PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 725–730 (1975)

Acid-Secretory Effects of Pentagastrin, Histamine, Urecholine, DBcAMP, and cGMP in Isolated Stomachs of Fed and Fasted Rats (38887)

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(Introduced by Virgil D. Wiebelhaus)

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During the course of experiments on acid secretion in the isolated rat stomach, it was observed that pentagastrin had no effect in the fasted rat but induced a marked stimulant response when in contact with the serosa of the stomach of a fed rat. Considering that basal secretion for the stomachs of both fasted and fed rats was found to be approximately the same in this system, the phenomenon of increased sensitivity of the fed stomach prompted us to investigate the effects of several other compounds; viz., histamine, urecholine, the dibutyryl derivative of adenosine 3′,5′-monophosphate (DBcAMP), and guanosine 3′,5′-monophosphate (cGMP).

Materials and Methods. Male Charles River rats (200–300 g) were killed by cervical dislocation, and the stomachs were quickly removed and placed in oxygenated secretory solution. The esophagus was ligated as close to the stomach as possible; another ligature was placed below the antrum. After a 1-cm cut along the rumen, the stomach contents were emptied before the stomach was turned inside out with a glass rod (Fig. 1). The stomach was rinsed gently in the oxygenated secretory solution while it was still on the glass rod. After the rinsing, another ligature was closed around the rumen onto the glass rod. The rod was finally withdrawn and the ligature snugly tied. As a final rinse, the mucosal surface of the stomach was washed gently with secretory fluid. Nutrient solution (2.5 ml) was injected into the everted stomach with a 27-gauge needle. The whole stomach was then placed in a 30-ml sintered-glass filter which contained 20 ml of secretory solution. A waterjacket around the filter maintained the temperature of the solution at 37°. Oxygen (100%) was bubbled up from the bottom of the filter at a rate sufficient to ensure adequate respiration and mixing.

Dikstein and Sulman (1) observed that when specific Ringer-type solutions were applied to the nutrient and secretory surfaces of the isolated, everted rat stomach, pH of the secretory solution dropped considerably in 2 hr. Instead of sampling pH, we continuously monitored the acid secretory rate over a 3- to 5-hr period by the pH stat method.

Acid secretion was monitored and titrated (0.1 *N* NaOH) with a Radiometer pH stat apparatus (pH Meter 26, Autotitrator 11, ABU 1B) according to the method described by Durbin and Heinz (2). In most experiments pH was maintained between 6.8 and 7.0.

We used solutions identical to those reported by Dikstein and Sulman except that we did not use histamine (100 μg/ml) in the secretory solution. We found that basal secretion of the fasted rat stomach was not influenced by this level of histamine. Both the nutrient (serosal) and secretory (mucosal) solutions were of the following composition (mmoles/liter): Na^+, 100; Cl^-, 100; K^+, 6.0; HPO_4^{2-}, 2.0; glucose, 80; in addition, the secretory solution contained Mg^{2+}, 2.0 mmoles/liter. The pH and osmolarity of the oxygenated solutions were approximately 7.4 and 300 mOsm/liter, respectively. Solutions of test compounds were buffered to pH 7.4 and were given at not more than 0.05 ml into the nutrient fluid and 0.4 ml into the secretory chamber.

The rats were maintained on Purina Lab Chow. Both fed and fasted animals were allowed water ad lib. until the start of testing each day at 10:00 AM. To insure food-free stomachs, fasted rats were taken off

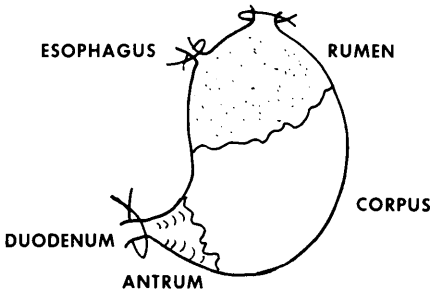

FIG. 1. Everted rat stomach with ligatures placed around esophagus, duodenum, and area of 1-cm incision in rumen.

feed at approximately 10:00 AM of the day prior to testing.

Pentagastrin, made up as a stock solution (1 mg/ml) was kept frozen until needed. Histamine diphosphate doses are expressed as free base. Secretion results are either expressed as microequivalents (μeq) of acid secreted per hr or, when compounds were given, as the percentage change from control hourly rate. Urecholine (bethanechol), prepared as a 1% solution of the hydrochloride salt, was made up in nutrient solution and buffered to pH 7.0. DBcAMP needed no buffer adjustment for the 0.5% solution made up in nutrient solution. cGMP was dissolved in nutrient fluid to a concentration of 0.1% and required a few drops of 0.1 *N* HCl to reach pH 7.4.

