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Research Article

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Effect of Histamine and Its Methyl Derivatives on Cyclic AMP Metabolism in Gastric Mucosa and Its Blockade by an H_2 Receptor Antagonist

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ABSTRACT In a cell-free system prepared from guinea pig gastric mucosa, histamine and N^{α}-methylhistamine produced dose-dependent stimulation of cyclic AMP formation and 1,4-methylhistamine had a minimal stimulatory effect. *N*-methyl-*N'*-(2-[5-methylimidazole-4-yl-methylthio]-ethyl)-thiourea (metiamide), a new H_2 receptor inhibitor, selectively blocked the stimulation of adenylate cyclase by histamine and its active methyl derivative but had no substantial effect on the basal adenylate cyclase activity or adenylate cyclase stimulated by sodium fluoride. Metiamide inhibited the histamine stimulation of adenylate cyclase at 1/100 the concentration of the histamine. Histamine, its methyl derivatives, and metiamide did not influence the activity of cyclic AMP phosphodiesterase from gastric mucosa. Therefore, histamine stimulates gastric mucosal adenylate cyclase via interaction with the H_2 receptor without influencing cyclic AMP breakdown, and *N*-methylation of histamine on the side chain preserves or even increases its stimulating ability. On the other hand, *N*-methylation in the ring nearly abolishes the ability of histamine to interact with the H_2 receptor.

INTRODUCTION

Both side-chain and ring methyl derivatives of histamine have been identified in the gastric mucosa and in the gastric juice during stimulation of secretion from canine Heidenhain pouches and in the acid secreted from isolated perfused canine stomachs (1, 2). The ring *N*-substituted methyl derivatives (1,4- and 1,5-methylhistamine or N^{τ}- and N^{τ}-methylhistamines) are physiologically inert (3); the products of side-chain methylation, *N*-methylhistamine and *N*-dimethylhistamine (N^{α}-MeH and N^{α}-Me_2H), are gastric secretagogues in the whole animal and are more active than histamine (4, 5). Thus, differential methylation of histamine in the mucosa may play a role in the regulation of gastric secretion (5).

It has also been proposed that histamine produces its secretory effect in the gastric mucosa by interaction with H_2 receptors (6). A specific H_2 receptor antagonist, burimamide, has been discovered by Black, Duncan, Durant, Ganellin, and Parsons (7). A less toxic antagonist, *N*-methyl-*N'*-(2-[5-methylimidazole-4-yl-methylthio]-ethyl)-thiourea (metiamide), with identical actions as burimamide, has recently become available (see Methods). These substances have been found (8) to block the in vivo secretory effects of N^{α}-MeH and N^{α}-Me_2H as effectively as they block those of histamine, indicating that histamine and its active methyl derivatives act at the same receptor site.

In both mammalian and nonmammalian species, cyclic AMP appears to act as an intracellular mediator of histamine's action on the oxyntic cells of gastric mucosa (9–12). A single dose of histamine can stimulate cyclic AMP production in preparations of gastric mucosa

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from guinea pigs and rats (9, 11), and a dose-dependent stimulation of adenylate cyclase by histamine has been observed in preparations from mucosa of *Necturus* (10). Such relationships have not been found with canine mucosa (13) although gastric juice of dogs contains cyclic AMP and the quantity present appears related to the secretion of H^+ (14).

We undertook this study in a cell-free system to determine whether histamine and its naturally occurring methyl derivatives, N^{α}-MeH and N^{τ}-MeH, affect the formation of cyclic AMP in membrane preparations from guinea pig gastric mucosa in a fashion parallel to their secretory actions in intact mucosa and whether a specific H_2 receptor antagonist blocks the effects of histamine and its derivatives on cyclic AMP formation. Because the breakdown of cyclic AMP by cyclic AMP phosphodiesterase may also regulate the amount of cyclic AMP present in the mucosa (15), we tested the effects of histamine, its methyl derivatives, and metiamide on this enzyme prepared from the mucosa.

