Effect of histamine on canine gastric mucosal adenylate cyclase

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The canine gastric acid response to histamine may be mediated by cyclic adenosine 3',5'-monophosphate (cyclic AMP). Stimulation of cyclic AMP formation by histamine in the fundic mucosa from several species (1, 6, 8, 13-15, 23-25, 29, 32, 33, 36) has been reported as evidence for such an effect. In the dog, however, stimulation of cyclic AMP in the gastric mucosa is controversial (16). In experiments conducted both in vitro and in vivo, Mao and his collaborators (20, 21) reported that histamine does not increase the formation of cyclic AMP in homogenates of canine gastric fundic mucosa. Furthermore, the parenteral administration of cyclic AMP or dibutyryl cyclic AMP either has had no effect (21) or has depressed the gastric acid secretion in the dog (17). In contrast, Bieck and his colleagues (2) have observed that histamine increases the concentration of cyclic AMP in biopsied gastric mucosal specimens as well as the amount of cyclic AMP in gastric juice of dogs. The same authors also showed that the peak output of cyclic AMP in gastric juice precedes the peak acid response to histamine and that theophylline potentiated the stimulatory effects of submaximal doses of histamine on the production of both cyclic AMP and HCl (2).

The missing link in the sequence of associations of cyclic AMP with HCl secretion in dogs is the absence of evidence that histamine stimulates the adenylate cyclase of isolated gastric mucosal preparations. In an attempt to fill this gap, we determined whether histamine and its active methyl derivatives, N"-dimethylhistamine and 4,5-methylhistamine (5, 12, 18), will stimulate the adenylate cyclase of cell-free preparations of canine mucosa when these are made in the same manner (8) as the cell-free preparations of guinea pig gastric mucosa, the adenylate cyclase of which is stimulated by histamine and its active analogues. When positive results were obtained, we tested the effects of N"-methylhistamine (an inactive histamine derivative), carbachol, pentagastrin, and metiamide (an H₂-receptor antagonist) (3).

MATERIALS AND METHODS

Full-thickness biopsy specimens of the proximal corpus (oxyntic gland area) and the distal antrum (pyloric gland area) were surgically obtained from the stomach of fasted, anesthetized (pentobarbital sodium, 30 mg/kg) mongrel male dogs. The identity of the mucosa from both areas was confirmed histologically. Tissue was removed specimens were stretched in a prefrozen Petri dish containing an ice-cold solution of 0.25 M sucrose, 5 mM tris(hydroxymethyl)aminomethane (Tris), 3 mM MgCl₂, and 1 mM EDTA, pH 7.4, and rinsed carefully. The fundic or antral mucosa was then dissected free from submucosal and muscular layers, weighed, and separated homogenized in a glass Teflon homogenizer for approximately 30-60 s (two strokes) in the above medium (1.6 wt/vol). The homogenate was filtered through a nylon mesh and centrifuged at 2,000 × g for 10 min. The sediment was resuspended in approximately 30 ml of the ice-cold medium without sucrose (5 mM Tris, 3 mM MgCl₂, and 1 mM EDTA, pH 7.4) and recentrifuged at 2,000 × g for 10 min. The washed sediment was resuspended in the sucrose-free medium.
and divided into 0.5-ml aliquots that were quickly frozen in solid CO₂ and stored at -80°C. This particulate fraction of homogenate, containing membrane fragments, served as the source of adenylate cyclase. In preliminary experiments, it was determined that quick freezing in solid CO₂ and storage at -80°C did not diminish either the basal activity of adenylate cyclase or its response to histamine. Therefore, to avoid variation in time between preparation of 2,000 × g pellets and assay for enzyme activity, the preparations were always first frozen, stored as described above, and assayed at a later date.