Results. Controls. Initially, all experiments were carried out with rats fasted overnight as recommended by Dikstein and Sulman. Subsequent studies were run with stomachs from either fed or fasted rats because their basal secretion rates were found to be approximately the same. With either preparation, the rate of acid output averaged between 30 and 34 μeq per hr and did not vary appreciably over 4 hr (see Table I).

Pentagastrin. Pentagastrin had a marked stimulant effect on acid secretion when added to the nutrient fluid of the fed rat stomach at a concentration of 3×10^{-5} *M* (Fig. 2). Acid output climbed progressively (+28, +57, +82, and +116%) over control rate in the 4-hr postdrug period. When the same dose of pentagastrin was added to the nutrient fluid of the fasted rat stomach, however, no increase in secretion was observed. Pentagastrin (3×10^{-5} *M*) was much less active when added to the secretory fluid. Acid secretion in the stomach of fed animals increased slightly (+7, +20, and +26%) over controls during the first 3 hr. Again, the fasted stomach was not stimulated by pentagastrin at this dose.

TABLE I. CONTROL ACID SECRETION—ISOLATED RAT STOMACH.

	Average μeq/hr (±SE) Hours				
	1	2	3	4	(*n*)
Controls (fasted)	31±4	31±3	32±3	32±4	9
Controls (fed)	30±2	29±2	29±2	30±3	9

Histamine. Histamine was studied in greater detail than any of the compounds tested. Results of a dose-response test in which acid output 3 hr after the administration of histamine to the secretory fluid indicated that peak activity occurred at a concentration of 4.5×10^{-5} *M* (5.0 μg/ml) (Fig. 3). In the fed rat at this dose, secretion was increased 86% above controls. A 39% increase was observed in the fasted rat also at this concentration. Activity was still pronounced (66%) at a concentration of 4.5×10^{-6} *M* (0.5 μg/ml) and fell off to 22% above controls at 0.9×10^{-6} *M* (0.1 μg/ml) of histamine in the fed rat. At these two doses, the increases in secretion were not statistically significant. These findings indicate a greater degree of sensitivity for histamine than that shown in a recent publication by Assem, Schild, and Wan (3) where they reported peak histamine responses occurring at doses between 12 and 50 μg/ml (base) for isolated rat fundic strips in an Ussing type of experiment.

In one experiment comparative effects of histamine were followed for 3 hr after a single dose was given to the nutrient and secretory fluids. At a concentration of 4.5×10^{-4} *M* on the nutrient side, histamine produced increases in secretion of +33, +46, and +65% over controls in the fed rat stomach (Fig. 4) over the 3 hr. No activity was seen in the fasted stomach. Exposure of the secretory surface to the 4.5×10^{-4} *M* concentration of histamine resulted

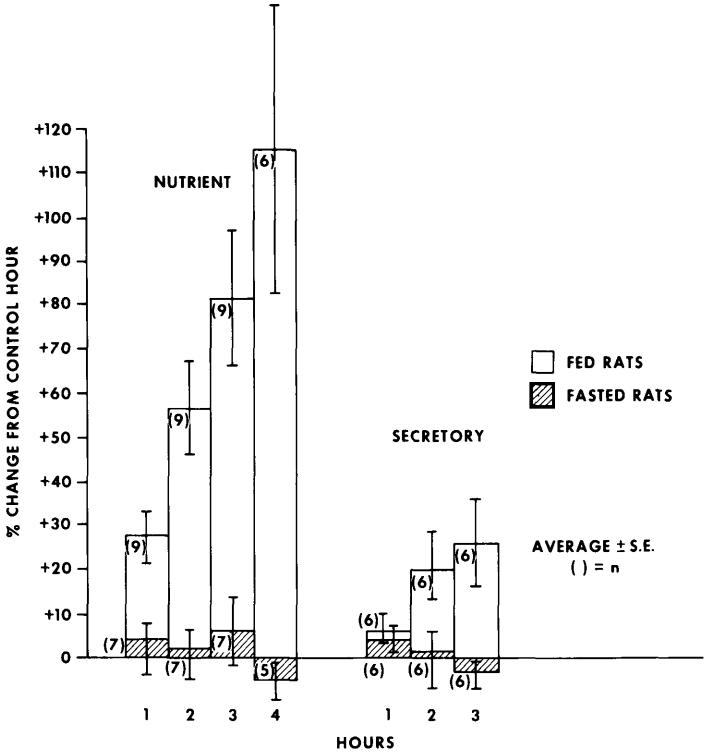

FIG. 2. Acid secretion in the fed and fasted rat stomach after the addition of pentagastrin (3×10^{-5} *M*) to the nutrient and secretory fluids.

in increases of +14, +30, and +51% over control secretion in the fed rat stomach. A slight, but progressive, decrease in acid output was seen in the stomachs of fasted animals at this dose.

