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EFFECT OF H_2 RECEPTOR ANTAGONISTS ON HISTIDINE DECARBOXYLASE ACTIVITY IN RAT GASTRIC MUCOSA*

DAVID V. MAUDSLEY, YUTAKA KOBAYASHI, LARRY BOVAIRD and MARK ZEIDEL

The Worcester Foundation for Experimental Biology, Inc., Shrewsbury, MA 01545, U.S.A.

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Abstract—Histidine decarboxylase activity in the gastric mucosa of the rat stomach is markedly increased by the H_2 receptor antagonists, burimamide and metiamide. The increase in enzyme activity is reduced by cycloheximide but not by actinomycin D. An inhibitor of histidine decarboxylase, 4-imidazolyl-3-amino-2-butanone (McN-A-1293), is also effective in reducing the enzyme activity stimulated by the H_2 receptor antagonists. The time course and the magnitude of the response of the enzyme with maximum doses of burimamide are similar to those obtained with pentagastrin. The histamine content of the mucosa is also reduced by burimamide and the data are discussed in relation to the hypothesis that H_2 receptor antagonists increase histidine decarboxylase activity through the release of endogenous gastrin.

BURIMAMIDE, a thiourea derivative of histamine, is a specific H_2 receptor antagonist.[1,2] It inhibits the increase in acid secretion produced by histamine and also by pentagastrin.[1] Results obtained with this drug, therefore, have provided support for the view that the actions of gastrin on acid secretion are mediated, at least in part, through the release of endogenous histamine.[3] Previously, however, we have demonstrated that the H_2 receptor antagonists, burimamide and metiamide, increase histidine decarboxylase activity in the glandular stomach,[4–6] and the present report further characterizes this effect.

METHODS

Female Sprague–Dawley rats (Charles River) weighing 150–175 g were used throughout. In most experiments, animals were starved 24 hr before use. Animals were killed by cervical dislocation and the stomachs removed and cleaned. For the preparation of histidine decarboxylase, only the glandular stomach was used. The tissue was minced with scissors, diluted 15:1 in 0·1 M phosphate buffer, pH 7·2, homogenized in a Polytron PT-10 and centrifuged at 10,000 *g* for 30 min. The supernatant solution was decanted and used for analysis.

Histidine decarboxylase was assayed as described previously.[7] Briefly, the reaction was carried out in a Warburg flask and the incubation mixture consisted of carboxyl-^{14}C-L-histidine (0·1 μCi containing 5 μg histidine), 10 μg pyridoxal phosphate, 1 ml enzyme extract and 0·1 M phosphate buffer, pH 7·2, to a final volume of 2·2 ml. Incubations were carried out for 2 hr at 37° in a shaking incubator, and the $^{14}CO_2$ evolved

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was absorbed on filter paper impregnated with Hyamine hydroxide. The reaction was stopped with 0·2 ml of 1 M citric acid, and shaking continued for a further 30 min to allow complete absorption of $^{14}CO_2$. The filter strips were removed, added to a toluene counting solution and counted in a liquid scintillation counter. All samples were assayed in triplicate, and enzyme activity is expressed as nmoles CO_2/g of tissue/hr.

Histamine contents were determined using the enzyme assay described by Kobayashi and Maudsley.[8] The incubation mixture contained 0·1 ml sample, 0·1 ml of a partially purified preparation of histamine *N*-methyl transferase obtained from guinea pig brain, 0·1 ml ^{3}H-*S*-adenosylmethionine (0·25 μCi), 0·2 ml of 0·05 M phosphate buffer, pH 7·2, to a final volume of 0·5 ml. Incubation time was 30 min in air at 37° in a shaking water bath after which the reaction was stopped with 0·5 ml of 2 N NaOH (NaCl saturated). The incubation mixture was then extracted with chloroform and ^{3}H-methyl histamine counted in a liquid scintillation counter.

In experiments involving drug treatment, animals were starved for 24 hr before use. All drugs were administered intraperitoneally unless otherwise indicated in the text, and the animals were killed 4 hr after drug treatment. The histidine decarboxylase inhibitor, 4-imidazolyl-3-amino-2-butanone (McN-A-1293), was a gift from Dr. R. J. Taylor of McNeil Laboratories, Inc., of Fort Washington, Pa. Burimamide and metiamide were gifts from Dr. J. W. Black of Smith, Kline & French Laboratories, Ltd., Welwyn Garden City, Herts., England.

RESULTS

Animals, starved for 24 hr, were injected intraperitoneally with 50 mg/kg of burimamide or 25 mg/kg of metiamide. Tissues were removed 2, 4 and 6 hr after injection and assayed for histidine decarboxylase activity. There was a rapid increase in enzyme activity produced by both drugs, and the time courses are shown in Fig. 1. By 6–8 hr after injection, the enzyme activity is returning to basal levels.