METHODS

Guinea pigs maintained on an ad libitum diet were killed by a blow on the head and exsanguination. The stomach was removed quickly, placed in ice-cold solution (0.25 M sucrose, 5 mM Tris, 3 mM $MgCl_2$, and 1 mM EDTA, pH 7.4), rinsed repeatedly to remove adherent contents, and cooled while the mucosal cells were scraped from the stomach walls. All subsequent preparations were done at 0°C. Histologic examination showed that the scrapings consisted almost entirely of mucosa with only occasional contamination by a tiny piece of muscularis mucosae. The mucosal tissue was homogenized in a glass-Teflon homogenizer, using a ratio of 1 g of tissue to 3–4 ml of the above solution and four or five strokes of the homogenizer. The homogenate was filtered through nylon mesh and centrifuged at 2,000 *g* for 10 min.

The supernate was collected, and the sediment was resuspended in 30 ml of ice-cold solution without sucrose (5 mM Tris, 3 mM $MgCl_2$, and 1 mM EDTA, pH 7.4) and centrifuged again at 2,000 *g*. The same procedure was repeated once more and the washed 2,000-*g* sediment was divided into portions that were used fresh or were frozen in Dry Ice and stored at −80°C. Washed 2,000-*g* sediments were prepared in the same way from mouse renal cortex and medulla. The supernate from the first centrifugation was spun down at 10,000 *g* for 60 min and this clear supernate was frozen and later assayed for cyclic AMP phosphodiesterase activity. Histamine and its derivatives were dissolved and diluted in 5 mM Tris solution, pH 7.4. Adenylate cyclase incubation was performed under conditions previously described (16). Chromatographic analysis was by a new method described by Bär (17).

Histamine and N^{τ}-MeH were purchased from Calbiochem (San Diego, Calif.); N^{α}-MeH was kindly given to us by Professor F. Mossini (University of Parma, Italy). We are indebted to Dr. J. W. Black (Research Institute, Smith, Kline and French Laboratories, Ltd., Welwyn Garden City, England) for supplies of metiamide. Synthetic [8-arginine]-vasopressin was from the Sigma Chemical Co., St. Louis, Mo.); bovine parathyroid hormone was a gift of Dr. C. D. Arnaud (Mayo Clinic).

Cyclic AMP phosphodiesterase activity was assayed by using the principle described by Thompson and Appleman (18). The enzyme preparation was incubated in a solution (total volume, 200 μl) of the following composition: 50 mM Tris HCl, 10 mM $MgSO_4$, 0.1 mM EDTA, and 5×10^{-4} M ^{3}H-labeled cyclic AMP. Enzymic action was stopped by heating in a boiling water bath. After the reaction mixture was cooled, 50 μl of king cobra venom (Sigma) solution (1 mg/ml) was added and the mixture was incubated for 15 min at 37°C. The second incubation was stopped by addition of a 1:3 slurry of AG 1 × 200–400 mesh, resin (Bio-Rad Laboratories, Richmond, Calif.) and then centrifuged. The tritium activity of nucleosides in aliquots of the supernate was counted in Bray's solution, and the activity of cyclic AMP phosphodiesterase was expressed in nanomoles of cyclic AMP destroyed per minute per milligram of protein. Histamine, its derivatives, and metiamide did not influence the binding of nucleotides on the resin or the breakdown of 5′-nucleotides to nucleosides in the second incubation.

Proteins in enzyme preparations were determined by the Lowry method (19). Significance of differences was evaluated by the paired *t* test.

