Effect of glucagon and pentagastrin on gastric mucosal potential difference in man¹

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SUMMARY Glucagon given as an intravenous injection of 2 mg (0.57 mmol) or as a constant intravenous infusion of 1.64 $\mu\text{g/kg/h}$ (0.47 nmol/kg/h) significantly increased gastric mucosal potential difference (PD) in man. Pentagastrin infusion of 2 $\mu\text{g/kg/h}$ (2.8 nmol/kg/h) dramatically reduced gastric PD in man. The effect of each of these hormones on PD was reversed by the administration of the other. Changes in PD induced by one hormone were not associated with reductions in blood levels of the other. There was an approximate correlation between changes in PD and pH of gastric aspirates, however, the patterns of PD and pH changes were at times dissimilar. This study indicates that administration of gastrointestinal hormones significantly alters gastric mucosal PD in man.

Few studies of the effect of gastrointestinal hormones on gastric mucosal potential difference (PD) in man or in animals have been reported (Andersson and Grossman, 1966; Vaughan *et al.*, 1967). In one study, crude gastrin was reported to decrease PD by a mean of 5 mV in the anaesthetised rat (Vaughan *et al.*, 1967) but the decrease in PD was not invariable. In a study of the effects of secretin (an inhibitor of gastric acid secretion) on the gastroduodenal PD profile in man (Andersson and Grossman, 1966) no significant changes in PD across the oxyntic glandular area of the stomach were reported.

The present study was designed to determine and compare the effect of the acid inhibitory hormone glucagon and that of the acid stimulator pentagastrin on gastric mucosal PD in man.

Methods

This study which was approved by the institutional Human Experimentation Committee, was performed on 18 healthy male volunteers, aged 21 to 27 years. All subjects studied had a fasting pH value of 3.0 or less.

Studies were performed on three groups, each of six subjects, to examine the following effects: group 1

—glucagon given as a single intravenous injection of 2 mg (0.57 mmol) within three minutes; group 2—pentagastrin administered as a constant intravenous infusion of 2 $\mu\text{g/kg/h}$ (2.8 nmol/kg/h); and group 3—glucagon given by constant intravenous infusion of 1.64 $\mu\text{g/kg/h}$ (0.47 nmol/kg/h). To examine the interrelationships of glucagon and pentagastrin on PD, in group 2 ($n = 6$) constant pentagastrin infusion (2 $\mu\text{g/kg/h}$) was discontinued after 60 minutes of infusion and followed by a single 2 mg intravenous glucagon injection; this was followed in 15 minutes by constant infusion of pentagastrin 2 $\mu\text{g/kg/h}$. In group 3 ($n = 6$), a constant infusion of glucagon was administered for 60 minutes and was then followed by a constant pentagastrin infusion of 2 $\mu\text{g/kg/h}$ for 30 minutes.

Parameters measured were: (1) gastric mucosal PD recorded continuously as previously described using intragastric and peripheral vein KC1-in-agar electrodes (Ivey *et al.*, 1975); a 30 minute baseline period was achieved with saline before drug administration. (2) pH of gastric aspirates obtained at 30 minute intervals and determined with a Beckman Expandometric pH meter. The pH was measured directly with a glass electrode. Conversion to hydrogen ion concentration was done by the table of Moore (1968) based on the sodium and potassium concentration of the gastric juice. Na^+ and K^+ concentrations were read on an (IL) Instrumentation Laboratory, Inc. flame photometer. In three subjects, studies were repeated after pentagastrin infusion (group 2) so that gastric aspirates could be obtained at five and 15 minutes after beginning the pentagastrin infusion. In an

¹This work was supported in part by the Medical Research Service of the Veterans Administration. We gratefully acknowledge the assistance and patient care facilities of the Clinical Research Center, University of Missouri, Columbia, Missouri. The paper was presented in part at the Annual Meeting of the Western Federation for Clinical Research, February 1977.