Adenylate cyclase activity was determined using a method (7) previously modified and employed for similar experiments with preparations of guinea pig mucosa (8). The enzyme preparation was incubated for 20 min at 37°C in a medium (total volume 50 µl) composed of 0.1 mM [α³²P]ATP (1.0 × 10⁶ cpm/tube, sp act, 10.0 Ci/mmol), 4 mM MgCl₂, 0.1 mM EDTA, 0.1% bovine serum albumin, 0.5 mM cyclic AMP, 25 mM creatine phosphate, 0.1 mg/ml creatine kinase (sp act, 100 IU/mg), and 40 mM tris-HCl buffer, pH 7.4. The incubation reaction was stopped by adding to the incubation mixture 5 µl of a solution composed of 25 mM ATP, 25 mM 5'-AMP, 25 mM cyclic AMP, and 250 mM KCl and by subsequently heating the mixture in a boiling water bath for 3 min. The mixture was cooled and centrifuged for 2 min. The formed [³²P]cyclic AMP was then separated from ATP and other radioactive materials by thin-layer chromatography, using cellulose polyethyleneimine thin-layer plates and 0.25 M lithium chloride as a developing agent (22). The concentration of protein in the enzyme preparation was determined by the method of Lowry and co-workers (19). The specific activity of adenylate cyclase is expressed as picomoles of cyclic AMP per minute per milligram of protein.

Histamine, the methylated derivatives of histamine, pentagastrin (N-t-butoxycarbonyl-β-Ala-Try-Met-Asp-Phe(NH₂)), carbachol, and NaF were dissolved and diluted in 5 mM Tris solution, pH 7.4. Adenylate cyclase activities in preparations from the gastric (fundic or antral) mucosa were assayed on paired basis without addition of agents (basal activity) or with addition of stimulants, antagonists, or both. Tested agents were added immediately before the assay was started. Each assay was done in triplicate. Results were evaluated using the Student t test either for paired or for group comparison.

The [α³²P]ATP was purchased from International Nuclear Corp. (Irvine, Calif.). Histamine, 4,5-methylhistamine, N⁴-methylhistamine, and synthetic pentagastrin were purchased from Calbiochem (San Diego, Calif.), and carbachol was purchased from Sigma Chemical Co. (St. Louis, Mo.). The N⁴-methylhistamine was a gift from Professor F. Mossini (Parma, Italy), and metiamide was kindly donated by the Smith Kline and French Laboratories, Welwyn Garden City, Eng.

RESULTS

Adenylate cyclase activity was found in cell-free fractions prepared from fundic and antral mucosae, and basal activity from both regions of the stomach did not differ significantly (Table 1). While NaF stimulated the mucosal adenylate cyclase from both regions, histamine significantly increased the activity only in the fundic mucosa preparations (Table 1). Not only histamine but also its active analogues, N⁴-dimethylhistamine and 4,5-methylhistamine (10⁻⁴ M), significantly (P < 0.001) increased adenylate cyclase activity (means ± SE) by 44.7 ± 6.6, 49.4 ± 6.7, and 34.0 ± 6.4%, respectively, over basal activity in the gastric fundic mucosa (Table 2). The degree of stimulation in terms of percentage increase over basal adenylate cyclase activity produced by histamine did not differ significantly from that generated by N⁴-dimethylhistamine or by 4,5-methylhistamine. In contrast, N⁴-methylhistamine (a histamine metabolite lacking secretagogue property) (10), carbachol, and pentagastrin had no significant effect on the adenylate cyclase activity of the gastric fundic mucosa (Table 2).

The stimulatory effect of histamine and N⁴-dimethylhistamine was dose-dependent in range of concentrations 10⁻⁸-10⁻³ M (Table 3). Maximal stimulation by both agents did not differ significantly (mean percent increase ± SE over basal activity with 10⁻³ M of histamine and 10⁻⁴ M of N⁴-dimethylhistamine were 58.1 ± 11.4 and 52.5 ± 11.4, respectively). The addition of metiamide (10⁻⁴ M) blocked the stimulation of fundic adenylate cyclase by both histamine and N⁴-dimethylhistamine, but basal and NaF-stimulated adenylate cyclase activities were not significantly altered by metiamide (Table 4).

