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Histamine, Pentagastrin, Methyl Xanthines and Adenyl Cyclase Activity in Acid Secretion (38414)

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Caffeine, histamine, and pentagastrin have been found to stimulate acid secretion in man, but previous studies have shown that caffeine is ineffective as a stimulant for acid secretion in the dog (1). Histamine in various combinations with pentagastrin or gastrin potentiates acid secretion in the dog (2), but it is debatable whether potentiation occurs in man (3–5).

The methyl xanthines increase cyclic AMP levels in tissue by inhibiting the enzyme phosphodiesterase which is responsible for its destruction. Harris *et al.* (6) found that, in the frog, gastric acid secretion was stimulated by cyclic AMP and suggested that pentagastrin may mediate its effect via that mechanism. If pentagastrin stimulates gastric acid secretion via cyclic AMP, it would seem likely that acid output in response to pentagastric plus a methyl xanthine would be greater per dose of stimulant than with pentagastrin alone; *i.e.*, the dose–response curve would shift to the left. Also these agents would be expected to stimulate adenyl cyclase in broken cell preparations in accord with the postulates of Sutherland (7).

The aim of the present study was to test this hypothesis by (a) studying the *in vivo* effects of the methyl xanthines on acid output stimulated by histamine or pentagastrin in man and dog and (b) by studying the effect of pentagastrin and histamine on adenyl cyclase activity in dog gastric mucosa *in vitro*.

Methods. Test substances. The amounts of the substances infused were as follows: caffeine (base) 10, 20, and 40 mg/kg^{-1}/hr^{-1}; aminophylline 5, 10, and 20 mg/kg^{-1}/hr^{-1}; pentagastrin 0.5, 1, 2, 4, and 8 μg/kg^{-1}/hr^{-1} with or without caffeine 10 mg/kg^{-1}/hr^{-1}; histamine dihydrochloride 0.0125, 0.025, 0.05, 0.1, 0.2 and 0.4 mg/kg^{-1}/hr^{-1} with or without caffeine 10 mg/kg^{-1}/hr^{-1}; pentagastrin 1, 2, and 4 μg/kg^{-1}/hr^{-1} with 10 or 20 mg/kg^{-1}/hr^{-1} aminophylline.

Animal preparations. Under pentobarbital anesthesia, four dogs were prepared with a gastric fistula (GF) and a vagally denervated pouch of the oxyntic gland area (Heidenhain). The Heidenhain pouch (HP) was drained by Gregory cannula. Experiments were started 4 weeks after the surgical procedures.

The animals were fasted 18 hr before each experiment. A polyethylene catheter (PE-50) was inserted into the peripheral hind-vein limb and 0.15 *M* NaCl was infused throughout the experiments using a peristaltic pump (Harvard Apparatus, Millis, MA) set to deliver 25 ml/hr. Gastric juice was collected for two 15-min periods to obtain basal levels of secretion and at the end of this period caffeine, pentagastrin, histamine, theophylline ethylenediamine (aminophylline), histamine plus caffeine, and pentagastrin plus caffeine or aminophylline were added to the saline to give the desired dosage. The infusion of the substances was continued for 2 hr and one dose only was tested each day.

Gastric juice was collected by gravity drainage from the GF and HP every 15 min, and the volume was recorded to the nearest 0.1 ml. Acid concentration was determined by titrating 0.2 ml of juice with 0.2 *N* NaOH to pH 7 using a glass electrode and an automatic titrator (Autoburet, Radiometer, Copenhagen). The mean acid output (mequiv/15 min) of the highest two consecutive 15-min periods in response to each dose was used to calculate the response to that particular dose.

Human studies. Studies were made in 16 male volunteers. Eight of the subjects were healthy students, and the remainder had either gastric or duodenal ulcer disease. After an overnight fast a 16 Fr gastric tube was positioned in the antrum under fluoroscopic control and the residual gas-

tric contents aspirated and discarded. Suction was maintained at a negative pressure of 40 mm Hg and was interrupted every 5 min, during which 10 ml of air were injected to insure patency of the tube. The subject remained supine and expectorated all saliva. An intravenous infusion of 0.15 *M* NaCl was started, and basal secretions were collected for three 10-min periods. The infusion was maintained by a peristaltic pump set to deliver 200 ml/hr. At the end of the 30-min basal period, either caffeine sodium benzoate, pentagastrin, or histamine acid phosphate was added to the infusion mixture to give the desired dosage. The dose of caffeine sodium benzoate is expressed in terms of caffeine base.

