Cyclic Nucleotides and the Regulation of Canine Gastric Acid Secretion

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The response of the cyclic nucleotide system (cAMP, cGMP, adenylate cyclase, guanylate cyclase, and specific phosphodiesterases) to two gastric acid secretagogues, histamine and acetylcholine, and two secretory inhibitors, prostaglandin E₂ and secretin, was studied in vivo and in vitro in canine gastric fundic mucosa. Histamine and acetylcholine in vivo failed to stimulate cAMP but significantly increased cGMP; in vitro they affected neither adenylate cyclase nor guanylate cyclase. Prostaglandin E₂ and secretin, however, increased cAMP in vivo and significantly stimulated adenylate cyclase in vitro. Specific phosphodiesterases were unaffected by these compounds. The changes, while not specifically localized to the acid-producing cells, are consistent with the suggestion that the control of canine gastric acid secretion may be mediated by changes in mucosal cAMP and cGMP.

Although the precise mechanism which regulates gastric acid secretion has not been elucidated, adenosine 3',5'-cyclic monophosphate (cAMP) has been reported to mediate gastric acid secretion in amphibians (1, 2). Since these early reports by Harris and coworkers, attempts have been made to correlate gastric acid secretion with cAMP metabolism in a number of mammalian systems. However, results have been conflicting and there is evidence both for and against cAMP as an intracellular mediator of acid secretion (3, 4). In addition to cAMP, guanosine 3',5'-cyclic monophosphate (cGMP) has also been implicated as a regulator of gastric acid secretion. Amer et al (5) showed that rabbit mucosal guanylate cyclase activity was increased by the secretagogues—gastrin, histamine, and acetylcholine. Acetylcholine has also been shown to increase cGMP concentration in the dog fundic mucosa although pentagastrin has no effect (6).

To date, most studies have focused on a role for cyclic nucleotides in the stimulation of gastric acid secretion. It may be equally important, however, to determine whether inhibition of acid secretion is related to changes in cyclic nucleotide concentrations. In this regard, the action of prostaglandins on acid secretion appears noteworthy. Prostaglandin E₁ (PGE₁) and prostaglandin E₂ (PGE₂) are potent inhibitors of both basal gastric acid secretion and secretion stimulated by food, gastrin, histamine, and cholinergic agents (7-11). These prostaglandins are present in the gastric mucosa of animals and man located anatomically at the level of acid secreting cells (12). The release of prostaglandin E-like material from gastric mucosa is increased by vagus nerve stimulation (13). These findings may suggest a possible role for prostaglandins in the control of gastric acid secretion although the exact mechanism of action is unknown. The aim of the experiments reported in this communication is two-fold: (1) to correlate the in vivo intraarterial (IA) infusion of two gastric acid secretory inhibitors (PGE₂ and secretin) and secretagogues (histamine and acetylcholine) with acid secretion and with mucosal

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and gastric juice cyclic nucleotide concentrations; and (2) to determine whether these agents in vitro affect the activities of the enzymes which are involved in cyclic nucleotide metabolism.

MATERIALS AND METHODS

*In Vivo* Studies. Fasted mongrel dogs of either sex weighing 12-18 kg were anesthetized with intravenous Sunital (25 mg/kg) following an 18-hr fast. An endotracheal tube was inserted, and the dog was maintained in a supine position under metahane anesthesia throughout the experiment. A laparotomy was performed, the splenic artery cannulated with PE 10 tubing, and the spleen removed. This allowed direct IA infusion of drugs to the fundus of the stomach. A portion of fundus with its arterial and venous blood supply intact was enclosed in a divided lucite ring (10 cm inside diameter) modified after the method of Moody and Durbin (14). This allowed for the collection of gastric juice from one half of the chamber and for obtaining biopsies of gastric mucosa from the other half during the IA infusion of drugs. The mucosal surface was washed with saline and allowed to equilibrate for 30 min before the infusion of drugs. Gastric mucosal biopsies were taken with a tonsillar biopsy punch at specific time periods before and during drug administration. The biopsies were quick-frozen in liquid nitrogen for the later determinations of cAMP and cGMP content. Gastric juice was collected every 15 min and measured for total volume; an aliquot was used to determine H+ concentration and output by titration to pH 7 with 0.1 N NaOH using an automatic titrator. Another aliquot of the juice was used for the determination of cyclic nucleotide concentration. Prostaglandin E2, secretin, histamine, and acetylcholine were administered intraarterially in all in vivo studies. Prostaglandin E2 (9 µg) was administered to the stomach over 2 min upon a background of secretion stimulated submaximally with histamine (40 µg/kg/hr, intravenously). Secretin was given to unstimulated mucosa as a bolus of 10 clinical units over 2 min followed by constant infusion of 1 unit/min for 30 min. Histamine was administered as a push bolus of 10 µg followed by infusion at 5 µg/min for 1 hr. All histamine concentrations are expressed as the free base. Acetylcholine chloride was administered as a bolus of 30 µg over 2 min followed by constant infusion at the rate of 5 µg/min for 1 hr. Atropine sulfate when used was given as one intravenous bolus (0.2 mg/kg) 15 min before the introduction of acetylcholine. The dosages of histamine and the acetylcholine were shown by prior testing to elicit satisfactory gastric acid secretion in the preparation. Prostaglandin E2 was administered during submaximal secretory stimulation with intravenous histamine so that the smallest effective dose (one that inhibited secretion and allowed recovery) could be used. This was important since PGE2 (unlike secretin) in larger dosages caused rapid deterioration in the viability of the preparation.

