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Comparison of the Effects of Secretory Stimulants and Inhibitors on Gastric Mucosal Adenylyl Cyclases of Various Species (37675)

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A controversial hypothesis about the regulation of gastric acid secretion maintains that upon binding to their respective receptors, secretory stimulants will then obligatorily activate an adenylyl cyclase of the parietal cell membrane, thereby initiating an intracellular accumulation of cyclic AMP (1-4). According to this concept the increase in intracellular cyclic AMP is an essential intermediate or "second messenger" for gastric secretagogues. There are conflicting reports in the literature concerning the validity of this hypothesis in which the gastric mucosa of a variety of different animal species were used (3, 4). In the present study we have investigated adenylyl cyclase activities from the gastric mucosae of four mammalian species in response to several secretory stimulants (histamine, carbachol and pentagastrin) and inhibitors (prostaglandin E_2, secretin and epinephrine).

Methods. Female Sprague-Dawley rats (80-100 g), female guinea pigs (400-600 g), male New Zealand white rabbits (2-4 kg) and male mongrel dogs (15-20 kg) were used in these studies. Animals were anesthetized and subjected to laparotomy and gastrotomy to expose the acid secreting mucosa. Mucosal scrapings from the corpus of the stomach were obtained after extensive washing from each species. Tissues were homogenized as 1 g wet weight in 10 ml of 0.32 *M* sucrose (enzyme grade (-50 m*M* Tris-Cl (pH 7.4, 4°) in a Duall type homogenizer using five strokes of a Teflon plunger at 1500 rpm. All homogenates were centrifuged at 600*g* for 20 min and the pelleted materials were then resuspended in 5 ml of the homogenizing medium. These particulate preparations were used for this study since we had previously found that most of the hormone and NaF sensitive adenylyl cyclase activity resided in this fraction of corpus mucosa (5). No unbroken cells were visible in this preparation by light microscopic investigation.

Adenylyl cyclase activities were measured according to Thompson *et al.* (6). Each assay contained 0.2 m*M* ATP, 0.5 μCi ^{32}P-a-ATP (specific activity 4-16 Ci/mmole), 5 m*M* $MgCl_2$, 1 mg phosphocreatine, 5 units creatine phosphokinase, 1 m*M* cyclic AMP, 7.5 m*M* theophylline, and 15 m*M* Tris-Cl (pH 7.4) in a total vol of 0.2 ml. Reactions were initiated by addition of particulate protein (700-850 μg) and the mixture was then incubated for 15 min in the presence or absence of drugs at 30°. The reaction was terminated by addition of 50 μl of 1 *M* acetic acid with vigorous stirring. The ^{32}p-cyclic AMP formed was purified using MnO_2 and Al_2O_3:$PbSO_4$ absorption chromatography (6). Activities were unaffected by solvents for the drugs. Adenylyl cyclase activities are expressed in our results as picomoles of cyclic AMP formed per min per mg protein. Protein was determined by the method of Schacterle and Pollack (7). All reactions were linear with the time of incubation and membrane protein used.

The hormones and drugs tested include histamine acid phosphate (Fisher), carbachol (Regis), pentagastrin (courtesy of B. Mallov, Ayerst), prostaglandin E_1 (courtesy of Dr. John Pike, Upjohn), synthetic secretin (Schwarz/Mann), *d*,1-epinephrine (Regis), tripelennamine (Ciba), chloropheniramine (U.S.V.), and metiamide (Smith, Kline and French), and dimethindine (Ciba).

Results. Figure 1 shows the effects of histamine (1 m*M*), carbachol (1 m*M*), pentagastrin (20 μ*M*), prostaglandin E_1 (0.1 m*M*), secretin (0.1 μ*M*), epinephrine (0.1 m*M*) and NaF (10 m*M*) on the fundic mucosal adenylyl cyclase activities of the 600*g* pellet from rat, dog, rabbit, and guinea pig. The amounts of drug or hormone employed

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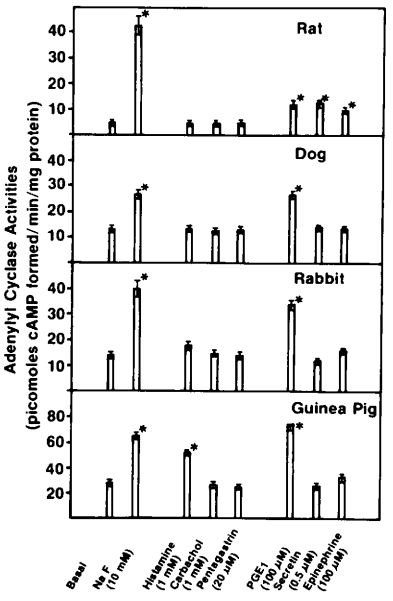

FIG. 1. Effects of secretagogues and Inhibitors of gastric acid secretion on mucosal adenylyl cyclase from various species. *= a significant difference from basal activity ($P < 0.05$).