Received for publication 17 July 1978

additional two subjects in group 2, a pH profile was obtained by continuous recording after pentagastrin injections by use of an intragastric pH probe (Beckman 151960, Cemar Micro-combination pH electrode). These last five additional studies were done separately so that accurate pH readings could be obtained without mechanical or electrical interference with the PD recordings. The pH values of these five subjects at five and 15 minutes after onset of pentagastrin infusion are shown by asterisks in Fig. 2. The doses of pentagastrin used as an infusion 2.0 µg/kg/h, and of glucagon as a single injection of 2 mg are similar to those used by Christiansen and Hendel (1973) in their study of interreactions of these two drugs on acid secretion and inhibition, except that these workers used 1.5 µg/kg/h pentagastrin. The dose of 2.0 µg/kg/h was chosen, as this more closely resembled the dose of pentagastrin 2.0 µg/kg/40 minutes, found by dose response curve to cause a maximal acid secretory response with continuous intravenous infusion (Prugh *et al.*, 1975).

Levels of plasma glucagon and serum gastrin in group 1 and of glucagon and pentagastrin in group 2 were estimated as pg/ml or pmol/l, using standard radioimmunoassay methods (McGuigan and Trudeau, 1970; Faloon and Unger, 1974). The glucagon levels were estimated using Unger's 30K glucagon antiserum highly specific for pancreatic glucagon (Faloon and Unger, 1974). The pentagastrin levels (pg/ml and pmol/l) were determined using antibodies to the carboxyl terminal tetrapeptide amide conjugated to bovine serum albumin as immunogen, with pentagastrin as the reference material (McGuigan and Trudeau, 1970). Therefore, these results are expressed as pmol/l (pg/ml) pentagastrin, are authentic for this compound, and do not represent immunological cross-reactivity with antibodies to the heptadecapeptide, but rather direct measurements of pentagastrin levels.

Results obtained were analysed statistically using paired and unpaired Student's *t* tests respectively.

Results

Figure 1 shows the effect of a single intravenous injection of glucagon on gastric mucosal PD. PD rose rapidly over 20 minutes from basal (mean $\pm$ SE) -48 ± 2 mV to -64 ± 2 mV ($P < 0.001$). A significant rise in gastric PD ($P < 0.001$) persisted for 120 minutes after injection. During this time, gastric pH also increased. The reduction in hydrogen ion concentration from baseline 20 ± 2 mmol/l to 2.0 ± 0.5 mmol/l was significant at all time periods ($P < 0.01$).

Figure 2 shows the effect of continuous intravenous infusion of pentagastrin on PD. At the end of one

Fig. 1 Effect of glucagon injection (GL) on gastric PD and pH. Values for PD and pH in this and subsequent figures are mean $\pm$ standard error. In this and subsequent figures PD is expressed on the vertical axis as negative millivolts. In the text an increase in electronegativity is termed a 'rise' in PD and a decrease a 'fall' in PD. Mean values for serum glucagon and gastrin in pg/ml during the study are shown above the PD and pH curves.

Fig. 2 Effect of pentagastrin (PNG) infusion on gastric PD and pH. Asterisks (*) refer to pH values obtained in five subjects studied separately to obtain values at five and 15 minutes, see text. Mean values for plasma glucagon and pentagastrin (in pg/ml) during the study are shown above the PD and pH curves.

hour the pentagastrin infusion was followed by a single injection of glucagon. Fifteen minutes later the pentagastrin infusion was started again. The figure shows a highly significant ($P < 0.001$) fall in PD within three minutes of starting pentagastrin; at the end of one hour glucagon injection caused a significant ($P < 0.01$) rise in PD. Reinfusion of pentagastrin again caused a highly significant fall in PD ($P < 0.001$). Figure 2 also illustrates some of the relationships between changes in PD and gastric pH. Initiation of pentagastrin infusion was followed by a dramatic drop in PD within 3–5 minutes. In five subjects restudied with pentagastrin infusion specifically to measure immediate effects on gastric pH, there was no detectable fall in pH at five minutes. In two of

these subjects, intragastric pH was monitored continuously after pentagastrin injection by a pH probe. In neither subject was a decrease in PD recorded within the first five minutes of injection. The pH profile by continuous recording closely resembled that shown in Fig. 2. In all subjects, the increase in hydrogen ion concentration after pentagastrin (reduction of pH) was significantly raised at 30 and 60 minutes. It should also be noted that, in the first 30 minutes after pentagastrin injection while gastric pH was still falling, gastric PD had recovered significantly ($P < 0.01$). At 30 and 60 minutes after pentagastrin infusion gastric pH was still maximally reduced, but gastric PD was returning toward or had returned to baseline.