Cyclic AMP and Cyclic GMP Determination. Frozen mucosal tissue samples each weighing >100 mg were homogenized in 6% TCA. After centrifugation, the TCA pellet was assayed for total protein concentration by the method of Lowry et al (15). The TCA supernatant was extracted 5 times with 10 ml of water-saturated diethyl ether, then applied to Dowex AG1-X8 (200-400 mesh) formate column (0.7 x 3 cm) previously washed with water. After sample application, the column was washed with 15 ml of water followed by 15 ml of 2 N formic acid to elute cAMP, then 25 ml of 4 N formic acid to elute cGMP. Both fractions were concentrated by lyophilization. Cyclic AMP content was determined by competitive binding to a cAMP-dependent protein kinase according to the method of Gilman (16).

Cyclic GMP was determined by radioimmunoassay according to the method of Steiner et al (17), with prior sccinylolation of the cyclic nucleotide (18). Sensitivity of the assay was to the level of 0.005 pmol cGMP. Tissue content of cAMP and cGMP were expressed as pmol/mg protein. [3H]cAMP and [3H]cGMP markers added during homogenization showed recovery of 70-80% for cAMP and 60-70% for cGMP.

Samples of gastric juice for determination of cyclic nucleotide concentration were treated with TCA, centrifuged, and the supernatant fractionated and assayed for cAMP and cGMP as described above. To confirm the binding specificity of the assays, samples were also treated with excess phosphodiesterase. In all gastric juice samples, there was no detectable cAMP or cGMP after phosphodiesterase treatment.

Assay for Adenylate and Guanylate Cyclase Activities. Fresh fundic mucosa was obtained from anesthetized dogs that were fasted for a period of 18 hr. The tissue was rinsed in cold saline and the glandular mucosa was scraped from the underlying muscularis mucosa and muscle layer with a scalpel. The mucosal scrapings were immediately homogenized in cold buffer containing 100 mM Tris HCl, pH 7.5, and 1 mM dithiothreitol (DTT) using a glass homogenizer fitted with a motor driven (8000 rpm) Teflon pestle (four to six strokes). Adenylate cyclase activity was determined in the total homogenate by measuring the amount of [32P]cAMP produced from [α-32P]ATP, using cellulose thin-layer chromatography for separation (19). The reaction mixture in a total volume of 0.05 ml, contained 40 mM Tris HCl, pH 7.5, 10 mM MgCl2, 0.2 mM cAMP, 1 mM 3-isobutyl-1-methylxanthine, 16 mM phosphoenolpyruvate, 3 units pyruvate kinase, 4 mM KC1, 1 mM [α-32P]ATP at 50 cpm/pmol, and 100-200 µg protein homogenate. The reaction was incubated at 37°C for 5 min and stopped by the addition of 5 µl of a mixture containing 50 mM EDTA and 10 mM cAMP and immediately heated for 3 min at 100°C. Guanylate cyclase activity was measured in the supernatant after the mucosal homogenate had been centrifuged at 12,000 g for 30 minutes. The reaction mixture contained 40 mM Tris HCl, pH 7.5, 4 mM MnCl2, 0.2 mM cGMP, 1 mM 3-isobutyl-1-methylxanthine, 15 mM creatine phosphate, 20 µg creatine phosphokinase, 1 mM [α-32P]GTP (50 cpm/pmol), and tissue supernatant. The incubation conditions and separation of cGMP were as described above for adenylate cyclase. Adenylate cyclase and guanylate cyclase activities were assayed as pmol cAMP or cGMP formed/mg protein/minute of incubation. The enzyme activities reported were linear with time of incubation and protein concentrations used.