Cyclic Adenosine Monophosphate and Human Gastric Acid Secretion*

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This report concerns gastric juice, plasma, and urinary levels of adenosine 3',5'-monophosphate (cAMP) in 27 subjects undergoing routine gastric analysis under maximum stimulation with betazole or pentagastrin. Cyclic AMP was measured by sensitive and specific radioimmunoassay. No increase in concentration of cAMP was noted in gastric juice, plasma, or urine following either betazole or pentagastrin stimulation. Betazole-stimulated human gastric acid secretion was associated with an increased cAMP output into the gastric juice ($P < 0.05$). There was no change in cAMP output following pentagastrin stimulation. The peak acid output produced by pentagastrin and betazole was similar. The lack of increase in cAMP concentration lends support to the concept that cyclic AMP is not a primary mediator in the stimulation of gastric acid secretion by betazole or pentagastrin in the human. The physiologic significance of the increase in cAMP output following betazole stimulation remains unresolved.

Studies in man and animal models, both *in vivo* and *in vitro*, have shown that the naturally occurring cyclic nucleotide, adenosine 3',5'-monophosphate (cAMP), mediates a variety of hormonal actions. The hormone, a polypeptide or a biologically active amine, travels from its site of origin to the cells of its target tissue where it interacts with a membrane-associated enzyme system, adenylate cyclase. This leads to the generation of cAMP which then mediates the effect of the hormone inside the cell (1–3).

Applying this concept to the study of gastric acid secretion has produced conflicting results. In amphibians, stimulated gastric acid secretion appears to be mediated by the intracellular effects of cAMP (4–6). Increased adenylate cyclase activity following histamine stimulation has been reported in canine, rabbit, and guinea pig gastric mucosa (7–9), as well as increased output of cAMP in canine and human gastric juice (7, 10, 11). Others, however, were unable to demonstrate a significant change in cAMP levels or adenylate cyclase activity in canine gastric mucosa following histamine stimulation (12, 13).

The role of cAMP in pentagastrin- and cholinergic-stimulated acid secretion is uncertain. Preliminary studies suggested that pentagastrin stimulates acid secretion by activation of mucosal adenylate cyclase (4, 14). Other studies, however, have failed to show a rise in mucosal or gastric juice cAMP under stimulation by pentagastrin or cholinergic drugs (8, 9, 11, 15–17).

In man, association between changes in levels of cAMP in biological fluids with hormonally induced changes in the composition of these fluids (18, 19) has provided evidence that this cyclic nucleotide is important as a mediator of the actions of these hor-

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TABLE 1. PLASMA AND URINARY LEVELS OF cAMP (MEAN ± SEM)

Plasma cAMP (pmol/ml)					*Urinary cAMP (μmol/g Cr)**			
Betazole		*Pentagastrin*			*Betazole*		*Pentagastrin*	
0	*30*	*0*	*30*	*60*	*Basal*	*Stimulated*	*Basal*	*Stimulated*
22.8 ± 2.5	25.1 ± 2.8	17.4 ± 1.8	19.1 ± 2.3	18.9 ± 1.9	5.8 ± 0.9	5.3 ± 0.5	3.7 ± 0.7	3.8 ± 0.9

*Basal specimens were obtained immediately before administering secretagogue. Stimulated specimens were obtained at the end of the 90-min poststimulation period.

mones. The purpose of this study was to quantify cAMP in gastric juice, first under basal conditions and then following maximum stimulation with betazole or pentagastrin to determine whether an association between gastric juice cAMP and these secretagogues exists. In addition, plasma and urinary cAMP were measured to detect any change which might result from back diffusion into the bloodstream with subsequent filtering into the urine.