Urecholine (bethanechol). Increased sensitivity of the fed, as opposed to the fasted, stomach was also seen when the cholinergic stimulant, bethanechol, was added to the nutrient fluid of the fed rat stomach at a concentration of 2.2×10^{-4} *M*. In a 5-hr period acid secretion was increased +13, +30, +44, +65, and +107% over controls (Fig. 5). Maximum change during this interval in the fasted rat stomach was +10% in the 5th hr. This cholinergic drug did not appear to be active from the secretory side. Cholinergic testing was done also with carbachol, but results with this compound were erratic and the experiments were discontinued.

DBcAMP. Because of the activity demonstrated by pentagastrin and histamine in the fed rat, one of the substances thought to be involved in the sequence of secretory events beyond histamine, viz., cyclic AMP (cAMP), was tested using the dibutyryl salt (DBcAMP). The compound was applied to gastric mucosa of fed and fasted rats at a concentration of 10^{-4} *M*, simultaneously, to both the nutrient and secretory surfaces of the stomach (Fig. 6). Acid secretion was measured for 4 hr. A progressive increase in secretion occurred (27, 47, 66, and 115%) in the fed rat stomach and essentially no activity was present in the fasted animals. The marked sensitivity to DBcAMP in the fed rat was very similar to the activity obtained with pentagastrin under the same conditions.

cGMP. When given simultaneously to the nutrient and secretory surfaces at the same concentration as DBcAMP, (10^{-4} *M*), cGMP showed marked stimulating effects—approximately twice that of DBcAMP (Fig. 6). During the 4-hr postdrug period, acid secretion increased +7, +41, +89, and +212% above controls in the fed rat.

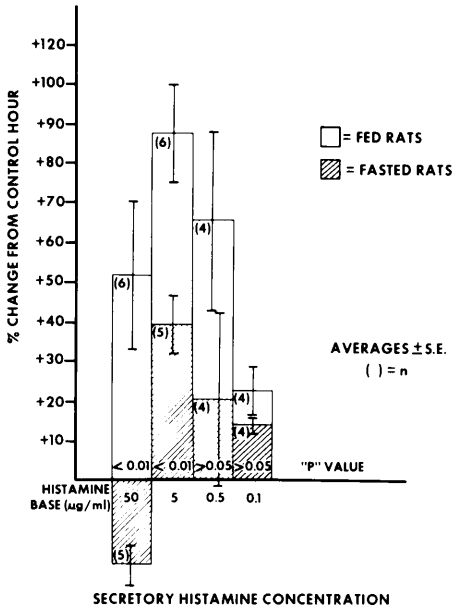

FIG. 3. Acid secretion in the fed and fasted rat 3 hr after the addition of histamine (base) to the secretory fluid at concentrations of 50, 5, 0.5, and 0.1 µg/ml (4.5×10^{-4}, 4.5×10^{-5}, 4.5×10^{-6}, and 0.9×10^{-6} *M*).

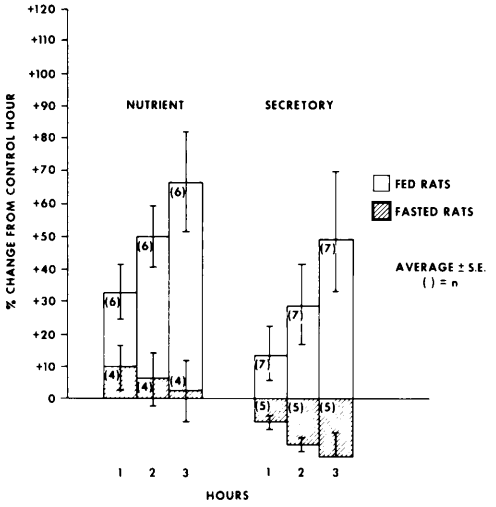

FIG. 4. Acid secretion in the fed and fasted rat stomach after the addition of histamine (4.5×10^{-4} *M*, 50 µg/ml, base) to the nutrient and secretory fluids.

Unlike DBcAMP, which was inactive in the fasted rat, administration of cGMP resulted in increases in secretion of +3, +16, +46, and +88% in fasted animals. It was apparent that, in the fed rat, the effects of cGMP were more potent but more variable than the responses to DBcAMP.

