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Comparison of the action of cholinomimetics and pentagastrin on gastric secretion in dogs

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1 Stimulation of acid secretion by muscarinic cholinomimetic agents depended on the periodic interdigestive activity of the stomach. This explains the peak and following fade.

2 Pentagastrin stimulated gastric secretion after a fixed interval and did not depend on the interdigestive activity.

3 Neither gastrin nor methacholine directly contracted the gallbladder in the doses used.

4 A sustained secretion of gastric acid and pepsin, such as follows a meal, required both hormonal stimulation and gastric distension.

5 The magnitude of the acid response from Heidenhain pouches following meals suggested that pentagastrin doses commonly used experimentally greatly exceed the physiological.

Introduction

It has been known for decades that the gastric secretory response which follows a constant intravenous infusion of a fixed dose of a cholinomimetic agent is not the expected plateau, but a peak followed by a progressive decline (Gray & Ivy, 1937). (This is referred to as fade.) Whether the gastric mucosa is vagally denervated or not seems to be immaterial. Pentagastrin or gastrin given under the same circumstances are said to exhibit the same phenomenon, (Sanyal & Watson, 1976) but here there is much less certainty. Interpretation of these experiments has proved difficult since precise information about the extent and nature of the fluid replacement is usually lacking. In many experiments of this kind, gastric and salivary secretions are copious.

We have noticed and so may have others without publishing it, that the elapsed time between the start of an infusion with a cholinomimetic agent and the peak secretory response is variable, but following pentagastrin it is less so. With either agent, when the peak is reached the secretion from the gastric fistula is very often bile-stained.

The following is an investigation of the fade phenomenon and an attempt to substantiate the impressions noted above.

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Method

Ten dogs of the Labrador retriever type weighing between 16 and 25 kg were used. Each, at a one-stage operation under ether anaesthesia, was furnished with a Heidenhain fundic pouch, a simple gastric fistula and a Thomas duodenal fistula. In five, the Thomas fistula was opposite the pancreatic duct and in the other five, opposite the common bile duct. All were used for the gastric studies but the pancreatic data are not given here.

All the experiments were conducted on animals between 2 and 24 months post-operative. After an 18 h fast the animals were held in Pavlov stands, the gastric and Thomas fistulae were opened and the pancreatic or common bile ducts cannulated, as appropriate. The cannulae used were plastic coeliac angiography catheters of appropriate diameter and length. When bile pressure rather than expelled volume was measured, a catheter with an inflatable balloon cuff around the open end was used.

Gastric fistula secretion was collected by simple drainage and the pouch secretion by the washout method of Magee & Nakajima (1968). All secretions were collected continuously and were measured and sampled at 10 min intervals. Gastric acid was titrated with a Radiometer (Copenhagen) automatic titrator. Pepsin was estimated by Anson's (1938) method and expressed as units (mg of tyrosine calculated to be

liberated by the pepsin in each 10 min secretion). Bile pressure was measured with a simple water manometer which was read continuously. One ml of bile was released each 10 min to compensate for the hepatic secretion. The average secretion has been found to be between 1 and 2 ml per 10 min (Magee *et al.*, 1952). In experiments in which bile was collected, the volume was measured every 10 min. In early experiments bile flow was measured. With the outflow at the level of the duodenal fistula, less than 1 ml per 10 min was secretion from the quiescent biliary tract.

A saline solution containing 150 mM NaCl and 8 mM KCl was given intravenously throughout every experiment and control. The volume was 2 ml min^{-1} or equal to that of the gastric fistula secretion in the previous 10 min if it exceeded 2 ml min^{-1}.

To begin with, experiments were started regardless of the clock time but when it became evident that there was a connection between spontaneous secretory and motor activity and the responsiveness of the secreting mucosa, procedures were timed in relation to spontaneous gastric activity peaks.

