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Failure of Urecholine to Potentiate the Pancreatic Response to Exogenous Stimuli.† (30371)

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Parasympathomimetic agents have been shown to potentiate the action of the hormone of the pyloric gland area, gastrin, on gastric secretion(1). It has frequently been suggested that potentiation between cholinergic effects and other stimuli might occur in the regulation of pancreatic secretion(2).

In this study we have attempted to determine whether there is potentiation by a stable choline ester, Urecholine, of pancreatic secretion induced by the normal hormonal stimulants of the pancreas.

Methods. Four mongrel dogs weighing between 14 and 21 kg were used. A chronic pancreatic fistula was created surgically in each animal by a method we have described (3). At a second operation, a Thomas cannula(4) was inserted into the most dependent portion of the stomach as a gastric fistula. At least 4 weeks were allowed for recovery from this procedure before experiments were undertaken.

After fasting for 18 hours, the pancreatic and gastric fistulas were opened and secretions collected for at least 30 minutes to confirm basal levels. A fine plastic catheter was introduced intravenously and an infusion of sterile 0.15 M NaCl maintained throughout each experiment by a Harvard peristaltic pump at a rate of 30 ml per hour. The stimulant under test was dissolved in the saline to give appropriate dose levels.

Gastrin was extracted from the pyloric gland area mucosa of hog stomachs by the method of Grossman and Gillespie(5). One large batch of gastrin was prepared of sufficient size for both parts of this study to ensure comparable dose-response curves. Similarly the secretin and Cecekin used came from the same batch numbers as obtained from the manufacturer (Vitrum, Stockholm).

Dose-response relations were studied for pancreatic and gastric secretion evoked by intravenous infusion of secretin, Cecekin, and gastrin. Each dose level was maintained for 60 minutes in the case of secretin and Cecekin, and for 75 minutes for gastrin. Secretions were collected every 15 minutes, and mean values of the last two 15-minute collection periods at any one dose level used to construct the dose-response curves shown in the Figures.

A dose-response curve was also determined

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for Urecholine (carbamoylmethylcholine chloride, Merck & Co., Inc.). On the basis of the pancreatic response to this ester alone, a dose of 1.0 mg Urecholine intravenously per hour was selected as a constant background on which dose-response curves were obtained for secretin, Cecekin, and gastrin. In this series the background infusion of Urecholine was started immediately: two 15-minute collections were then made, and the last collection with Urecholine alone was used to represent the control response shown in the Figures. Then graded doses of secretin, Cecekin, or gastrin were infused along with the continuous Urecholine infusion, and calculation of the response made as described for the control series.

The pancreatic and gastric responses to a continuous infusion of 1.0 mg Urecholine per hour for 4 hours were also determined in a separate experiment.

The concentration of hydrochloric acid in the gastric juice was found by titrating samples to pH 7.0 with 0.2 N NaOH with an Autoburet (Radiometer, Copenhagen). The pancreatic juice was refrigerated overnight, and centrifuged at 18,000 rpm for 3 minutes if not clear to inspection. Protein concentration was estimated by diluting the juice 1:100 with distilled water, and reading the optical density at 280 mμ in a Zeiss spectrophotometer; this was compared with a standard solution of bovine serum albumin. No attempt was made to estimate individual enzyme activities, as Keller *et al*(6) have shown that these bear a constant relationship to the total protein concentration. The bicarbonate concentration was measured by adding 0.5 ml pancreatic juice to 1.0 ml of 0.1 N HCl and backtitrating the mixture to pH 7.0 with 0.2 N NaOH with the Autoburet. In the Figures the gastric responses are given in mEq of hydrochloric acid, and the pancreatic responses in ml, mEq bicarbonate, and mg protein respectively secreted per 15 minutes.

Statistical analysis. In the diagrams the mean and standard error of the mean are given for each dose level. The differences between the means at each dose level of secretin, Cecekin, or gastrin and the same dose level with the background Urecholine stimulation have been compared by the t test for non-paired values. In those instances in which the difference between the means was found to be significantly different ($p<0.05$), a star has been inserted in the Figure. Where this is not present, no significant difference was found between the means ($p>0.05$).

Results. Urecholine alone (Fig. 1 and 2). The relationship between the dose of Urecholine and the gastric and pancreatic response is shown in Fig. 1. Acid output by the stomach rose to reach a maximal level with 2.0 mg Urecholine per hour and fell with 4.0 mg per hour. With the latter dose level 2 of the 4 dogs consistently exhibited signs of restlessness, salivated freely, and retched. This is similar to the responses to Urecholine described by Gillespie and Grossman(1), and to Mecholyl described by Gray and Ivy(7).