In six of the 16 subjects, a dose-response curve for caffeine was determined by infusing caffeine intravenously in doses of 3 and 6 mg/kg^{-1}/hr^{-1}, the dose being doubled after 60 min. On another occasion a dose of 9 mg/kg^{-1}/hr^{-1} was infused for 1 hr.

In 10 other subjects, gastric acid output in response to pentagastrin and histamine with or without caffeine was tested. In five of these subjects, pentagastrin 6 μg/kg^{-1}/hr^{-1} with or without caffeine (4 mg/kg^{-1}/hr^{-1}) and in the other five subjects histamine acid phosphate (0.04 mg/kg^{-1} /hr^{-1}) with or without caffeine (4 mg/kg^{-1} /hr^{-1}) were infused intravenously for 120 min on separate occasions. Benadryl (diphenhydramine hydrochloride), 100 mg, was given intravenously as a single dose immediately before the histamine infusion.

Gastric juice was collected at 10-min intervals. The volume was measured to the nearest 1 ml, and the concentration of acid was measured by titrating a 0.2-ml sample of gastric juice with 0.2 *N* NaOH to pH 7.0 using a glass electrode and an automatic buret (Autoburet, Radiometer, Copenhagen). The peak acid output/30 min was calculated by summing the highest three consecutive 10-min periods.

Statistics. Differences between acid output in response to histamine alone and histamine plus caffeine or pentagastrin alone and pentagastrin plus caffeine were tested by a nonpaired *t* test (8). Tests for significance of differences between acid output in response to pentagastrin with or without caffeine and in response to histamine with or without caffeine were made in the human subjects by a paired *t* test (8).

Adenyl cyclase assay. When the above studies were completed, the dogs were anesthetized and sections of the oxyntic gland and pyloric gland area were removed immediately. The mucosa was dissected from the underlying submucosa and muscle layers, cut in strips, and placed in ice-cold 50 m*M* Tris buffer, pH 7.4, containing 6 m*M* $MgCl_2$, minced with scissors, then disrupted in a Polytron. Final homogenization was performed in a Dounce homogenizer with a loose pestle. The extract was filtered through four layers of cheesecloth, centrifuged for 10 min at 2000*g* and the pellet from this was suspended in the above buffer, centrifuged again, and resuspended in 0.5 ml of buffer.

Adenyl cyclase activity was determined by measuring the conversion of α^{32}P-ATP to cyclic 3′,5′-AM^{32}P (9). The composition of the assay mixture was as follows: 1.2 m*M* ATP-α-^{32}P (25–30 cpm/pmole), 6 m*M* $MgCl_2$, 60 m*M* theophylline, 0.04% albumin, 50 m*M* tris - HCl, pH 7.4, 50 m*M* phosphoenolpyruvate, 0.05 mg pyruvate kinase, and 0.4–0.7 mg extract in a final volume of 0.065 ml. The incubation was performed for 3 min at 37°, and was terminated by rapid addition of 0.1 ml of a diluting solution containing 100 m*M* ATP, 50 m*M* cyclic AMP, and 30,000 cpm of ^{3}H-cyclic AMP followed by heating at 100° for 3 min. Both radionuclides were obtained from New England Nuclear Corp. Cyclic 3′,5′-AM^{32}P was isolated from other ^{32}P-labeled compounds by chromatography on Dowex 50W-X2, 200–400 mesh, and two $BaSO_4$ precipitations. ^{3}H and ^{32}P radioactivities of the $BaSO_4$ supernatants were counted in a Nuclear Chicago Mark II liquid scintillation spectrometer. Total cyclic AM^{32}P formed was between 35–45%. A blank for each experiment was prepared by boiling the extract then adding the α-^{32}P-ATP. This value, which ranged between 2–5 pmoles per tube, was then subtracted from each experimental value. Protein was determined by the method of Lowry *et al.* using bovine serum albumin as standard (10). The concentrations of histamine and pentagastrin in the assay were 10^{-4} and 2.4×10^{-5} *M*, respectively.