The pattern of response of histidine decarboxylase to the H_2 receptor antagonists is very similar to that observed for gastrin, insulin or in response to food.[3,4] Furthermore, the increase in histidine decarboxylase activity produced by burimamide is inhibited by cycloheximide (10 mg/kg) but not by actinomycin D (1 mg/kg) (Table 1). An inhibitor of histidine decarboxylase, McN-A-1293 (200 mg/kg), is also effective

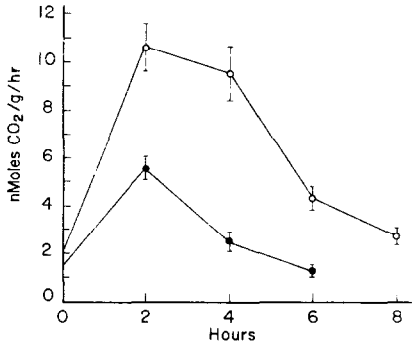

FIG. 1. Time course for the response of histidine decarboxylase to burimamide (50 mg/kg) and metiamide (25 mg/kg). Each point is the mean of five animals ± S.E.M. Burimamide, upper curve; metiamide, lower curve.

TABLE 1. EFFECT OF INHIBITORS ON THE RESPONSE OF HISTIDINE DECARBOXYLASE TO BURIMAMIDE STIMULATION

Treatment	Histidine decarboxylase activity (nmoles CO_2 g^{-1} hr^{-1} ± S.E.M.)
Control	2·13 ± 0·35
Burimamide (50 mg/kg)	5·40 ± 1·14
Burimamide + cycloheximide (10 mg/kg)	1·13 ± 0·31
Burimamide + McN-A-1293 (200 mg/kg)	1·11 ± 0·27
Burimamide + actinomycin D (1 mg/kg)	5·21 ± 0·91

in reducing the enzyme activity produced in response to burimamide. All drugs were given intraperitoneally immediately before giving burimamide, and the histidine decarboxylase activity was determined 4 hr later.

Figure 2 shows the effect of different doses of burimamide on histidine decarboxylase activity. Tissues were studied 4 hr after a single injection and a maximum response was obtained with a dose of 100 mg/kg. In subsequent experiments, it was found that a dose of 200 mg/kg often produced a lower response than 100 mg/kg. At high doses, therefore, the response of histidine decarboxylase tends to be reduced. Also shown in Fig. 2 is the effect of different doses of pentagastrin, and this demonstrates that the maximum response of histidine decarboxylase to pentagastrin administration is similar to that obtained with burimamide.

Another feature of the response to the H_2 receptor antagonists is that, after repeated doses, histidine decarboxylase activity remains at high levels. In the experiment illustrated in Table 2, animals were injected with 25 mg/kg of metiamide at time 0, and histidine decarboxylase was determined 4 and 8 hr later. In some animals, the first injection of metiamide was followed by a second dose (25 mg/kg) at 4 hr and the histidine decarboxylase activity determined 4 hr later, i.e. 8 hr after the initial

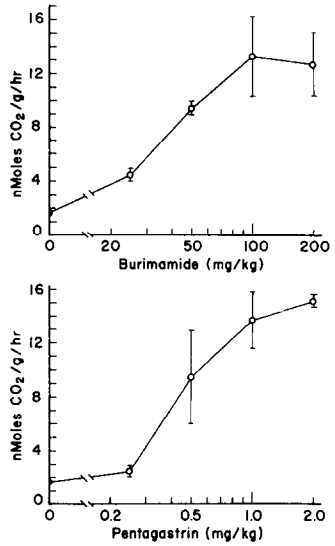

FIG. 2. Dose–response curves for pentagastrin and burimamide. Enzyme activity was determined 4 hr after injection, and the values are the mean ± S.E.M. (n = 5).

TABLE 2. EFFECT OF SINGLE AND REPEATED DOSES OF METIAMIDE ON HISTIDINE DECARBOXYLASE ACTIVITY IN GASTRIC MUCOSA*

Treatment	Histidine decarboxylase activity (nmoles g^{-1} hr^{-1} ± S.E.M.)
Control	1·85 ± 0·54
Metiamide, single dose, 4 hr	5·72 ± 0·71
Metiamide, single dose, 8 hr	1·72 ± 0·24
Metiamide, two doses, 8 hr	6·44 ± 0·85

* Test animals were injected with 25 mg/kg, i.p., of metiamide, and histidine decarboxylase activity was determined 4 and 8 hr later. Five animals also received an additional dose (25 mg/kg) at 4 hr, and histidine decarboxylase activity was determined 4 hr later.

injection. In animals given a single dose of metiamide, histidine decarboxylase activity declines substantially between 4 and 8 hr but, in animals given two doses, the decline in enzyme activity is prevented. Similar effects were observed with burimamide.

A characteristic of some compounds and procedures which increase histidine decarboxylase activity is the concomitant mobilization of histamine from the stomach. This is evidenced as a decrease in the histamine content of the mucosa which returns within a few hr to basal levels. The results in Table 3 show that histamine found in the mucosa, on an equivalent weight basis, is approximately twice that found in the underlying muscle. It is also clear that the reduction in histamine content 1 hr after refeeding starved animals or after a single injection of pentagastrin occurs only in the mucosa. The histamine content in the muscle layers is unaltered. When burimamide (50 mg/kg) was injected and the tissues were removed 1 hr later, there was a reduction in the histamine content of the mucosa similar to that observed with refeeding.