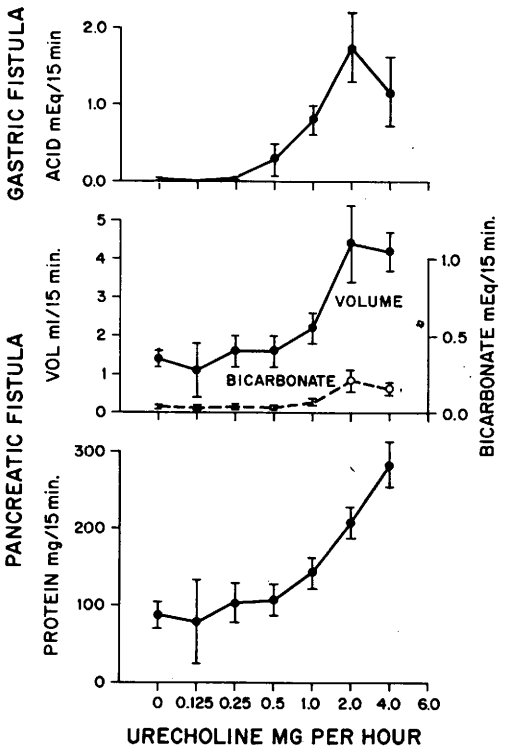
Urecholine was a weak stimulant of pancreatic volume flow and bicarbonate output, but was effective in promoting protein output. A dose of 1.0 mg Urecholine per hour was just greater than the threshold level for pancreatic secretion, and was therefore selected as a background dose for the other studies described. This dose had previously been shown to produce maximal potentiation of gastric secretion(1).

Fig. 2 shows a control study with a continuous infusion of Urecholine (1.0 mg per hour throughout). This confirms that this dose level caused a small but definite rise in gastric and pancreatic secretion.

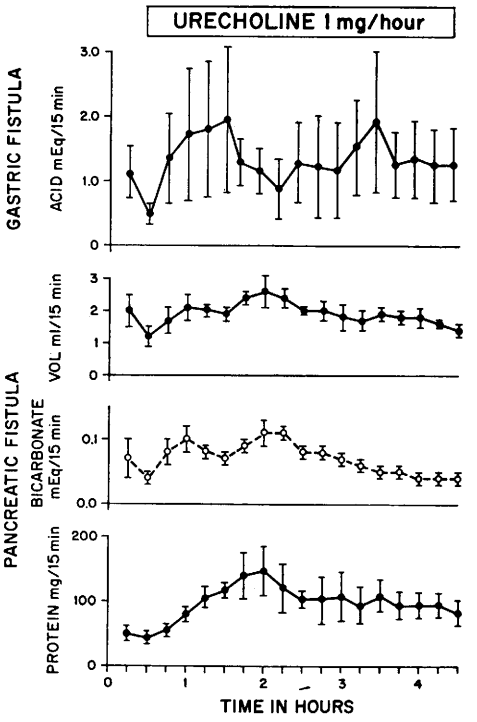
Secretin (Fig. 3). There was no gastric secretory response to secretin alone. The background Urecholine stimulation caused a small gastric secretory response to appear, which diminished as the dose of secretin was increased. This suggests that the gastric stimulatory effect of Urecholine, like that of other gastric secretory stimulants, is inhibited by secretin(8).

The pancreatic response to secretin alone was similar to that previously described for this form of pancreatic fistula in the dog(3). There was no significant difference in the response when the background of Urecholine was added.

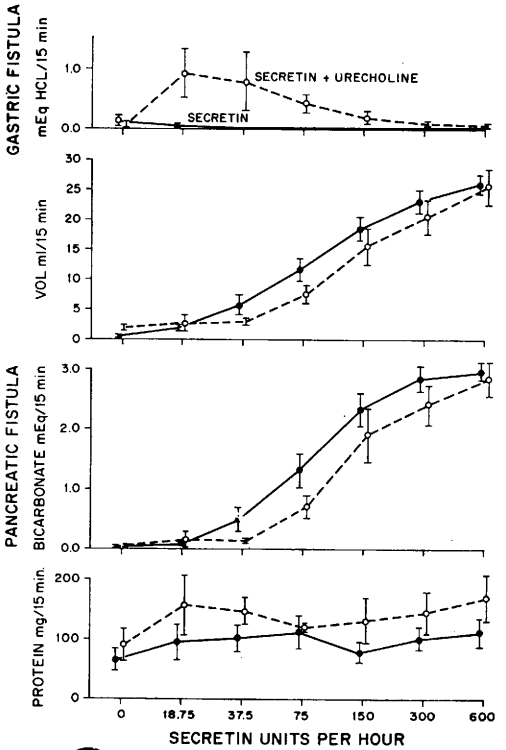
Cecekin (Fig. 4). The gastric secretory response to this preparation was irregular, as had been previously reported(3). The Figure shows an apparent increase in secretion