were previously determined by dose-response curves to be maximally stimulating concentrations were applicable. The basal and hormone stimulated enzyme activities shown were from single representative experiments on one stomach of each species. They are representative of at least five experiments on each species where up to three agents were tested, on each, but not all agents simultaneously as shown here. The adenylyl cyclase activities of each species do not represent the maximum velocity as they were not determined at saturating substrate concentrations. However, in separate experiments, the affinities of the adenylyl cyclase activities of the four species were shown to be similar (Km $\cong$ 0.2 mM ATP) and the stimulating agents (histamine, prostaglandin E_1 and NaF) were shown to alter only the maximum velocity and not the Michaelis–Menten constant; therefore, the specific activities as indicated are representative and comparable.

Of the four species tested the guinea pig adenylyl cyclase had the highest basal specific activity. The guinea pig enzyme was also the only system significantly stimulated by histamine, although the rabbit mucosal adenylyl cyclase did show slight stimulation in some experiments as indicated in Fig. 1. None of the enzymes tested responded to the other gastric acid secretory stimulants, carbachol or pentagastrin. Of the secretory inhibitors tested, prostaglandin E_1 stimulated the adenylyl cyclase activity of all four species. Secretin and epinephrine activated adenylyl cyclase only in the rat. The sodium fluoride data are included for comparative purposes, being representative of a non-receptor requiring catalytic site activator.

The specificity of the activation of guinea pig mucosal adenylyl cyclase was further tested using histamine antagonists. The histamine H_1-receptor antagonists, tripelennamine and dimethindine, decreased the apparent affinity of the adenylyl cylcase system for histamine (3 μM to 12 and 20 μM, respectively). Each agent also slightly decreased the maximum stimulation by histamine and showed modest inhibition of basal adenylyl cyclase activity (Fig. 2). Results were similar with a third H_1-receptor antagonist, chlorpheniramine. The histamine H_2-receptor antagonist, metiamide, also diminished the apparent affinity of mucosal adenylyl cyclase for histamine but to a far greater extent (<800 μM) than the H_1-receptor antagonists (Fig. 2). Using 100 μM histamine, the H_1-receptor antagonists concentrations

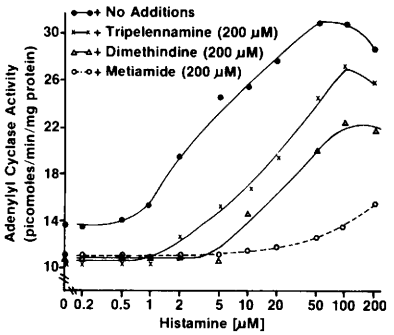

FIG. 2. Inhibition of the histamine activation of guinea pig gastric mucosal membrane adenylyl cylcase activity by histamine H_1- and H_2-receptor antagonists. Guinea pig fundic mucosal membranes were prepared and adenylyl cyclase activities were measured as described in Methods. The concentrations of the histamine antagonists were 200 μM. Reactions were incubated for 15 min at 30° using 0.2 mM ATP and enzyme activities are expressed as picomoles of cyclic AMP formed per min per mg protein. Values are the mean triplicates.

that produced half maximal inhibition were all in the same range (≅ 0.3 m*M*) but were much greater than that for the H_2-receptor antagonist, metiamide, (≅ 0.01 m*M*) (Fig. 3).

Discussion. The results of this study indicate that particulate gastric mucosal adenylyl cyclase activity can be stimulated by both inhibitors and activators of gastric acid secretion depending upon the species studied. This apparent species variability could contribute considerably to the problem of verifying or disproving the theory that cyclic nucleotides are necessary intermediates in the mechanism of action of gastric acid secretagogues. Our results constitute the first systematic study of adenylyl cyclase responsiveness to these agents in several species in which the enzyme has been localized to a particulate cell fraction. Some of our results confirm portions of previous studies of others (2, 9–17) and some conflict with published data (9–11, 13, 16), although the conflicting reports could involve methodological shortcomings of previous studies, as we have recently reviewed in detail (4). The lack of histamine stimulation in the rat study was not due to the loss of H_2-receptor since H_2-receptor binding has been identified in a similar particulate fraction from rat gastric mucosa (18).