Balloon distension of the stomach was effected by introducing a toy balloon through the gastric fistula. It was inflated with 200 ml of water. The thin tube used for inflation allowed collection of juice from the fistula. If the balloon were inflated in the antrum the dogs immediately vomited. It is likely, since vomiting was rare in these experiments, that the balloon remained in the fundus. To keep the stomach and duodenum alkaline in some experiments $NaHCO_3$ (150 mM) was pumped into the stomach at 3 ml min^{-1}. It dribbled out through the Thomas fistula continuously. If the pH of the effluent showed signs of falling, the rate was increased. The vago-sympathetic trunks were blocked in the neck with adrenaline-free 2% lignocaine HCl (Astra Pharmaceutical Products Inc., Worcester, Mass). Success was evidenced by 100% increase in heart rate, bilateral Horner's syndrome and low pepsin concentrations in the gastric fistula samples. Failed attempts, which were quite frequent, served as controls.

In the initial experiments with cholinomimetics, zero time was taken as the time of the peak secretion. This was done for analysis when the variability in the interval between the onset of cholinomimetic injection and peak secretion became obvious and calculations based on the time the injection was begun therefore became meaningless. With pentagastrin there was no such variability, so that zero could be taken as the start of the injection and peaks occurred at 30 ± 3 min later. With pentagastrin, sample 4 was taken as full stimulation. Decline from these points, if any, was tested by determining the significance of the least square regression line, secretion versus time. Slopes before and after treatments were compared statistically. Total acid and

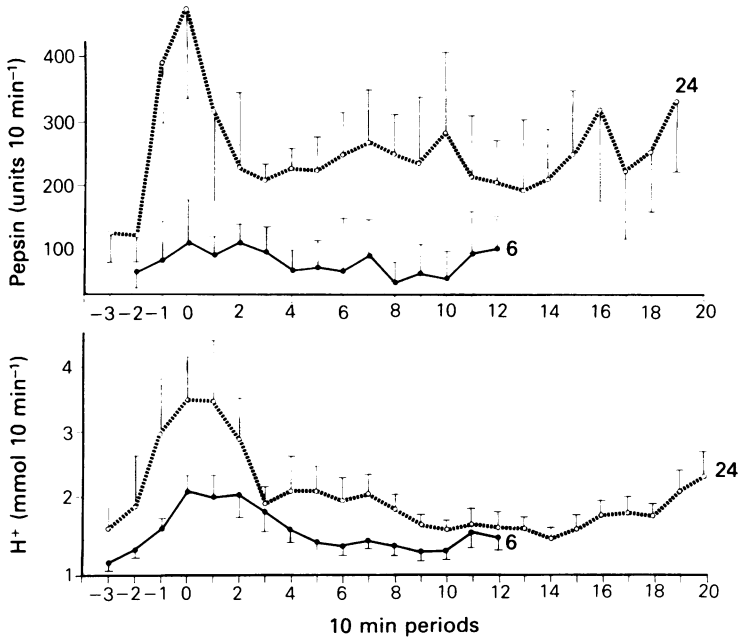

Figure 1 The effect of continuous intravenous methacholine at two infusion rates (24 μg kg^{-1} h^{-1} and 6 μg kg^{-1} h^{-1}) on pepsin (upper curves) and on acid (lower curves) output from the gastric fistula. Each point is the mean of observations in 8 dogs; vertical lines show s.e.mean.

Table 1 Slopes* of regression lines from peak secretion to sampe 10 thereafter indicating rate of decline of secretion

	Fistula		*Pouch*	
Drug and dose ($\mu g\,kg^{-1}\,h^{-1}$)	*H+*	*Pepsin*	*H+*	*Pepsin*
Pentagastrin 6	NS	NS	−26	NS
Pentagastrin 1.5	−202	NS	−14	NS
Pentagastrin	NS	NS	−25	NS
Pentagastrin + balloon	NS	NS	NS	NS
Pentagastrin 1.5 + HCO_3^-			−17	−2
Methacholine 24	−24	−50	−66	−4
Methacholine 24 + balloon	−313	−292	−62	NS
Methacholine 24 + HCO_3^-			−36	NS
Food			NS	NS
Food + HCO_3^-			NS	NS
Urecholine 100	−169	NS	−48	NS

The dose for pentagastrin plus balloon and pentagastrin is not given as doses between 6 and 1.5 $\mu g\,kg^{-1}\,h^{-1}$ were used to obtain comparable secretory rates among the dogs. The experiments were paired for analysis. *Calculated by method of least squares. Level of significance for r, $P < 0.05$; NS, not significant.

pepsin secreted in the 100 min following peak secretions were compared also by analysis of variance. In our animals 100 min was found to be the duration of one complete interdigestive cycle (Magee & Naruse, 1983).