Discussion. These experiments demonstrate that stimulation of acid secretion from the isolated stomach of the fed, but not the fasted rat, is remarkably sensitive not only to pentagastrin, but also to histamine, urecholine, DBcAMP, and cGMP. Why recent food intake should catalyze the activity of these compounds, is unclear. If one would try to implicate factors such as stretching, increased fatty acid production, or higher levels of histidine decarboxylase as a common denominator for the enhanced activity of the fed rat to these agents, it becomes difficult to rationalize the observation that basal secretion of the fed and fasted rat is approximately the same. Of these possibilities, it is tempting to implicate, as yet in some unclear fashion, the enzyme histidine decarboxylase for part of

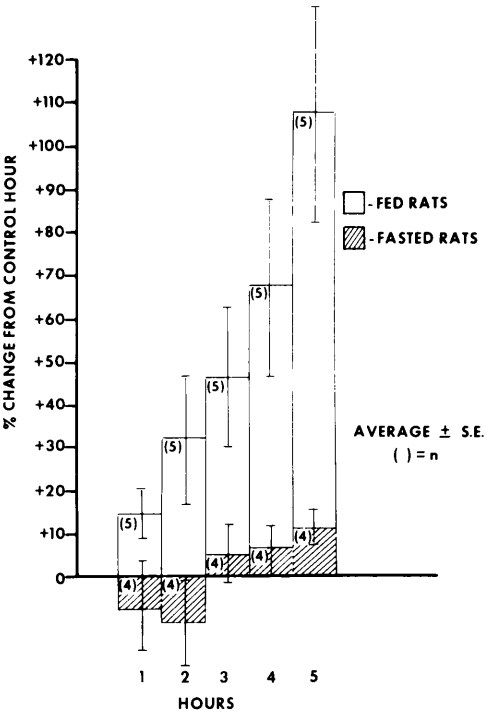

FIG. 5. Acid secretion in the fed and fasted rat stomach after the addition of urecholine (2.2×10^{-4} *M*) to the nutrient fluid.

the differences observed. As is known, fasting decreases and food elevates histidine decarboxylase in the gastric mucose (4, 5). Although it appears difficult to correlate

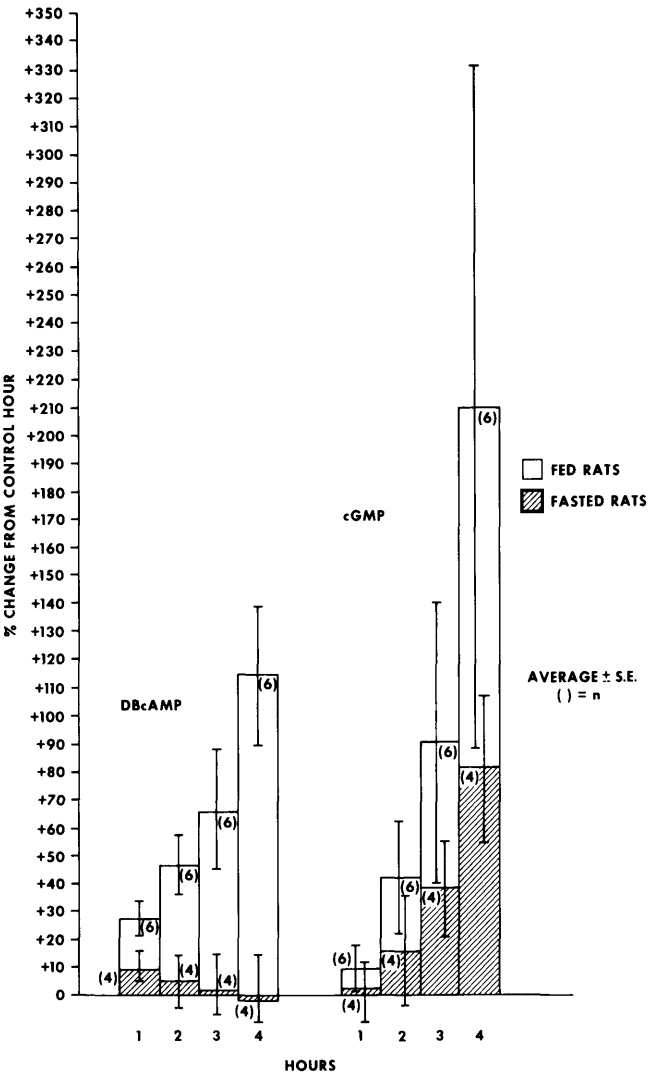

FIG. 6. Acid secretion in the fed and fasted rat stomach after the addition of DBcAMP and cGMP (10^{-4} *M*) to the nutrient and secretory fluids.