RESULTS

At concentrations ranging from 10^{-6} to 10^{-3} M, histamine produced a dose-dependent stimulation of cyclic AMP formation (Fig. 1). The side-chain methyl derivative, N^{α}-MeH, produced similar activation and its ability to stimulate adenylate cyclase appears to be greater than that of histamine. In contrast, the ring methyl derivative, N^{τ}-MeH, had no stimulatory effect except at the highest concentration (10^{-3} M), and that stimulation was slight. Stimulation of the mucosal adenylate cyclase by 10 mM NaF exceeded that by 10^{-3} M histamine or 10^{-3} M N^{α}-MeH.

At a concentration of 10^{-3} M, metiamide produced a slight inhibition of basal activity of adenylate cyclase but had no significant effect on the adenylate cyclase stimulated by fluoride (Table I). However, it completely blocked the stimulation of gastric mucosa adenylate cyclase by both histamine and N^{α}-MeH. At 10^{-5} and 10^{-4} M, this compound progressively inhibited the adenylate cyclase stimulated by 10^{-3} M histamine or 10^{-3} M N^{α}-MeH without significant effect on basal adenylate cyclase activity (Fig. 2). Metiamide did not inhibit the stimulation of renal medullary adenylate cyclase by vasopressin or of renal cortical adenylate cyclase by parathyroid hormone.

The mean (±SE) activity of the cyclic AMP phosphodiesterase in the extracts of gastric mucosa was 2.39 ±0.46 ($N = 4$) nmol cyclic AMP/min per mg protein and was not significantly influenced by either histamine or its methylated derivatives at 10^{-3} M. Metiamide at 10^{-3} M had no effect on this cyclic AMP metabolizing enzyme.

DISCUSSION

The stimulation of cyclic AMP formation by both histamine and N^{α}-MeH and its complete blockade by a

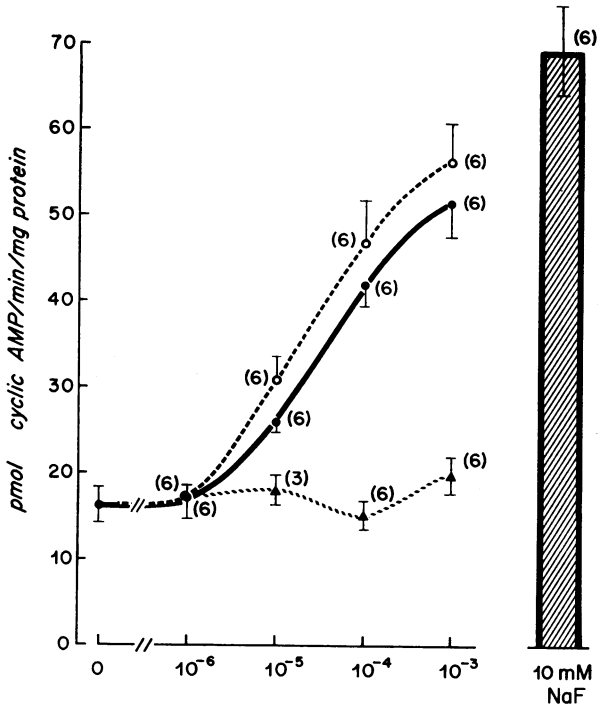

FIGURE 1 Effect on gastric mucosal adenylate cyclase of increasing concentrations of histamine (●——●), N^{α}-MeH (----), and N^{τ}MeH (▲---▲) and of 10 mM NaF. Abscissa: molar concentration. Each point is mean±SE; number of experiments is indicated in parentheses. Mean value at 10^{-3} M N^{τ}-MeH is significantly different from control; all values for histamine and N^{α}-MeH at $> 10^{-6}$ M are significantly different from control ($P < 0.01$).