Pentolinium tartrate (Wyeth Laboratories, Inc., Philadelphia, Pa.), a ganglionic blocking agent, was given (1 $mg\,kg^{-1}$) intramuscularly. All of the other agents used were given by intravenous infusion. They were: methacholine chloride (2-acetyl β-methycholine, Aldrich Chemical Co., Inc., Milwaukee, Wisc.) 6 and 24 $\mu g\,kg^{-1}\,h^{-1}$; bethanechol chloride (Urecholine chloride) 100 $\mu g\,kg^{-1}\,h^{-1}$; pentagastrin (Peptavalon, Ayerst Laboratories Inc., New York), 1.5 and 6 $\mu g\,kg^{-1}\,h^{-1}$. The latter dose in our laboratory is approximately maximal. all the other doses of stimulants used were less than maximal. These doses were used so that comparison could be made with previous experiments; they produce neither copious salivation nor gastric bleeding. The maximal dose of pentagastrin gave pouch acid secretion comparable to that of 6 mg $kg^{-1}\,h^{-1}$ of methacholine. In some instances cholecystokinin (CCK Kabi, Stockholm, Sweden) was used: 0.4 Ivy units kg^{-1} as a single injection or 100 ng $kg\,h^{-1}$. In the feeding experiment 450 g of a proprietary dog food was given. It was collected and measured as it left the Thomas fistulae.

Table 2 The time (min) to peak secretion of pouch and fistula HCl from the start of the intravenous infusion shown

Dog. no.	691	909	955	645	763	960	1650
Methacholine 24 $\mu g\,kg^{-1}$		50	40	90	10	60	50
Methacholine 6 $\mu g\,kg^{-1}$	30	70	30	40		100	
Urecholine 100 $\mu g\,kg^{-1}\,h^{-1}$	100	20	50	20	90	80	
Methacholine + balloon		50	70	30	50	70	50
Pentagastrin 1.5 $\mu g\,kg^{-1}\,h^{-1}$	40	30	20	40	30	20	40

Results

Cholinomimetics

Methacholine and urecholine produced copious peak secretion from both the gastric fistula and the Heidenhain pouch (urecholine acid μmol 515 ± 110 and 1687 ± 289 and pepsin units 129 ± 48 and 557 ± 176 from pouch and fistula, respectively per 10 min. The acid secretion declined significantly and rapidly over the succeeding 160 min (Figures 1 and 2, Table 1). Pepsin was obviously stimulated by methacholine (24 $\mu g\,kg^{-1}\,min^{-1}$); its output from the pouch was maintained with urecholine, but declined slightly to methacholine. Pepsin declined significantly from the fistula during methacholine stimulation (Table 1). Declining volumes of secretion, however, made pepsin secretion from the fistula difficult to interpret since it is washed out by parietal cell secretion (Figure 1). With both of these agents the elapsed time from the start of the infusion to the peak secretion varied from 30–100 min (Table 2).

In every instance in which they were measured, gallbladder contraction or bile expulsion coincided with, or preceded, by about 10–20 min, the start of gastric secretion from either pouch or fistula (Figure 2). As secretion declined so did biliary pressure and expulsion. Since gallbladder contraction may have simply been due to the entry of gastric acid into the duodenum, a series of experiments was conducted in which methacholine 24 $\mu g\,kg^{-1}\,h^{-1}$ was given while sufficient $NaHCO_3$ was pumped through the stomach to keep its pH neutral. In this instance also, gallbladder contraction preceded by 10–20 min or coincided with peak secretion from the Heidenhain pouch and declined as the biliary pressure fell (Figure 3); alkalinization did not change the rate of decline