MATERIALS AND METHODS

Twenty consecutive patients undergoing routine gastric analysis (10 with active duodenal ulcer, 10 with benign gastric ulcer) and seven normal volunteers were studied. A radiopaque nasogastric tube was placed in the gastric antrum under fluoroscopic control. Specimens of gastric secretion were obtained by continuous suction over 15-min periods. Suction was broken manually every 5 min, and air was injected to clear the tube and prevent the mucosa from sealing the tube opening. All studies were done after an overnight fast of at least 8 hr. Subjects were placed in the left lateral decubitus position and encouraged to expectorate all salivary secretions. Following a 1-hr period, pentagastrin* (6 μg/kg) was administered by subcutaneous injection to the first 13 subjects, and betazole† (1.5 mg/kg) was given subcutaneously to 14 additional subjects. Six additional 15-min collections were then obtained over the following 90-min period. Gastric secretions collected in this manner were clear, with no bilious appearance to suggest contamination by duodenal fluid. The volume of each sample was accurately recorded and the pH determined by a Beckman Expandomatic SS-2 pH meter. Total acidity was determined by titration of an aliquot of each sample against 0.1 N NaOH to a phenolphthalein endpoint. Normal volunteers and patients with gastric and duodenal ulcers were distributed equally in the pentagastrin and betazole groups.

*In the form of Peptavlon, Ayerst Laboratories, New York, New York.

†In the form of Histalog, Eli Lilly and Company, Indianapolis, Indiana.

Blood and urine samples were obtained prior to and following stimulation. Blood specimens were drawn into chilled EDTA-containing vacutainers and were immediately centrifuged at 3000 rpm at 4° C. Urine was freshly voided prior to and at the end of the stimulation period and kept refrigerated until processed. Radioactive cAMP ([^{3}H]cAMP, 40,000 cpm) was then added to 5-ml aliquots of gastric juice and plasma. The pH of the gastric juice samples was adjusted to 6.2 with dry $KHCO_3$. Ultrafiltrates of gastric juice and plasma were immediately prepared by centrifugation at 3500 rpm in the Amicon Corporation Centriflo system utilizing Diaflo ultrafilters with 50,000 mol wt retention. Resulting ultrafiltrates were clear and showed no precipitate after addition of 10% TCA to small aliquots. An aliquot of the ultrafiltrates (0.1 ml) was counted in 10 ml of Aquasol in a Nuclear Chicago Model 6848 liquid scintillation counter to determine recovery of cAMP, which averaged 98%. Ultrafiltrates and urine samples were diluted in sodium acetate buffer and assayed utilizing a sensitive and specific radioimmunoassay according to the method of Steiner et al (20). This assay has been shown to be capable of accurately measuring cAMP without the use of chromatographic techniques to isolate or purify the compound. The final assay dilution in each tube for gastric juice ultrafiltrates was 1 : 50; for plasma ultrafiltrates, 1 : 20 to 10 : 50; and for urine samples, 1 : 1000 to 1 : 20,000. Bound and free ^{125}I ligands were separated by ammonium sulfate precipitation. Anticyclic AMP antiserum, [^{125}I]succinyl cAMP tyrosine methyl ester, and cAMP standard were obtained from Schwarz/Mann, Orangeburg, New York.

Salts are known to interfere with cyclic nucleotide radioimmunoassays, and varying concentrations of salts were present in the clinical samples of gastric juice because of titration of HCL present in them with dry $KHCO_3$. To determine whether this affected the results obtained, an experiment was designed in which solutions varying in HCL content from 10^{-2} to 10^{-6}, each containing 4 pmol/ml cAMP, were titrated to pH 6.2 using dry $KHCO_3$. A 100-μl aliquot was assayed in duplicate in the radioimmunoassay for cAMP. No effect was noted with variations of salt concentrations in this range.

In preliminary experiments the concentration (picomole/tube) of cAMP in gastric juice and plasma ultrafiltrates, in final assay dilutions of 1 : 20, 1 : 50, and 1 : 100,