GASTRIC FISTULA
ACID mEq/15 min
2.0
1.0
0.0
PANCREATIC FISTULA
VOL ml/15 min.
5
4
3
2
1
0
VOLUME
BICARBONATE
1.0
0.5
0.0
BICARBONATE mEq/15 min.
PROTEIN mg/15 min.
300
200
100
0
0 0.125 0.25 0.5 1.0 2.0 4.0 6.0
URECHOLINE MG PER HOUR

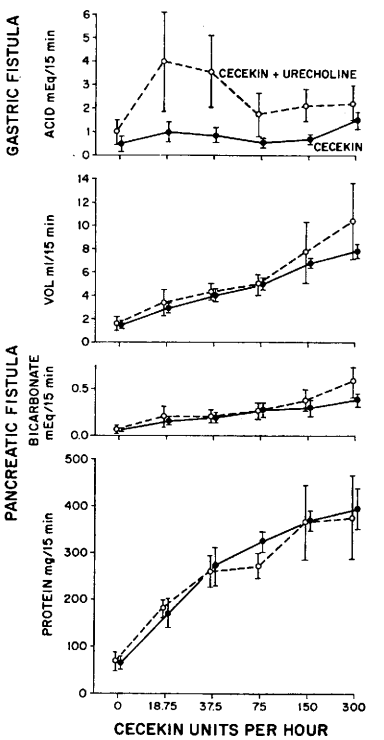
1


URECHOLINE 1 mg/hour
GASTRIC FISTULA
ACID mEq/15 min
3.0
2.0
1.0
0
PANCREATIC FISTULA
VOL ml/15 min
3
2
1
0
BICARBONATE mEq/15 min
0.1
0.0
PROTEIN mg/15 min
200
100
0
0 1 2 3 4
TIME IN HOURS

2


GASTRIC FISTULA
mEq HCL/15 min
1.0
0.0
SECRETIN + URECHOLINE
SECRETIN
VOL ml/15 min
30
25
20
15
10
5
0
PANCREATIC FISTULA
BICARBONATE mEq/15 min
3.0
2.0
1.0
0.0
PROTEIN mg/15 min.
200
100
0
0 18.75 37.5 75 150 300 600
SECRETIN UNITS PER HOUR

3


GASTRIC FISTULA
ACID mEq/15 min
6
5
4
3
2
1
0
CECEKIN + URECHOLINE
CECEKIN
VOL ml/15 min
14
12
10
8
6
4
2
0
PANCREATIC FISTULA
BICARBONATE mEq/15 min
0.5
0.0
PROTEIN mg/15 min
500
400
300
200
100
0
0 18.75 37.5 75 150 300
CECEKIN UNITS PER HOUR

4

FIG. 1. Gastric and pancreatic dose response curves to Urecholine alone. In this and subsequent Figures vertical lines indicate standard error of mean. Mean of 12 experiments in 4 dogs.

FIG. 2. Gastric and pancreatic responses to a continuous intravenous infusion of Urecholine, 1.0 mg per hour. Mean of 5 experiments in 3 dogs.

FIG. 3. Gastric and pancreatic dose response curves to secretin. In this and subsequent Figures dotted line shows dose response curve of stimulus superimposed on a background stimulation of Urecholine, 1.0 mg intravenously per hour. Mean of 7 experiments with secretin alone, and 4 with secretin plus Urecholine in 4 dogs.

FIG. 4. Gastric and pancreatic dose response curves to Cecekin alone, and Cecekin plus Urecholine. Mean of 11 experiments with Cecekin alone, and 4 experiments with Cecekin plus Urecholine.

when the background of Urecholine was used: however when compared statistically the differences between the two curves were not significant. There was no significant difference between the pancreatic responses with and without Urecholine, and both were similar to the dose response curves previously described for Cecekin(3).