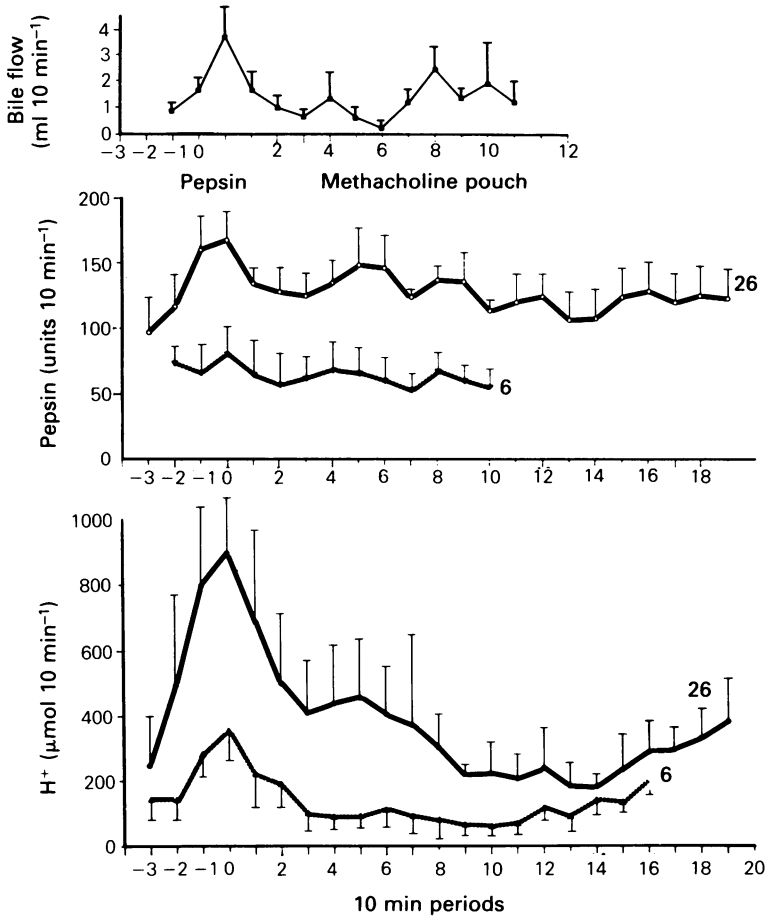

Figure 2 The effect of continuous intravenous methacholine at 24 μg kg^{-1} h^{-1} and 6 μg kg^{-1} h^{-1} on the pepsin concentration (middle curves) and acid (lower curves) secretion from 8 Heidenhain pouch dogs; mean values are given with vertical lines showing s.e.mean. The upper curve shows the mean concomitant bile flow from the 5 biliary fistula animals with both doses. The mean of the last 4 acid collections, in the 5 bile fistula animals at either dose, was significantly higher than that of samples 9, 10, 11, and 12.

significantly. More pouch acid per 100 min and less pepsin were produced after alkalinization than before. One hundred minutes later the biliary pressure rose once more, but pouch acid secretion did not. If the methacholine stimulation requires spontaneous activity, a peak is to be expected at 100 min intervals with fade between. In a few animals a small peak was seen at 100 min but in most the second peak was not seen but a third one was significant at 200 min (Figure 2).

Pentagastrin

Unlike cholinomimetics, copious secretion started with pentagastrin quite uniformly 20–40 min after the beginning of the infusion with either 1.5 or 6 μg kg^{-1} h^{-1}. Pouch acid declined significantly from sample 4 (Figure 4) with either infusion rate but, in the fistula, only with the lower one (Table 1). Gallbladder contraction started within 10 min of pentagastrin injection, reached a peak about 10 min behind the gastric peak and was maintained thereafter. When the experiment was repeated with intragastric neutralization, as used in the methacholine experiments, it became evident that the gallbladder maintained its own spontaneous interdigestive 100 min periodicity (Figure 5). The prompt gallbladder contraction which followed pentagastrin, infused at either 1.5 μg kg^{-1} min^{-1} or 6 μg kg^{-1} min^{-1}, could be prevented also by both bilateral vagal block (Figure 6) and ganglionic