Results. Animal studies. Acid output in response to caffeine and aminophylline. Caffeine and aminophylline at all doses were ineffective as stimulants of acid output as both failed to increase acid output above basal values.

Effect of pentagastrin and pentagastrin plus caffeine or aminophylline. Acid output from the gastric fistula (GF) in response to pentagastrin

increased with increasing dose, and highest acid output was found with a dose of 8 $\mu g/kg^{-1}/hr^{-1}$ (Fig. 1). In a few studies using a greater dose (16 $\mu g/kg^{-1}$ $/hr^{-1}$), acid output did not increase significantly. The highest output in response to pentagastrin from the Heidenhain pouch was obtained with a dose of 4 $\mu g/kg^{-1}$ $/hr^{-1}$ (Fig. 1). When caffeine (10 $mg/kg^{-1}/hr^{-1}$) was infused with the various doses of pentagastrin, there was no significant increase in acid output from the GF or the HP (Fig. 1). Acid output from the HP decreased with the higher doses of pentagastrin plus caffeine, but the decrease was not statistically significant (Fig. 1). In limited studies with pentagastrin plus aminophylline (1, 2, and 4 $\mu g/kg^{-1}/hr^{-1}$ pentagastrin + 10 or 20 $mg/kg^{-1}/hr^{-1}$ aminophylline) acid output was not significantly different with the agents in combination than with pentagastrin alone.

Acid output in response to histamine and histamine plus caffeine. In response to histamine, acid output from the GF increased with increasing dose, and the highest output was in response to 0.2 mg/kg^{-1} $/hr^{-1}$ of histamine (Fig. 2). A similar response was found with the HP (Fig. 2). When histamine plus caffeine (10 $mg/kg^{-1}/hr^{-1}$) was infused, acid output from the GF was greater at all dose levels and this reached statistical significance at doses of 0.1, 0.2, and 0.4 $mg/kg^{-1}/hr^{-1}$ of histamine (Fig. 2). Although acid output from the HP in response to caffeine plus histamine was greater than with histamine alone, it did not reach statistical significance (Fig. 2).

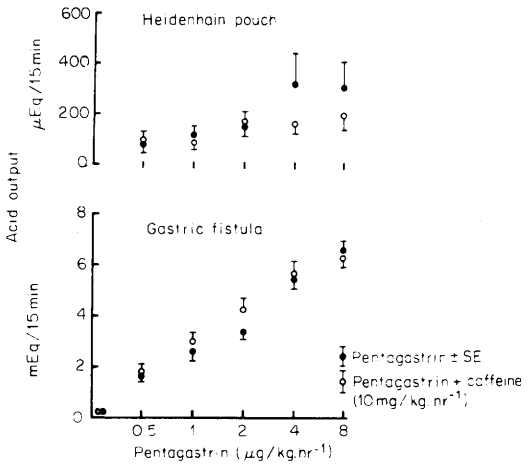

FIG. 1. Acid output from the gastric fistula and Heidenhain pouch in response to pentagastrin alone and pentagastrin plus caffeine. Each point is the highest mean acid output at each dose and represents 16 experiments in four dogs. The vertical bars are the SE.

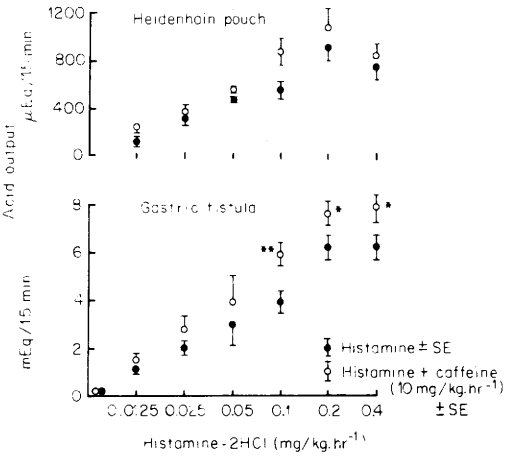

FIG. 2. Acid output from the gastric fistula and Heidenhain pouch in response to histamine dihydrochloride alone and in combination with caffeine. Mean of 16 experiments in four dogs. The symbol ** indicates $P < 0.01$ and * indicates $P < 0.05$.

