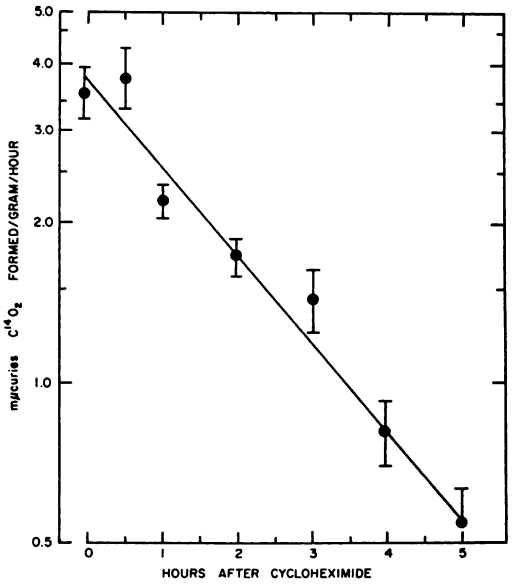

FIG. 8. *Effect of cycloheximide on gastric histidine decarboxylase of freely feeding rats*

Groups of 6 rats received cycloheximide and were killed at varying times as described in the text. Data are plotted on a semilogarithmic scale and represent the mean ± standard error of the mean.

gastric histidine decarboxylase in greater detail, fasted rats received 10 units of gastrin and were killed at 7 time intervals between 3 and 8 hr after gastrin injection, and histidine decarboxylase activity of their stomachs was measured (Fig. 9). Between 3 and 8 hr after gastrin injection, enzyme activity decreased exponentially with a half-life of about 2.1 hr. The rate of decline of enzyme activity was similar to that observed in fed rats treated with cycloheximide (Fig. 8).

In another experiment, fasted rats received 10 units of gastrin. After 90 min, one group received cycloheximide (50 mg/kg) and the other saline. Both groups were killed 5 hr after gastrin treatment and their stomachs were assayed for histidine decarboxylase activity (Fig. 10). Enzyme activity in cycloheximide treated rats at this time had fallen to fasting levels and was markedly lower ($p < 0.001$) than that of control rats. If cycloheximide lowers enzyme activity by inhibiting the synthesis of new enzyme molecules, these results sug-

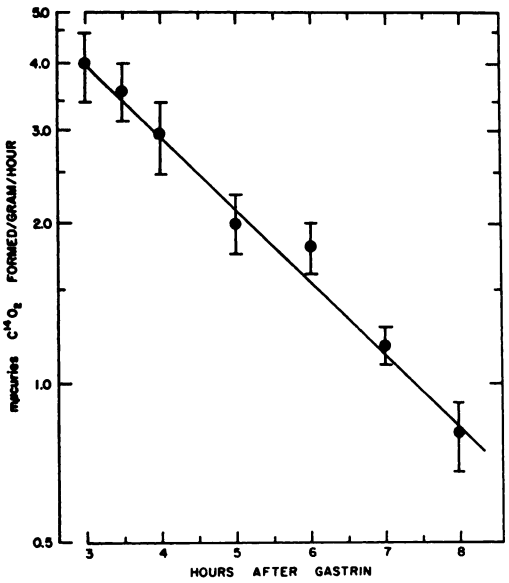

FIG. 9. *Rate of decline of gastric histidine decarboxylase after gastrin*

Groups of 5 rats were killed between 3 and 8 hr after gastrin administration and their stomachs assayed for histidine decarboxylase activity. Data are plotted on a semilogarithmic scale and represent the mean ± standard error of the mean.

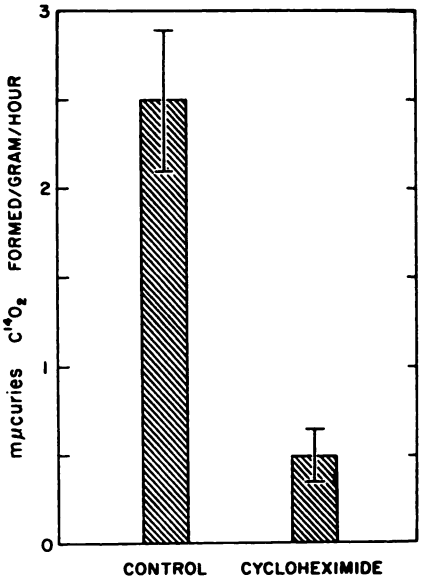

FIG. 10. *Acceleration by cycloheximide of the decline of gastrin-activated gastric histidine decarboxylase*

At 90 min after gastrin administration, groups of 7 rats received saline or cycloheximide and were killed 5 hr after gastrin. Vertical bars indicate the standard error of the mean.

gest that 90 min after gastrin treatment new enzyme synthesis is continuing.

DISCUSSION

The increase of gastric histidine decarboxylase activity produced by gastrin is among the most rapid among mammalian enzymes. The time course observed in this study is similar to that described by Kahlson *et al.* (6), who noted that the increase in gastric histamine-forming capacity took place earlier after gastrin than after refeeding fasted rats. Insulin also increases gastric histidine decarboxylase activity with a time course similar to that observed upon refeeding (9–11), a result suggesting that the effects of insulin and refeeding may be mediated through gastrin release.