Figure 3 shows the effect of glucagon infusion on gastric PD. A similar rise in PD occurred as with the injection (from -42 ± 1.5 mV to -58 ± 3 mV, $P < 0.001$) though the peak rise was delayed to 45 minutes. Pentagastrin infusion was followed by a marked fall in PD within three minutes from -58 ± 3 mV to -34 ± 1 mV, $P < 0.001$). While the mean gastric pH was raised after glucagon infusion the change in hydrogen concentration failed to achieve statistical significance. The fall in hydrogen concentration after the onset of pentagastrin infusion was significant ($P < 0.01$).

Glucagon injection induced significant hyperglucagonaemia (Table 1), but did not change serum

Fig. 3 Effect of glucagon infusion (GL) alone and followed by pentagastrin infusion on gastric PD and pH.

gastrin levels significantly. Pentagastrin infusion was followed by persistent hyperpentagastrinaemia, but was not associated with significant changes in plasma glucagon levels (Table 2). Comparison of plasma glucagon values (Table 1) during the period of significantly raised PD (Fig. 1) indicates that a significant rise in PD is maintained throughout the period of hyperglucagonaemia, including the two hour levels of 534 pg/ml (1861 pmol/l). This value is not far above blood glucagon levels observed after a meal (190 to 426 pg/ml or 660 to 1485 pmol/l) by Christiansen *et al.* (1976), which suggests that glucagon may affect PD at physiological levels.

Table 1 Blood hormone levels after intravenous injection of 2 mg (573 µmol) glucagon (Mean $\pm$ SE)

	Time (minutes)					
	0	15	30	60	90	120
Plasma glucagon						
pg/ml	128 $\pm$ 7	18,344 $\pm$ 1300*	4,250 $\pm$ 990*	2,625 $\pm$ 730*	703 $\pm$ 110*	534 $\pm$ 68*
pmol/l	446 $\pm$ 24	63,929 $\pm$ 4,531*	14,811 $\pm$ 3,450*	9,148 $\pm$ 2,544*	2,450 $\pm$ 383*	1,861 $\pm$ 237*
Serum gastrin						
pg/ml	24.1 $\pm$ 2.0	22.9 $\pm$ 2.0	24 $\pm$ 2.3	22.8 $\pm$ 2.3	27.0 $\pm$ 2.0	29.3 $\pm$ 2.3
pmol/l	52.5 $\pm$ 4.4	49.9 $\pm$ 4.4	52.3 $\pm$ 5.0	49.7 $\pm$ 5.0	58.8 $\pm$ 4.4	63.8 $\pm$ 5.0

* $P < 0.001$ vs 0 value. Units are expressed in terms of conventional units pg/ml, and SI units pmol/l.

Table 2 Blood hormone levels after constant intravenous infusion of 2 µg/kg/h (2.8 nmol/kg/h) Pentagastrin (Mean $\pm$ SE)

	Time (minutes)			
	0	15	30	60
Plasma glucagon				
pg/ml	90 $\pm$ 7	103 $\pm$ 7	103 $\pm$ 7	80 $\pm$ 7
pmol/l	314 $\pm$ 24	359 $\pm$ 24	359 $\pm$ 24	279 $\pm$ 24
Serum pentagastrin				
pg/ml	36 $\pm$ 5	474 $\pm$ 101*	368 $\pm$ 61*	317 $\pm$ 21*
pmol/l	28 $\pm$ 4	365 $\pm$ 78*	283 $\pm$ 47*	244 $\pm$ 16*

* $P < 0.005$ vs 0 value.