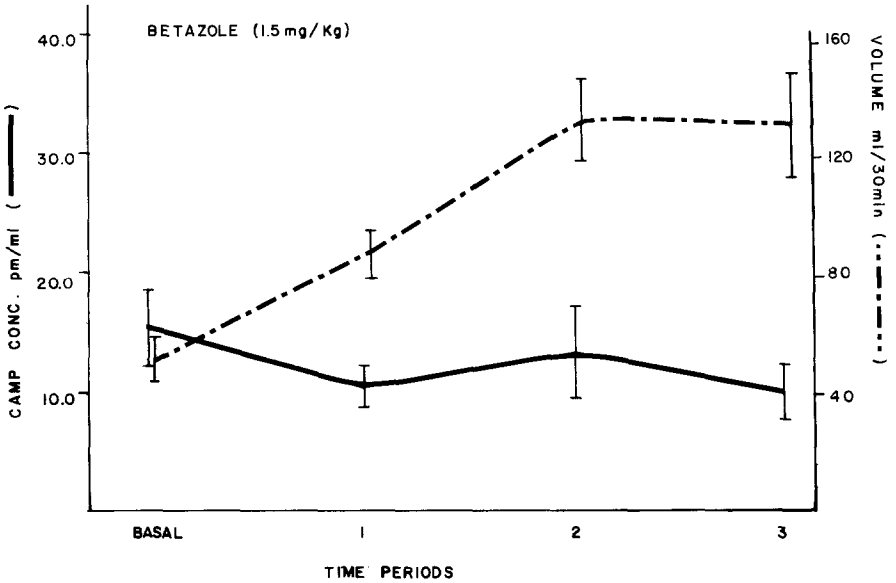

Fig 1. The concentration (pmol/ml) of cAMP following maximum betazole stimulation. Each point represents the mean ± SE of 14 patients. Numbers on the abscissa represent 30-min time periods.

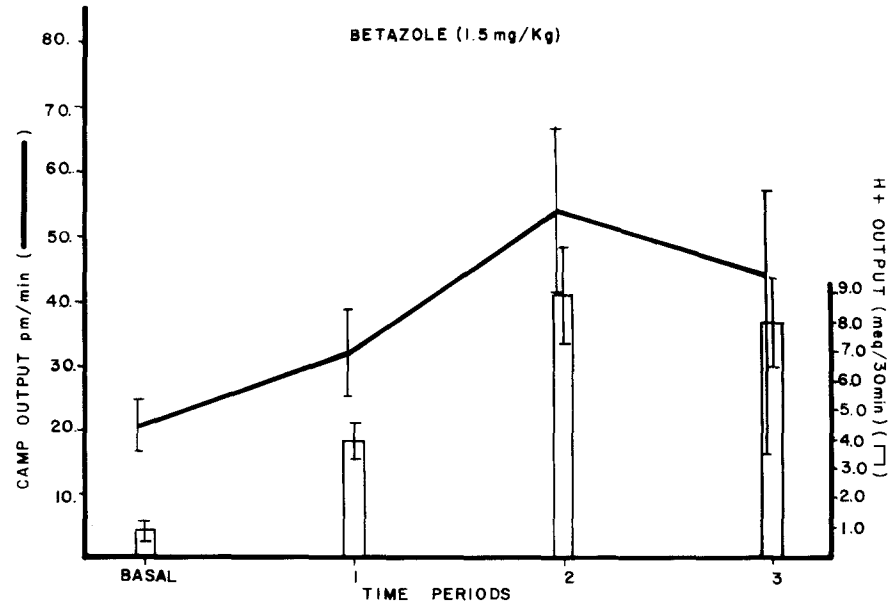

Fig 2. The output (pmol/min) of cAMP (mean ± SE) following maximum betazole stimulation in 14 patients. cAMP output increases in all subjects ($P < 0.05$).