### Table 1. Effect of histamine and NaF on formation of cyclic AMP in preparations of canine fundic and antral gastric mucosa

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of observations</th>
<th>Fundic mucosa</th>
<th>Antral mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine, 10⁻⁴ M</td>
<td>20</td>
<td>7.5 ± 0.4</td>
<td>12</td>
</tr>
<tr>
<td>NaF, 10⁻² M</td>
<td>8</td>
<td>42.9 ± 5.6*</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SE. * Significantly different from basal (control) conditions; P < 0.001.

### Table 2. Effect of histamine, histamine analogues, carbachol, and pentagastrin on adenylate cyclase activity of canine gastric fundic mucosa

<table>
<thead>
<tr>
<th>Compounds Added</th>
<th>No. of observations</th>
<th>Without addition (basal)</th>
<th>With addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>20</td>
<td>7.5 ± 0.4</td>
<td>20</td>
</tr>
<tr>
<td>N⁴-Me-H</td>
<td>10</td>
<td>7.1 ± 0.6</td>
<td>10</td>
</tr>
<tr>
<td>4,5-Me-H</td>
<td>7</td>
<td>6.6 ± 0.7</td>
<td>7</td>
</tr>
<tr>
<td>N⁴-Me-H</td>
<td>6</td>
<td>7.8 ± 0.7</td>
<td>6</td>
</tr>
<tr>
<td>Carbachol</td>
<td>6</td>
<td>7.8 ± 0.7</td>
<td>6</td>
</tr>
<tr>
<td>Pentagastrin</td>
<td>6</td>
<td>7.8 ± 0.7</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SE. * All drugs added at concentration of 10⁻⁴ M. † Significantly higher than basal values (Student t test for paired data); P < 0.001.
Histamine and canine gastric mucosa, adenylate cyclase

Histamine increases the formation of cyclic AMP in cell-free fractions of homogenate, containing membranes, prepared from the acid-secreting gastric fundic mucosa of dogs and that this effect is dose-dependent. These findings contrast with those of Mao and co-workers (21), who reported that the adenylate cyclase present in the canine fundic mucosa is insensitive to histamine but responds to NaF. The difference in results is most likely attributable to differences in methods. As suggested by Kimberg (16), the vigorous 2-min homogenization employed by Mao and his coauthors (21) could have damaged histamine-receptor sites or its coupling with adenylate cyclase, a possibility that would explain the lack of stimulation by histamine in their investigation. Such damaging effect of mechanical treatment would not alter the response of the preparation to unspecified stimulation by NaF, which is much less sensitive to the inactivating effect of homogenization (27, 28). In our experiments, the mucosal scrapings were purposefully prepared using a relatively gentle homogenization procedure—homogenizing for 30 s or less in loose Teflon pestle-glass homogenizer. Thus, our findings demonstrate that histamine stimulates adenylate cyclase activity from the canine gastric fundic mucosa, as observed in other mammals such as guinea pigs (8, 13, 14, 29), rats (1, 6, 15, 23, 25, 33), and rabbits (36).