Adenyl cyclase activity in dog gastric mucosa. The results of adenyl cyclase determinations on antral and fundic mucosa of dog stomach are given in Table I. Although a good preparation of cyclase was obtained as indicated by the substantial base (unstimulated) activity and the expected response to sodium fluoride, no stimulation resulted when either histamine (10^{-4} *M*) or pentagastrin (2.4×10^{-5} *M*) was added. In the experiment illustrated (one of five performed) the responses to histamine and pentagastrin were lower than the basal activity, although this was not a uniform finding. In another experiment, the extract was centrifuged at 10,000*g* after removal of 2000*g* pellet. The pellet from this higher-speed centrifugation and the resulting supernatant were checked for basal, histamine, pentagastrin, and fluoride stimulatable cyclase. In the 10,000*g* pellet, basal and fluoride stimulatable activity resembled that of the 2000*g* pellet, but histamine and pentagastrin again had no effect. No substantial cyclase activity was found in the 10,000*g* supernatant faction.

Human studies. Acid output in response to intravenous caffeine alone and in combination. Acid output in response to intravenous caffeine increased with all doses of caffeine (Table II). Little difference was found in acid output between 3, 6, and 9 $mg/kg^{-1}/hr^{-1}$ of caffeine. Nausea was found frequently with 6 and 9 mg/kg^{-1} $/hr^{-1}$, and for this reason 4 $mg/kg^{-1}/hr^{-1}$ of caffeine was used in combination with pentagas-

TABLE I. Effect of Pentagastrin, Histamine and Sodium Fluoride on Adenyl Cyclase Activity.

Agents	Fundus	Antrum
	(p moles/mg protein/3 min)	
Unstimulated	41.1	38.8
Pentagastrin	37.7	17.1
Histamine	39.7	13.2
Sodium fluoride	366.5	278.6

trin and histamine. The mean peak acid output (± SE) in response to pentagastrin (6 $\mu g/kg^{-1}/hr^{-1}$) was 14.24 ± 1.66 mequiv/30 min and did not significantly differ ($P > 0.05$) from that in response to pentagastrin plus caffeine (13.96 ± 1.55 mequiv/30 min) (Table II). In response to histamine, peak acid output was 18.3 ± 1.75 mequiv/30 min, and this did not significantly increase ($P > 0.05$) when caffeine was added (19.38 ± 2.23 mequiv/30 min) (Table II).

Discussion. In suggesting that a certain physiological or biochemical effect of a hormone (effector) is mediated by cAMP, Sutherland *et al.* (7) have suggested that the following criteria be met: (a) Adenyl cyclase in broken cell preparations should be stimulated by the hormone (effector) in question, (b) hormonal stimulation should result in an increased cAMP level in the target tissue, (c) drugs which inhibit phosphodiesterase, such as methyl xanthines, should potentiate submaximal effects of the hormones, and (d) cAMP, or biologically active derivatives thereof, should mimic the effects of the hormone.

Harris *et al.* (6) found that cyclic AMP stimulated acid output in frog gastric mucosa and that methyl xanthines increased the mucosal content of cyclic AMP and this increase preceded the secretory response. Thus, in frog mucosa there is some evidence for a casual relationship between cyclic AMP and gastric secretion.

In the present experiments dose-response curves for acid output were made in response to histamine, pentagastrin, caffeine, and aminophylline (Figs. 1 and 2). Caffeine and aminophylline did not stimulate acid output in the dog whereas when caffeine was infused into volunteer subjects acid output was increased (Table II). These findings confirm an old observation made by Roth and Ivy (1). When caffeine or aminophylline was infused with submaximal and maximal doses of pentagastrin, no shift in the dose-response curve was found for either the HP or GF (Fig. 1). Thus, criterion (c) of Sutherland *et al.* (7) was not satisfied. Similar but limited studies in man using caffeine and pentagastrin confirmed the results obtained in the dog (Table II). Furthermore, Cohen and colleagues (11) using submaximal doses of pentagastrin in four subjects did not find any effect of caffeine on acid output stimulated by pentagastrin.