DISCUSSION

There is now a diverse group of substances and procedures which increase histidine decarboxylase activity in the gastric mucosa of the rat. A common feature is that they affect acid secretion either by stimulation or inhibition of the secretory process. The stimulating effects of insulin, 2-deoxyglucose and gastrin on both acid secretion and histidine decarboxylase have been known for some time,[9] but in recent

TABLE 3. EFFECT OF REFEEDING, PENTAGASTRIN OR BURIMAMIDE ON THE HISTAMINE CONTENT OF THE GLANDULAR STOMACH*

Treatment	Histamine content (μg/g ± S.E.M.)	
	Mucosa	Muscle
Control	21·1 ± 2·4	10·3 ± 2·0
Food	16·1 ± 1·6	8·8 ± 0·9
Control	24·4 ± 3·4	11·7 ± 0·5
Pentagastrin	15·0 ± 0·8	10·0 ± 0·8
Control	22·2 ± 2·8	11·8 ± 1·2
Burimamide	16·9 ± 2·0	9·9 ± 1·3

* Tissues were removed 1 hr after treatment. Each value is the mean of five animals.

years it has become increasingly apparent that substances which inhibit acid secretion such as SC15396 (antigastrin),[10–12] atropine[13–15] and, under certain circumstances, prostaglandin E_1[16] also increase histidine decarboxylase activity. The increased level of histidine decarboxylase produced by the H_2 receptor antagonists, burimamide and metiamide, therefore, is possibly a reflection of inhibition of acid secretion rather than due to some other property of these drugs. Since compounds which increase histidine decarboxylase activity constitutes such a chemically diverse group, a common mechanism of action is very attractive. Hakanson *et al.*[16] have suggested that increases in histidine decarboxylase produced by inhibitors of acid secretion are linked to the circulating levels of gastrin, i.e. raising the intragastric pH increases the release of gastrin. If this is applicable to the enhancement of histidine decarboxylase activity produced by burimamide and metiamide, then the characteristics of the enzyme and its response should be similar to those obtained with pentagastrin. Insofar as it has been studied, this appears to be the case. Histidine decarboxylase activity raised by either burimamide or pentagastrin administration is found in the same region of the stomach; the characteristics *in vitro* of the enzyme are similar, while the enzyme activity *in vivo* increased by either pentagastrin or burimamide is effectively reduced by cycloheximide but not by actinomycin D. The high rate of turnover of the enzyme implied by the results with cycloheximide probably accounts for the wide variations in levels which are frequently observed in studies on histidine decarboxylase. The decarboxylase inhibitor, McN-A-1293, is also effective in reducing the enzyme activity stimulated by burimamide. This drug has been reported to be an effective inhibitor of histidine decarboxylase and to be more specific than NSD1055.[17–19] Other similarities between burimamide and pentagastrin are that the maximum responses are similar and the histamine content is reduced to a comparable extent by both drugs. The results are in accord with, but do not prove that increases in histidine decarboxylase activity by burimamide and metiamide are mediated through endogenous gastrin. Moreover, it is implicit in this interpretation that these drugs should increase the circulating levels of gastrin, and antrectomy should abolish the stimulation of histidine decarboxylase by burimamide and metiamide. To our knowledge, these experiments have not yet been carried out.

In attempting to understand the role of histidine decarboxylase, we have pointed out the striking similarity between luteinizing hormone (LH) stimulation of ornithine decarboxylase in the ovary and gastrin stimulation of histidine decarboxylase in the mucosa.[6] Briefly, the activity of both enzymes is sharply raised under physiological stimulation—histidine decarboxylase by food and ornithine decarboxylase during late proestrus. The responses are hormone specific and the pattern of enzyme response is very similar. The induction of both enzymes is reduced by inhibitors of protein synthesis, and both enzymes have high rates of turnover.[20] The similarity between the two enzymes and their products strongly suggests that in some cases histamine and putrescine are fulfilling broadly similar roles in their respective tissues, a possibility first raised by Russell and Snyder[21,22] but subsequently rejected. If the analogy to putrescine is correct, it suggests that the gastrin stimulation of histidine decarboxylase in the rat stomach is simply another example of the hormonal regulation of a diamine-forming decarboxylase in its target tissue. This, in turn, would imply that the regulation of histidine decarboxylase is associated with the trophic actions of gastrin. These actions are now thought to be independent of the effects

of gastrin on acid secretion.[23] There are, however, some differences between ornithine decarboxylase and histidine decarboxylase which should be noted. First, actinomycin D does not inhibit the increase in histidine decarboxylase activity, whereas there is some evidence that actinomycin D does affect the hormonal stimulation of ornithine decarboxylase.[24] Second, repeated doses of metiamide prevented the decline in enzyme activity which normally occurs between 4 and 5 hr after a single injection. Continued presence of human chorionic gonadotrophin (HCG), however, does not prevent the decline in activity of ornithine decarboxylase in the ovary.[25] The molecular mechanisms involved in the regulation of the two enzymes may be different, at least in detail, and further studies are required to determine whether the analogy between histamine and putrescine is justified.

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