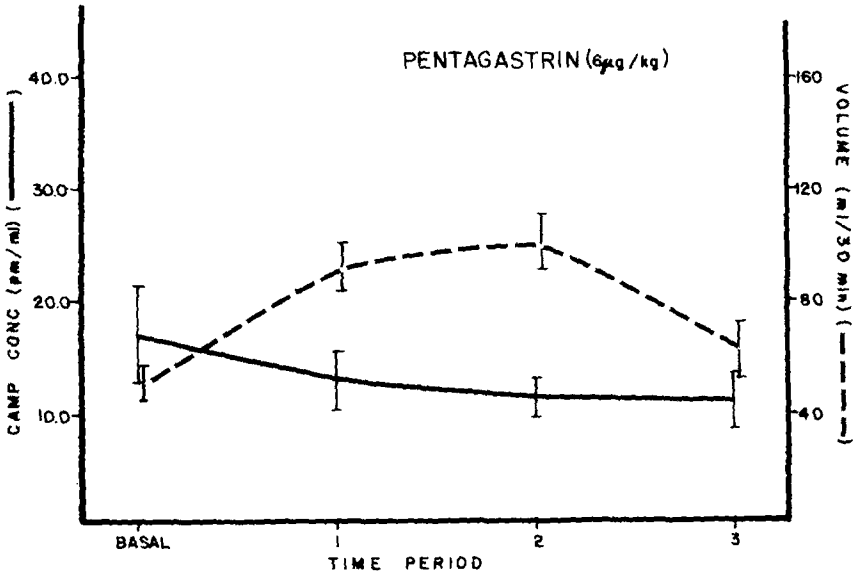

Fig 3. Concentration of cAMP (mean ± SE) following maximum pentagastrin stimulation in 13 subjects. Following pentagastrin stimulation, cAMP falls slightly below basal levels during maximum gastric juice volume output. This change is not significant ($P > 0.05$).

were found to parallel the standard curve in the radioimmunoassay, indicating immunochemical identity between the standard cAMP and the material in the biological samples being measured as cAMP. Known amounts of cAMP added to gastric juice and plasma samples in the assay were recovered quantitatively. Phosphodiesterase blanks were run with each specimen.

The intraassay coefficient of variability was 8%. All determinations were performed in duplicate, and all samples from an individual subject were measured in the same assay. Statistical analysis was performed by Student's *t* test (21). All patients and volunteers gave informed consent, and this study was approved by the Human Investigation and Research Committee of the National Naval Medical Center, Bethesda, Maryland.

RESULTS

Mean basal acid output and mean basal gastric juice concentration of cAMP in the subjects undergoing betazole and pentagastrin stimulation were not statistically different. Basal plasma concentrations and urinary excretion of cAMP were comparable in the two subject groups and did not change following stimulation (Table 1). During betazole stimulation, mean acid output increased from a basal value of 2.0 ± 0.2 mEq/hr to a peak output of 18.0 ± 0.8 mEq/hr. The concentration of cAMP did not change significantly (Figure 1). Cyclic AMP output, expressed as picomoles/minute, rose following betazole ($P < 0.05$) (Figure 2).

During pentagastrin stimulation, mean basal acid output rose from 1.2 ± 0.1 mEq/hr to a peak output of 18.0 ± 0.7 mEq/hr. The concentration of cAMP in the gastric juice declined during the first 30 min following pentagastrin stimulation (Figure 3). However, this change did not reach statistical significance. The output of cAMP remained unchanged following pentagastrin (Figure 4).

DISCUSSION

It appears well established that the adenylate cyclase–cAMP system mediates histamine and gastrin stimulation of acid secretion in amphibians (4–6, 22).

Studies in mammals have yielded varying results. In the rat, histamine and pentagastrin have been shown to increase gastric mucosal adenylate cyclase and cAMP content (15, 23, 24) and cAMP and dibutyryl cAMP increase acid secretion (25). Other studies have suggested a decrease in acid secretion following dibutyryl cAMP (26) and stimulation of adenylate cyclase *in vivo* by pentagastrin but not *in vitro* (27). In the rabbit, gastric fundic adenylate cyclase was stimulated by histamine but not by pen-