increased total tissue histamine levels with elevated concentration of histidine decarboxylase (6), the recompartmentalization or redistribution of histamine in the fed animal might contribute to the increased sensitivity of the compounds in these tests. For instance, it is thought that gastrin might exert its effect by releasing histamine from a gastrin-sensitive histamine pool (7, 8). It is also possible that the absence of or diminished response in fasted rats to stimuli in the present study could be due to reduced serum gastrin levels in fasted animals. Wilder and Hohnke (9) reported that gastrin levels in the fed rat were three times greater than those in 24-hr fasted rats.

From the enhanced secretion produced by DBcAMP in these tests, it appears likely that the effects of pentagastrin, histamine, and urecholine are related to an activation of cyclic AMP via the adenyl cyclase system. Although Ruoff and Sewing (10) reported that 24-hr fasted rats were not significantly different in mucosal levels of cAMP from fed animals, results of a later study by Glick *et al.*, (11) showed that in the area of the chief cells of fed rats, there was a 100%

greater concentration of cAMP than in the equivalent area of the fasted rat.

There is gathering evidence that cGMP may be strongly involved in the cholinergic stimulation of acid secretion (12). Our observations of the marked secretory effect produced by bethanechol and cGMP in the fed rat support the possible involvement of this nucleotide in the final events of acid secretion in this species. Difference in the response to secretagogues in this study also might be related, not to any function of the stomach itself, but to a prior conditioning of the mucosa by one or more of several intestinal substances (secretin, glucagon, cholecystokinin–pancreozymin, etc.) known to have an inhibitory effect on acid secretion.

The histamine dose-response section of this study serves to emphasize that compound concentration is critical in the appraisal of secretory activity and that the "stat" doses used for the evaluation of other compounds in this test may not represent their optimal concentrations.

The isolated stomach of the fed rat appears to be a simple but sensitive *in vitro* test system for studying basic secretory mechanisms in the rat. Secretion with several known stimulants can be achieved without a "resting period" as is the case with frogs, or without special elaborate pretreatment. The secretory response to cGMP, and its relationship to cholinergics and possibly other secretagogues, seems worthy of further investigation. These experiments also raise questions in the choice of the fed or fasted animal for studies on secretion, absorption, or transport along other portions of the gastrointestinal tract.

Summary. Pentagastrin, histamine, urecholine, DBcAMP, and cGMP were all potent stimulants of acid secretion in the isolated stomach of the fed rat. With the exception of histamine and cGMP, all of these compounds were inactive when the stomach of the fasted rat was used. Pentagastrin was most effective when given on the serosal surface while histamine was equally potent whether it was added to the mucosal or serosal surface of the isolated stomach. The test would appear to be a relatively simple, but effective system for studying the basic mechanisms of action of several important secretagogues in the rat.

1. Dikstein, S., and Sulman, F. G., Biochem. Pharmacol. **14**, 355, 1965.
2. Durbin, R. P., and Heinz, E., J. Gen. Physiol. **41**, 1035, 1958.
3. Assem, E. S. K., Schild, H. O., and Wan, B. Y. C., J. Physiol. **234**, 75, 1973.
4. Kahlson, G., Rosengren, E., Svahn, D., and Thumberg, R. J. Physiol. (London) **174**, 400, 1964.
5. Kahlson, G., Rosengren, E., and Thumberg, R., J. Physiol. (London) **190**, 455, 1967.
6. Weidle, E., and Sewing, K.-Fr., J. Pharm. Pharmacol. **25**, 234, 1973.
7. Aures, D., Hakanson, R., and Schauer, A., Eur. J. Pharmacol. **3**, 217, 1968.
8. Kim, Y. S., and Glick, D., Gastroenterology **55**, 657, 1968.
9. Wilder, D. E., and Hohnke, L. A., Physiologist **17**, 359, 1974.
10. Ruoff, H.-J., and Sewing, K.-Fr., Scand. J. Gastroenterol. **8**, 241, 1973.
11. Glick, D., Katsumata, Y., and von Redlich, D., J. Histochem. Cytoche . **22**, 395, 1974.
12. Eichhorn, J. H., Salzman, E. W., and Silen, W., Proc. 59th Annu. Clini. Conf., Amer. Coll. Surg. **24**, 363, 1973.

Received Dec. 16, 1974. P.S.E.B.M., 1975, Vol. 149.