When pentagastrin was tested *in vitro* for activation of adenyl cyclase activity in dog gastric fundic and antral mucosa, no effect could be demonstrated (Table I). Thus, criterion (a) of Sutherland *et al.* was not satisfied. Perrier and Laster (12) also could not stimulate adenyl cyclase activity in guinea pig mucosa with gastrin. These findings together with the *in vivo* studies suggest that pentagastrin stimulation of acid secretion may not be mediated by cyclic AMP. Nakajima *et al.* (13) using Necturus have found that pentagastrin stimulates the parietal cells by a

TABLE II. Acid Output in Response to Intravenous Caffeine, Caffeine plus Histamine, and Caffeine plus Pentagastrin.

Agent	Dose	Acid output
Basal secretion		1.47 ± 0.50
Caffeine[a]	3.0 $mg/kg^{-1}/hr^{-1}$	5.49 ± 2.68
Caffeine	6.0 $mg/kg^{-1}/hr^{-1}$	6.46 ± 2.77
Caffeine	9.0 $mg/kg^{-1}/hr^{-1}$	7.61 ± 2.72
Pentagastrin[b]	6.0 $\mu g/kg^{-1}/hr^{-1}$	15.24 ± 1.66
Pentagastrin + Caffeine[b]	6.0 $\mu g/kg^{-1}/hr^{-1}$ + 4 $mg/kg^{-1}/hr^{-1}$	13.96 ± 1.55
Histamine[c]	0.04 $mg/kg^{-1}/hr^{-1}$	18.30 ± 1.75
Histamine + caffeine[c]	0.04 $mg/kg^{-1}/hr^{-1}$ + 4 $mg/kg^{-1}/hr^{-1}$	19.88 ± 2.23

[a] Mean ± SE of one study in each of six subjects for each dose of caffeine.
[b] Mean ± SE of one study in each of five subjects.
[c] Mean ± SE of one study in each of five subjects. The subjects were not those used for the pentagastrin study.

mechanism different from that of the methyl xanthines. The present studies in mammalian species are consistent with the conclusions of these workers.

In contrast to pentagastrin, caffeine shifted the histamine dose-response curve to the left in dogs and increased the maximal acid output significantly ("potentiation") (Fig. 2). Thus, criterion (c) of Sutherland *et al.* was satisfied. Previous studies in the dog with histamine and caffeine using limited doses are consistent with the findings of the present study (14). We are unable to explain our failure to demonstrate stimulation of adenyl cyclase activity by histamine despite evidence from other workers that it does so in guinea pig gastric mucosa (12) and heart muscle (15). However, Mao *et al.* (16) were unable to increase mucosal adenyl cyclase activity in the dog *in vivo* and *in vitro* with histamine. Thus, there is evidence in the dog that criterion (a) of Sutherland *et al.* is not satisfied.

There are conflicting studies about the role of cyclic AMP in acid secretion (17). Bieck and co-workers (18) found increased quantities of cyclic AMP in gastric juice in man (after betazole stimulation) and dogs (after pentagastrin or histamine). In contrast, Amer (19) found that gastrin and histamine decreased levels of cyclic AMP by stimulating phosphodiesterase in rabbit gastric mucosa. Thus it is difficult at present to determine whether or not mammalian gastric secretion is related to or independent of changes in cyclic AMP.

Summary. Studies of acid secretion were made in four dogs with a gastric fistula (GF) and Heidenhain pouch (HP) in response to various doses of histamine, pentagastrin, caffeine, aminophylline, histamine plus caffeine, pentagastrin plus caffeine, and aminophylline. Similar but limited studies were made in 16 volunteers using histamine or pentagastrin with or without caffeine. It was found that pentagastrin-stimulated acid output was not increased by the methyl xanthines in dog and man. Acid output in response to histamine was potentiated by caffeine in the dog but not in the human studies. Neither caffeine nor aminophylline when infused alone stimulated acid output in the dog. At the end of the animal experiments studies were made *in vitro* of adenyl cyclase activity in fundic and antral mucosa. Adenyl cyclase activity in dog gastric mucosa was not stimulated by histamine or pentagastrin.

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