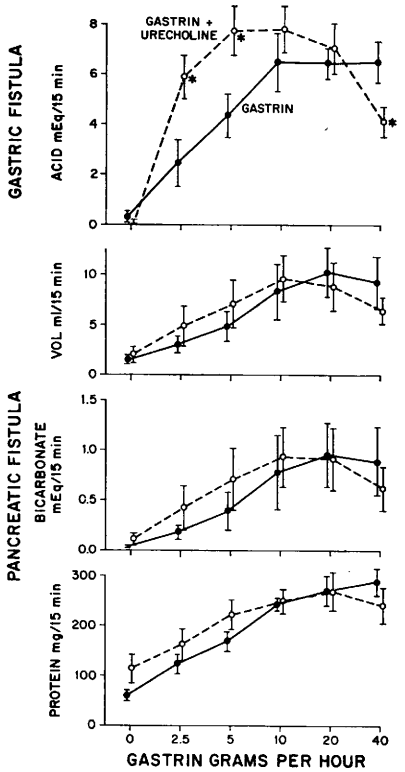


FIG. 5. Gastric and pancreatic dose response curves to gastrin alone, and gastrin plus Urecholine. Stars indicate a significant difference between the 2 curves ($p<0.05$) as tested by the t test for non-paired values. The peak output of acid by the stomach with gastrin alone did not differ significantly from peak output of gastrin plus Urecholine (difference 1.30 mEq, $t = 1.05$, $p>0.10$). Mean of 8 experiments with gastrin alone and 8 experiments with gastrin plus Urecholine in 4 dogs.

Gastrin (Fig. 5). The gastric response to gastrin plus Urecholine was significantly higher than to gastrin alone at low dose levels, yet the peak response to gastrin alone (40 g gastrin per hour) did not differ significantly from the peak response to gastrin plus Urecholine (10 g gastrin per hour). This is in agreement with the report of Passaro and Grossman(9) that the maximal responses of a gastric fistula to gastrin and gastrin plus Urecholine were not significantly different. However Andersson and Grossman(10) have recently demonstrated that the maximal response of an innervated fundic pouch to gastrin can be increased by Urecholine: the difference in these findings may be related to the absence of duodenal acidification in the studies with the gastric fistulas.

Figure 5 also shows that the response of the stomach to 40 g gastrin per hour plus Urecholine was significantly smaller than the response to 40 g gastrin alone. This indicates that the whole dose-response curve was shifted to the left by addition of the background Urecholine so that the inhibitory effect of large doses of gastrin(5,10) became apparent at lower dose levels.

The pancreatic responses to gastrin alone and to gastrin plus Urecholine were similar, and both were comparable to dose-response curves previously described for this preparation of gastrin(3).

Discussion. Although parasympathomimetic agents have been recognized as stimulants of pancreatic secretion(11), there have been few attempts to observe the effects of combining these agents with other forms of pancreatic stimulation.

Lin and Ivy(12) injected small doses of methacholine and Urecholine during the pancreatic response to continuous secretin and found little change in the rate of secretion.

However they reported some augmentation of the response in a few experiments in which small doses of secretin were used. This report is not necessarily contradictory with the findings of the present study, as the augmentation observed at low dose levels by Lin and Ivy may have been due simply to the additive effect of the parasympathomimetic agent itself on pancreatic volume flow. Fig. 1 and 2 show that Urecholine itself was a weak stimulant of volume flow.

Lin and Grossman(13) found no evidence of potentiation of single injections of a preparation of pancreozymin by a simultaneous injection of methacholine, although additive effects were observed. We have confirmed this using a continuous background of Urecholine added to the pancreatic response to graded doses of Cecekin, which is a preparation of intestinal mucosa containing, in addition to pancreozymin, cholecystokinin and secretin-like activity.

Gastrin has recently been recognized as a potent stimulus for pancreatic secretion(14) and Harper(15) has reviewed the present evidence for a gastric phase of pancreatic secretion. Fig. 4 shows that the background Urecholine stimulation markedly augmented the gastric secretory response to gastrin, as has been previously reported(1). However, the parasympathomimetic agent had no apparent effect on the pancreatic response in the same experiment.

This study has shown that Urecholine, as a continuous background infusion at a rate of 1.0 mg per hour, had no effect on the pancreatic reponses to graded doses of secretin, Cecekin, or gastrin. It is possible that other dose levels of this drug, or other forms of cholinergic or cholinomimetic stimulation might reveal some augmentation of the pancreatic response to hormonal extracts. In fact, Brown, Harper and Scratcherd have briefly reported(16) that the pancreatic response to secretin in the anesthetized cat can be augmented by electrical stimulation of the vagus nerves.

Summary. The gastric and pancreatic secretory responses to gastrin, secretin, Cecekin, and Urecholine have been studied in conscious dogs with gastric and pancreatic fistulas. Dose response curves to secretin, Cecekin, and gastrin were compared with similar responses obtained using a background of Urecholine stimulation. Although augmentation of the gastric secretory response to gastrin by Urecholine was confirmed, no augmentation of the pancreatic responses to these stimuli was seen.

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