Except in the guinea pig, the results of these investigations do not support the hypothesis that gastric secretion is initiated by a secretagogue activating particulate adenylyl cyclase on the parietal cell (1–3). Even in the guinea pig mucosa, the other two secretagogues failed to activate this enzyme under our experimental conditions, although it is possible that pentagastrin and the cholinergic agonist could utilize cyclic AMP to mediate their actions on gastric acid secretion by causing the release of histamine (19). This thesis, as far as cyclic AMP is concerned, appears applicable only in the guinea pig stomach. Our studies using antagonists of histamine action indicate that metiamide, an H_2-receptor blocker, is competitive with histamine activation of mucosal adenylyl cyclase and that metiamide has an inhibition constant at least an order of magnitude greater than the three H_1-re-

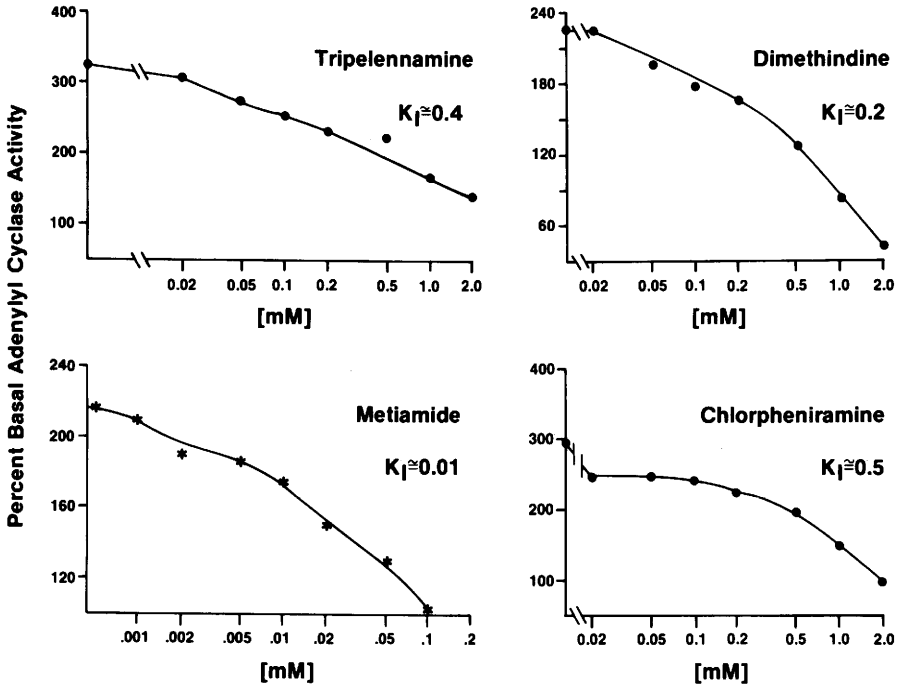

FIG. 3. Inhibition of histamine stimulated gastric mucosal membrane adenylyl cyclase activity by histamine antagonists. Enzyme preparation and assay conditions were as indicated in the legend for Fig. 2. All drugs were tested using 100 μM histamine.

ceptor antagonists tested. However, it is not clear from our preliminary studies whether or not the stimulation of adenylyl cyclase by histamine in the guinea pig can occur via a specific histamine H_2-receptor mechanisms, principally because the three H_1-receptor antagonists tested also competed with histamine, albeit with much lower apparent affinities. Other studies have also shown that H_1-receptor antagonists inhibit histamine stimulation of mucosal adenylyl cyclase (14, 17). In addition, all of the antagonists tested inhibited basal and maximally stimulated activity, which suggests a potential nonspecificity of the histamine activation.

Since these crude particulate preparations from each mammalian species contain some parietal cell plasmallemma and the enzyme of only one of the four species tested was activated by histamine, it would appear that cyclic AMP may not be a universal mediator of the actions of gastric acid secretagogues. We cannot determine from these studies whether the guinea pig response represents a true species variance or whether the heterogeneity of the mucosal preparation has contributed to our results. Studies on pure cell preparations from each species will be necessary to differentiate between these possibilities.

The only agent which activated mucosal adenylyl cyclase from each species was prostaglandin E_1, an inhibitor of gastric acid secretion. Since parietal cell membranes are in each adenylyl cyclase preparation, it may even be possible that cyclic AMP is associated with the mechanism of inhibition of gastric acid secretion by prostaglandin E_1. However, each preparation also contains other cell types, any of whose enzymes might be stimulated by the prostaglandin. Recently, an E-type prostaglandin, dibutyryl cyclic AMP and theophylline were shown to stimulate active transport of sodium across gastric epithelial cells (20).

Summary. Adenylyl cyclase activities were measured in mucosal 600*g* particulate preparations from the fundic stomach of rats, dogs, rabbits and guinea pigs. *In vitro* incubation with prostaglandin E_1 and NaF activated the enzyme in all species. Pentagastrin and carbachol were ineffective activators in any of the species tested. Histamine activated mucosal adenylyl cyclase activity in the guinea pig but not in any of the other species tested. Histamine antagonists did not conclusively demonstrate the specificity of histamine activated adenylyl cyclase. Our results do not support the universal hypothesis that activation of adenylyl cyclase is the first step in the mechanisms of action of gastric acid secretagogues. Our findings do indicate that species variability and mucosal tissue heterogeneity constitute difficult problems that must be solved prior to the elucidation of the mechanisms of regulation of acid secretion.

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