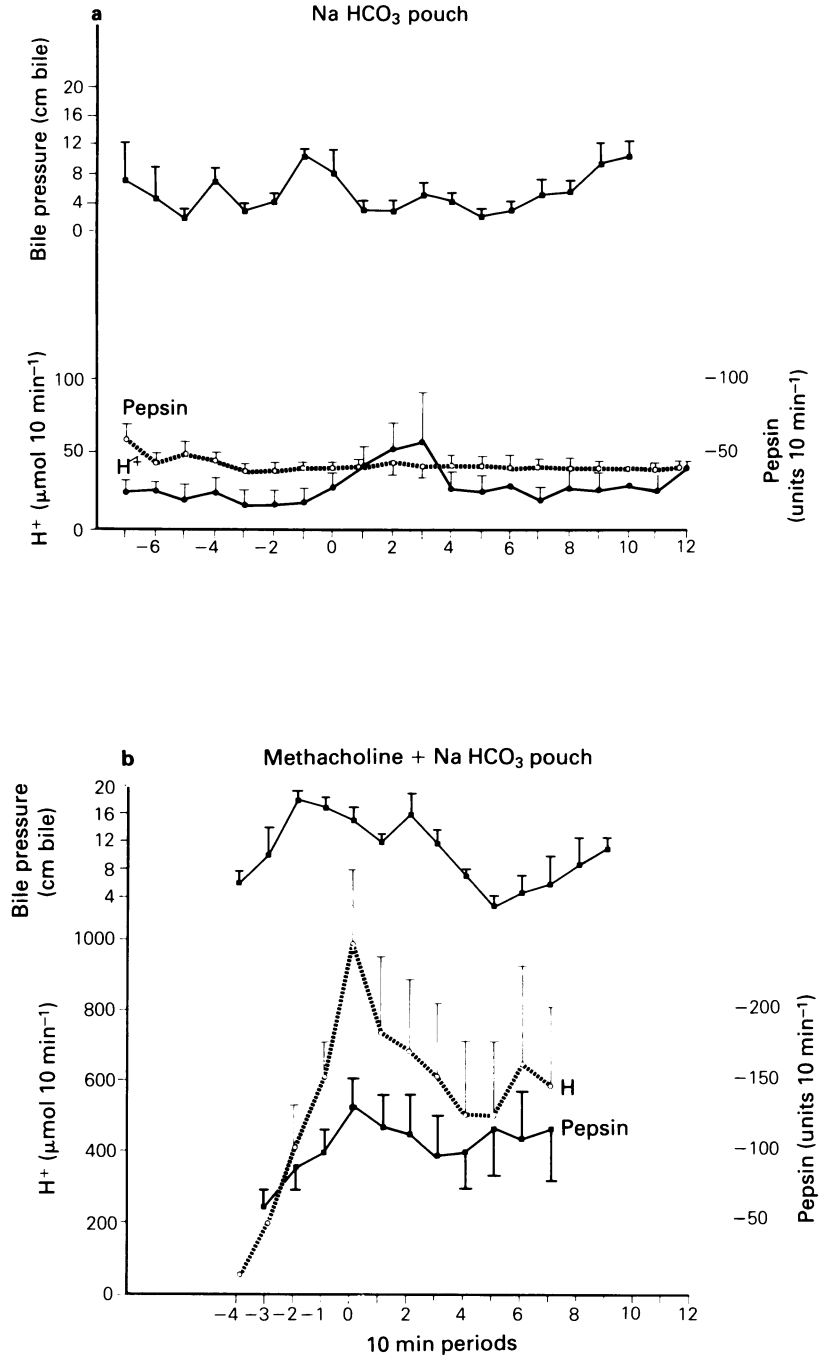

Figure 3 The effect of continuous intravenous administration of methacholine at 24 $\mu g\,kg^{-1}\,h^{-1}$ with concomitant alkalinization of the stomach and duodenum on Heidenhain pouch acid and pepsin (b), above in ascending order the simultaneous record of the biliary pressure. The control pouch acid and pepsin secretion with alkalinization only and its concomitant biliary pressure (a). Eight dogs were used; mean values are given with vertical lines showing s.e.mean.