Discussion

This study showed that, in the doses used, administration of glucagon increases and pentagastrin significantly reduces gastrin PD. The effect evoked by each of these hormones was reversed by the other. A marked and significant increase in gastric PD occurred after constant intravenous infusion of glucagon in a dose of $1.64 \mu\text{g/kg/h}$ (0.47 nmol/kg/h). This dose has been reported as near physiological (Christiansen *et al.*, 1976). It suggests that glucagon affects PD at physiological levels. Unfortunately, as we did not measure plasma glucagon levels during glucagon infusion at this dose, we cannot say whether plasma glucagon was at a physiological level or not in our subjects. While the studies reported herein with pentagastrin were not within the physiological range, in very recent studies (Ivey *et al.*, 1978) by infusing human gastrin I we have found a significant fall in PD with a serum gastrin level well within the physiological range ($85 \pm 30 \text{ pg/ml}$; $185 \pm 64 \text{ pmol/l}$).

At the hormone doses used in our studies, neither glucagon nor pentagastrin infusion significantly altered blood gastrin or glucagon levels respectively. These results make it unlikely that the effects of either hormone on PD were mediated through changes in blood levels of the other.

Pentagastrin increases acid secretion, whereas glucagon inhibits basal and pentagastrin stimulated gastric acid output (Christiansen and Hendel, 1973; Christiansen *et al.*, 1976). It is possible that the effects of these hormones on PD are direct reflections of their actions on gastric acid secretion by formation of liquid-junction potentials between secreted acid and luminal contents. According to this hypothesis, agents which increase gastric acid secretion cause a decrease in PD, while those which inhibit gastric acid secretion cause an increase in PD. This hypothesis cannot be correct, as certain inhibitors of gastric acid secretion—for example, the orally effective prostaglandin 16 16-dimethyl PGE_2 —cause a fall in PD (Bolton and Cohen, 1976; Dajani *et al.*, 1978). Moreover, such a hypothesis must take into account that the change in PD after intravenous pentagastrin infusion in our study was dramatic and prompt, reaching its peak at 3.5 minutes after starting infusion (Fig. 2). In this period, no changes in the pH of gastric luminal contents were detected even with continuous recording using a pH probe. Subsequently, in our study PD gradually recovered while gastric pH decreased, so the pattern of pH and PD changes was different (Fig. 2). The rise in PD with onset of acid secretion probably reflects the known ability of intragastric acid to raise gastric PD in man (Ivey *et al.*, 1976; Ivey and MacKercher, 1977).

Further evidence against formation of liquid-junction potentials as the sole explanation of the effect of these gastrointestinal hormones on PD would be evidence of similar effects on PD in tissues which cannot secrete acid. In a separate study (Tarnawski *et al.*, 1977) in rats, we have found that pentagastrin causes the same reduction in PD in ligated forestomach (non-acid secreting mucosa) pouches as in the gastric fundus (acid secreting mucosa). In the human colon, pentagastrin has significantly increased the number of spike potentials (Snape *et al.*, 1977). Such studies indicate that the effect of gastrointestinal hormones on PD does not simply reflect effects on pH. Effects such as modification of active ion transport and permeability of the mucosa to passive ionic diffusion (Cohen, 1974) should be considered.

In piglets, Forte *et al.* (1977) reported definite reduction of electrical PD and resistance within 30 seconds of histamine administration, whereas H^+ secretion was first observed four to five minutes after the secretagogue was given. They postulated that the fall in PD and resistance was due to increased apical surface area in the parietal cells as a result of proliferation of secretory canaliculi. In unpublished studies we have found by morphometric analysis that pentagastrin causes the same increase in canalicular surface area at the time of fall in PD in man and before detectable change in pH. On the other hand, glucagon injection significantly decreases canalicular membrane area at the time PD is increased (Ackman *et al.*, 1978).

In conclusion, pentagastrin and glucagon exert marked and significant effects on gastric mucosal PD in man. The physiological importance of this effect and its mechanism remain to be established.

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