Histamine stimulates gastric acid secretion by interacting with H2-receptors (3, 9), presumably on the parietal cell membranes (26, 31), and recently Preiss and Code (30) have shown that some of the histamine-active methyl derivatives act on the same H2-receptors. Our finding that metiamide selectively blocked the increased formation of cyclic AMP produced by histamine and by Nø-dimethylhistamine without altering basal or NaF-stimulated adenylate cyclase activities is consistent with this hypothesis. It cannot be ascertained from the present experiments whether or not histamine and its active analogues are acting on parietal cells, but the absence of antral stimulation by histamine suggests that parietal cells in fundic mucosa are the responsive elements. Such contention is in agreement with the localization of histamine-sensitive adenylate cyclases reported in isolated parietal cells of other species (26, 31, 32). Similar results were noted in rabbits (34) and guinea pigs (8, 14). Sachs and colleagues (34) found, in addition, that the H2-receptor antagonists, diphenhydramine (10-5 M) or mepyramine maleate (10-5 M), were also capable of blocking the histamine-stimulated adenylate cyclase activity. The effect appeared to be nonspecific for histamine-stimulated activity because these compounds also equally affected basal and NaF-stimulated adenylate cyclase activities (34, 36). In guinea pigs, a lesser concentration (10-4 M) of the H2-receptor blocking agent, diphenhydramine, has been found to have no effect on the formation of cyclic AMP induced by histamine, whereas under the same conditions the H2-antagonist blocked the action of histamine (14). In preliminary work, we have found that the presence of the H2-receptor blocker, pyrilamine (10-4 M), does not affect basal or histamine-stimulated (10-4 M) adenylate cyclase activity in canine gastric fundic mucosa (unpublished observations).

In our study, carbachol and pentagastrin failed to stimulate the formation of cyclic AMP in the gastric fundic mucosa. Similar in vitro results have been obtained by others in the rat (1), guinea pig (13, 29), and rabbit (36). The extent of adenylate cyclase stimulation by equimolar doses of histamine and its analogues (in

**TABLE 3. Stimulation of adenylate cyclase from gastric fundic mucosa by histamine and by Nø-dimethylhistamine**

<table>
<thead>
<tr>
<th>Drug Conc</th>
<th>Adenylate Cyclase Activity, nmol cyclic AMP/min per mg protein</th>
<th>5% increase over basal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of observations</td>
<td>Histamine</td>
</tr>
<tr>
<td>10-6 M</td>
<td>10</td>
<td>10.8 ± 4.1*</td>
</tr>
<tr>
<td>10-5 M</td>
<td>10</td>
<td>27.0 ± 5.6*</td>
</tr>
<tr>
<td>10-4 M</td>
<td>10</td>
<td>50.0 ± 10.6*</td>
</tr>
<tr>
<td>10-3 M</td>
<td>10</td>
<td>55.1 ± 11.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. * Significantly higher than basal activity (Student t test for paired data); P < 0.05. No statistical difference between histamine and Nø-dimethylhistamine.

**TABLE 4. Inhibition of histamine and Nø-dimethylhistamine stimulation of fundic mucosa adenylate cyclase by metiamide**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Adenylate Cyclase Activity, μmol cyclic AMP/min</th>
<th>5% decrease over basal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of observations</td>
<td>Without antagonist</td>
</tr>
<tr>
<td>Basal activity</td>
<td>0</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td>Histamine, 10-5 M</td>
<td>9</td>
<td>11.6 ± 0.8*</td>
</tr>
<tr>
<td>Nø dimethylhistamine, 10-5 M</td>
<td>4</td>
<td>6.7 ± 0.4</td>
</tr>
<tr>
<td>NaF, 10-4 M</td>
<td>3</td>
<td>53.8 ± 8.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE. * Significantly greater than basal activity; P < 0.02.

**DISCUSSION**

Our results indicate that histamine increases the formation of cyclic AMP in cell-free fractions of homogenate, containing membranes, prepared from the acid-secreting gastric fundic mucosa of dogs and that this effect is dose-dependent. These findings contrast with those of Mao and co-workers (21), who reported that the adenylate cyclase present in the canine fundic mucosa is insensitive to histamine but responds to NaF. The difference in results is most likely attributable to differences in methods. As suggested by Kimberg (16), the vigorous 2-min homogenization employed by Mao and his coauthors (21) could have damaged histamine-receptor sites or its coupling with adenylate cyclase, a possibility that would explain the lack of stimulation by histamine in their investigation. Such damaging effect of mechanical treatment would not alter the response of the preparation to unspecified stimulation by NaF, which is much less sensitive to the inactivating effect of homogenization (27, 28). In our experiments, the mucosal scrapings were purposefully prepared using a relatively gentle homogenization procedure—homogenizing for 30 s or less in loose Teflon pestle-glass homogenizer. Thus, our findings demonstrate that histamine stimulates adenylate cyclase activity from the canine gastric fundic mucosa, as observed in other mammals such as guinea pigs (8, 13, 14, 29), rats (1, 6, 15, 23, 25, 33), and rabbits (36).