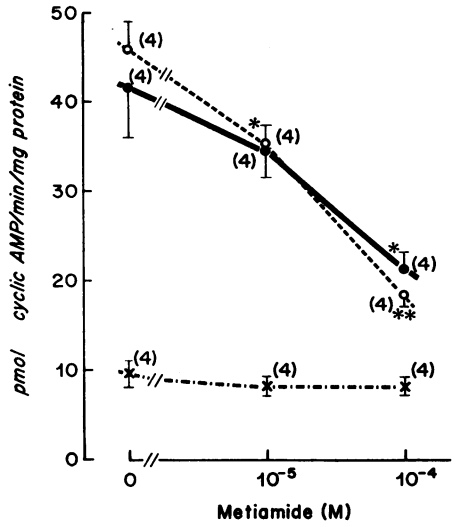

FIGURE 2 Effect of metiamide on gastric mucosal adenylate cyclase. Adenylate cyclase activity: basal (×----×); stimulated by 10^{-3} M histamine (----); stimulated by 10^{-3} M N^{α}-MeH (●——●). Data shown as mean±SE with number of experiments in parentheses. * = inhibition significant at $P < 0.05$; ** = inhibition significant at $P < 0.005$.

specific H_2 receptor antagonist, metiamide, which itself did not influence significantly the basal activity of adenylate cyclase or the stimulation of it by NaF, provide the basis for suggesting that histamine and its active derivative, N^{α}-MeH, stimulate cyclic AMP formation via action on H_2 receptors in membranes of the gastric mucosal cells. This antagonist appears to be a powerful inhibitor of the H_2 receptor in this in vitro system because measurable inhibition of the stimulation of adenylate cyclase occurred at inhibitor concentrations that were 1% of those of the active compounds. The specificity of metiamide (the inhibitor) is supported by the finding that it did not influence the vasopressin-sensitive adenylate cyclase of renal medulla or the parathyroid hormone-sensitive adenylate cyclase of renal cortex.

Imidazole inhibits the cyclic AMP-induced secretion by gastric mucosa of frog, and the suggestion was made that this drug acts by stimulation of cyclic AMP phosphodiesterase, thereby increasing the breakdown of cyclic AMP within the mucosa (20, 21). Since the structure of histamine and its derivatives includes an imidazole ring, we examined the possibility that histamine, its methyl derivatives, and metiamide may also alter the catabolism of cyclic AMP in the mucosa we used. Our results show that histamine and its derivatives have no differential effect on cyclic AMP breakdown, ruling out the possibility that their different actions on secretion are due either to stimulation or to inhibition of cyclic AMP breakdown.

Histamine and N^{α}-MeH, therefore, would produce a net accumulation of cyclic AMP in guinea pig gastric mucosa by stimulation of its formation. The minimal, if any, effect of metiamide on basal and NaF-stimulated activity of adenylate cyclase and on cyclic AMP breakdown also indicates that the blocking effect of this substance on gastric secretion is due to interference with the H_2 receptor in the gastric mucosa.

TABLE I

Effect of Metiamide on Gastric Mucosal Adenylate Cyclase

Condition	Activity	
	Control	With 10^{-3} M metiamide
	*pmol/min per mg protein**	
Basal activity	12.5±1.2 (3)‡	9.2±0.7 (3)
With 10^{-4} M histamine	39.2±4.2 (3)	10.7±1.2 (3)
With 10^{-4} M N^{α}-MeH	39.3±4.2 (3)	10.0±0.6 (3)
With 10 mM NaF	65.8±6.5 (3)	62.7±14.9 (3)

* Mean ±SE.

‡ Number of experiments in parentheses.

Thus, the results of this study indicate that histamine produces a dose-dependent stimulation of cyclic AMP formation in the guinea pig gastric mucosa via its interaction with H_2 receptors and has no effect on cyclic AMP breakdown. Methylation of histamine at the N^{α} position of the side chain preserves and even increases the ability to interact with the H_2 receptor, while methylation at N^{τ} of the ring almost completely abolishes the stimulating action on adenylate cyclase. We propose that metiamide, a new H_2 receptor antagonist, inhibits gastric secretion by blocking the stimulation of cyclic AMP formation by histamine and its active methyl derivatives, at least in the gastric mucosa of the guinea pig.

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