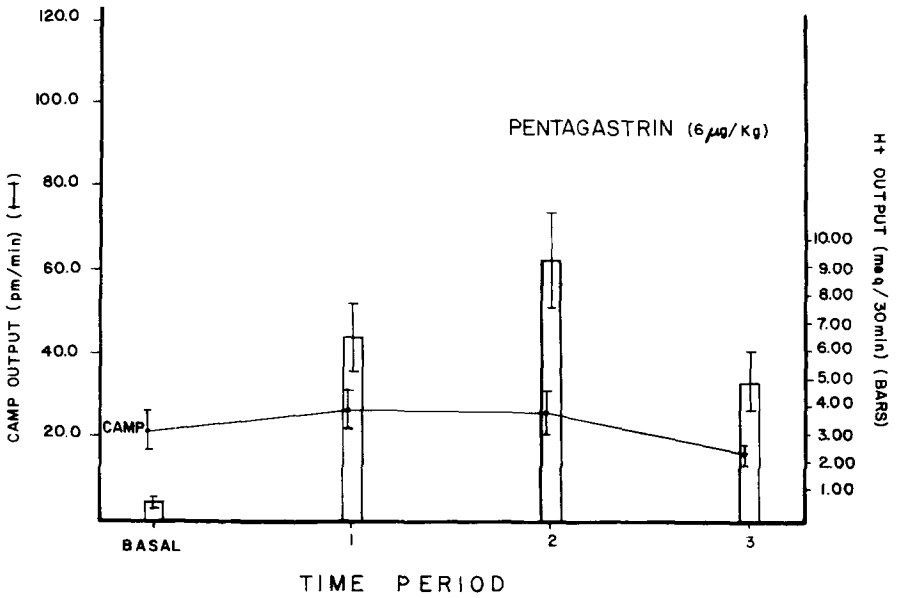

Fig 4. Cyclic AMP and H^+ output (mean ± SE) following maximum pentagastrin stimulation in 13 subjects showed that cAMP output does not rise.

tagastrin *in vitro* (28). Studies with different phosphodiesterase inhibitors suggested that acid secretion was inhibited by increased cAMP (29). *In vitro* studies indicate that cAMP and related agents stimulate acid secretion from rabbit fundic mucosa (30). In the guinea pig, histamine and betazole stimulate mucosal adenylate cyclase, while gastrin does not (9, 31). Metiamide, a specific histamine H_2-receptor blocker which inhibits histamine-stimulated gastric acid secretion, also blocks histamine stimulation of adenylate cyclase (32). In the dog, some studies have shown increased mucosal levels of cAMP following histamine and increased output of cAMP in gastric juice following histamine or pentagastrin (7). Methyl xanthine augments the acid secretory response to pentagastrin (33). Other studies have failed to show increased cAMP or adenylate cyclase activity in the mucosa (12, 13), and dibutyryl cAMP did not increase acid secretion (13). In man, methyl xanthine augments submaximally stimulated gastric acid secretion by histamine and pentagastrin (34, 35). One study of a small number of subjects showed an increase in cAMP output in gastric juice following betazole (7). Other studies suggested similar findings (10, 11). No increase in gastric mucosal cAMP following pentagastrin was noted in another study (16). The recent study by Talev (36) suggests that neither betazole, histamine, nor pentagastrin cause increases in gastric mucosal or gastric juice cAMP concentrations. Some of the variations between these results can possibly be ascribed to species differences, but many cannot be resolved simply.

The present study indicates that betazole-stimulated human gastric acid secretion is associated with a rise in cAMP output in gastric juice, while pentagastrin-stimulated acid secretion is not. This may indicate that the increased cAMP output following betazole is derived from a nonparietal cell source, either from a cell which is acted upon by betazole but not pentagastrin, from interstitial fluid in the wall of the stomach, or from plasma. The known effects of histaminic compounds of increasing vascular permeability would make the latter possibility quite feasible. An alternative explanation would be that betazole action on the parietal cell in stimulating acid secretion is mediated through adenylate cyclase, while that of pentagastrin is not. The study in humans of mucosal cAMP levels following histamine (36), suggests that this is not the case. Also, the lack of increase in concentration of cAMP in gastric juice, plasma, or urine following betazole stimulation, as reported here, would make this explanation unlikely. The increase in cAMP

concentration is several-fold in studies of changes in cAMP concentration resulting from hormonal action where it has been clearly demonstrated that cAMP is acting as a second messenger, ie, changes in cAMP concentration in urine following administration of parathyroid hormone, or in plasma following glucagon administration. Thus, this study suggests that cAMP does not play a dominant mediatory role in betazole or pentagastrin stimulation of human gastric acid secretion.

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