Nø-dimethylhistamine, which occurs naturally in canine gastric mucosa and gastric juice (4), and 4,5-methylhistamine, a specific H2-agonist, significantly increased the formation of cyclic AMP in the gastric fundic mucosa, whereas N7-methylhistamine had no such effect. Thus, the methylated derivatives Nø-dimethylhistamine and 4,5- nor-methylhistamine, known to stimulate gastric acid secretion in the conscious dog (5, 18), also stimulate the formation of cyclic AMP in the canine gastric fundic mucosa. In contrast, the adenylate cyclase of the gastric fundic mucosa is insensitive to N7-methylhistamine, which does not stimulate gastric acid secretion in vivo (4, 10). Similar corresponding structural-activity relationships of histaminic compounds on the formation of cyclic AMP have been observed in mammalian cerebral cortex (35) and more recently in the acid-secreting gastric mucosa of guinea pigs (8). The present study also demonstrates a striking parallelism between the effect of histamine and its derivatives on cyclic AMP formation and gastric HCl secretion in the same species.

Histamine stimulates gastric acid secretion by interacting with H2-receptors (3, 9), presumably on the parietal cell membranes (26, 31), and recently Preiss and Code (30) have shown that some of the histamine-active methyl derivatives act on the same H2-receptors. Our finding that metiamide selectively blocked the increased formation of cyclic AMP produced by histamine and by Nø-dimethylhistamine without altering basal or NaF-stimulated adenylate cyclase activities is consistent with this hypothesis. It cannot be ascertained from the present experiments whether or not histamine and its active analogues are acting on parietal cells, but the absence of antral stimulation by histamine suggests that parietal cells in fundic mucosa are the responsive elements. Such contention is in agreement with the localization of histamine-sensitive adenylate cyclases reported in isolated parietal cells of other species (26, 31, 32). Similar results were noted in rabbits (34) and guinea pigs (8, 14). Sachs and colleagues (34) found, in addition, that the H2-receptor antagonists, diphenhydramine (10-5 M) or mepyramine maleate (10-3 M), were also capable of blocking the histamine-stimulated adenylate cyclase activity. The effect appeared to be nonspecific for histamine-stimulated activity because these compounds also equally affected basal and NaF-stimulated adenylate cyclase activities (34, 36). In guinea pigs, a lesser concentration (10-4 M) of the H2-receptor blocking agent, diphenhydramine, has been found to have no effect on the formation of cyclic AMP induced by histamine, whereas under the same conditions the H2-antagonist blocked the action of histamine (14). In preliminary work, we have found that the presence of the H2-receptor blocker, pyrilamine (10-4 M), does not affect basal or histamine-stimulated (10-4 M) adenylate cyclase activity in canine gastric fundic mucosa (unpublished observations).

In our study, carbachol and pentagastrin failed to stimulate the formation of cyclic AMP in the gastric fundic mucosa. Similar in vitro results have been obtained by others in the rat (1), guinea pig (13, 29), and rabbit (36). The extent of adenylate cyclase stimulation by equimolar doses of histamine and its analogues (in
terms of percent increase over basal activity) is less than that observed for analogous preparations from rodent species (8, 23, 34). This finding is likely due to the fact that, in canine gastric mucosa, oxyntic cells account for less than 50% of the total cell population (11).

In conclusion, our present findings support the view that canine gastric acid secretory response to histamine and its active analogues is due to interaction of these agents with H₂-receptors and is subsequently mediated by the increase in the generation of "second-messenger" cyclic AMP.

REFERENCES