Levine and Watts (7, 8) suggested that enhancement of histidine decarboxylase activity may be due to the removal of a soluble inhibitor. It would be expected that such an inhibitor in the stomachs of fasted rats would lower histidine decarboxylase activity in mixtures of enzyme preparations from fasted and fed rats. Our finding that enzyme activity of such mixtures is additive suggests the absence of a soluble inhibitor in fasted animals. Since enzyme activity in stomachs of fasted or fed rats did not change after dialysis, it is unlikely that a dialyzable inhibitor is present. We did not observe an increase in enzyme activity after Sephadex chromatography as had been reported by Levine and Watts (7). The difference between our results may reflect differences in enzyme preparations, as we found considerable enzyme activity in preparations similar to those in which Levine and Watts found negligible activity.

In all incubation mixtures pyridoxal phosphate was added in excess (7) indicating that the increase in enzyme activity after gastrin was not the result of a change in cofactor concentration.

After gastrin administration, gastric histidine decarboxylase activity increased very rapidly to a peak activity between 2 and 3 hr after hormonal treatment. The

decline to basal levels of activity was exponential and somewhat slower than the initial increase of enzyme activity. There are numerous mechanisms whereby an hormonal stimulus may increase an enzyme's activity. The enzyme may be transformed from inactive to an active form, synthesis of new enzyme molecules may take place, or the rate of enzyme degradation may be diminished. Segal and Kim (12) and Berlin and Schimke (13) have shown that if an increase in enzyme activity results from cessation of enzyme breakdown, the return to basal enzyme level after hormonal stimulus is withdrawn should be faster than the hormonally produced rise. If we apply this principle to the effect of gastrin on gastric histidine decarboxylase, the very rapid rise in histidine decarboxylase activity after gastrin and the subsequent, slower, exponential decline suggests that gastrin does not influence this enzyme primarily by causing a cessation of enzyme degradation.

Cycloheximide and puromycin completely prevented the increase in activity of histidine decarboxylase after gastrin, possible by interfering with new protein synthesis. Although amino acid incorporation into protein was not studied in our preparations, the large doses of cycloheximide and puromycin employed are known to cause almost complete shutoff of protein synthesis in several rat tissues. Inhibition of protein synthesis by cycloheximide and puromycin might influence the degradation as well as the synthesis of gastric histidine decarboxylase. Moreover, other actions of these drugs, such as a liberation of hepatic glycogen (14) might be involved in its effects on this enzyme activity.

Cycloheximide treatment of freely feeding rats results in a rapid exponential fall in gastric histidine decarboxylase with a half-time of 1.8 hr. If this were due to inhibition of new protein synthesis, the results suggest a very rapid turnover for gastric histidine decarboxylase. Only a few other mammalian enzyme possess as rapid turnover rates. After puromycin treatment or after peak activation by hydrocortisone tryptophan pyrrolase declines with a half-life of 2.5 hr (15). Between 3 and 8 hr after gastrin treatment, histidine decarboxylase activity falls with a half-life of about 2.1 hr, similar to the half-life of enzyme activity of fed rats receiving cycloheximide. This similarity suggests that little new enzyme synthesis occurs 3 hr after gastrin administration. The accelerated decline of histidine decarboxylase activity when cycloheximide is given 90 min after gastrin indicates that at this time new enzyme synthesis is still proceeding.

Actinomycin D failed to prevent enzyme activity enhancement after gastrin; this finding suggests that the effect of gastrin is not dependent on the formation of new messenger RNA. Actinomycin D caused a small enhancement of enzyme activity increase.

Schayer (16) reported that stimuli which provoke inflammation also increase histidine decarboxylase activity in a variety of tissues. This action is prevented by cycloheximide and puromycin, but not by actinomycin D.

Moog (17) found that actinomycin D accelerates the appearance of intestinal phosphatase in young mice, while Rosen *et al.* (18) reported an increase in tyrosine transaminase and several other enzymes following small doses of actinomycin D.

Garren *et al.* (15) reported that actinomycin D prevented the decline of hepatic tryptophan pyrrolase which had been induced in adrenalectomized rats by hydrocortisone. In preliminary experiments that could not be consistently replicated we found that actinomycin D tended to prevent the fall of histidine decarboxylase activity after gastrin-induced rise. When given before hydrocortisone, actinomycin D prevents induction of tryptophan pyrrolase and tyrosine transaminase (15). There is no marked increase in hepatic tryptophan pyrrolase or tyrosine transaminase of adrenalectomized rats until about 2 hr after hydrocortisone. Activity of gastric histidine decarboxylase, however, is considerably increased within 30 min after gastrin treatment. Thus the actinomycin D-sensitive phase of tryptophan pyrrolase and tyrosine transaminase induction occurs during the

lag phase, which is not present in the gastrin induction of histidine decarboxylase.

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