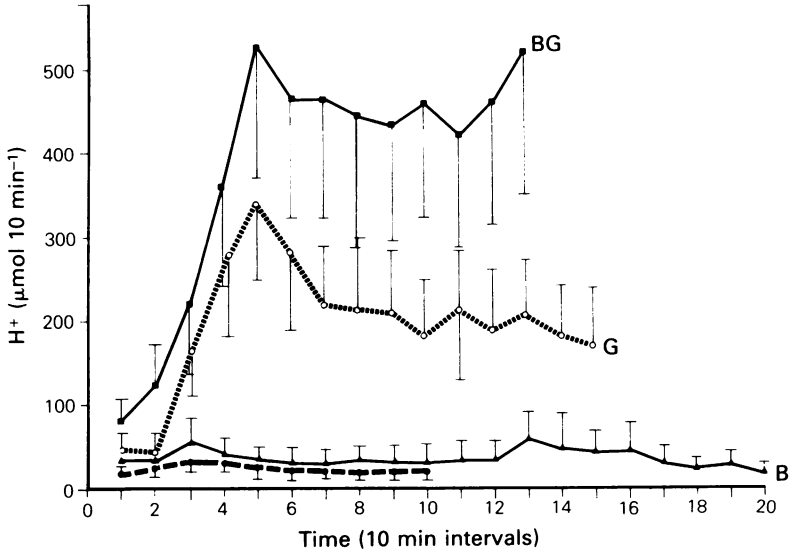

Figure 4 The mean effect of pentagastrin on Heidenhain pouch acid secretion when given alone (G) and with a distending balloon (BG) in the main stomach. Infusion rates of between 1.5 and 6 mg $kg^{-1} h^{-1}$ were chosen to give comparable control secretion among the dogs. The experiment was paired. The lowest graph is basal secretion (unlabelled) and above it the control with the distending balloon alone (B). Seven dogs were used.

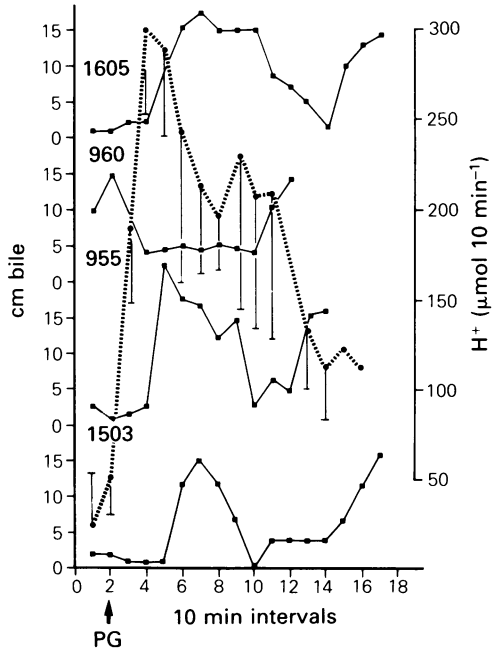

Figure 5 The mean effect of pentagastrin (1.5 μg $kg^{-1} h^{-1}$ with alkalinization of the stomach and duodenum on the acid secretion from the Heidenhain pouches of 5 dogs (interrupted line) superimposed on the concomitant individual changes in biliary pressure for 4 of these dogs (continuous lines) to show random peaking of biliary pressure. 1605, 960, 955 and 1503 are dog numbers; each has its own scale (0–15 cm bile) on the left. s.e.mean shown by vertical lines.

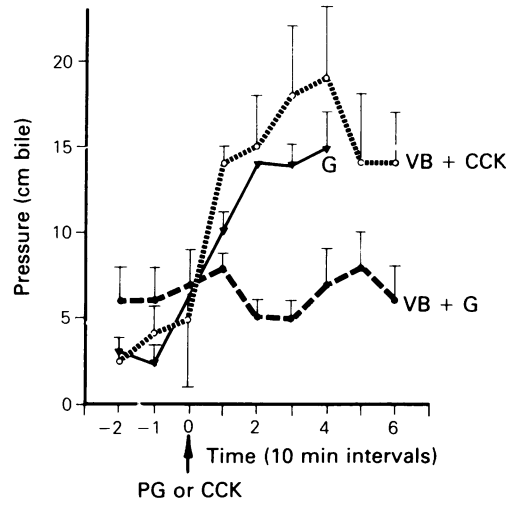

Figure 6 The mean effect of bilateral cervical vago-sympathetic block on the gallbladder pressure in response to pentagastrin or cholecystokinin (CCK). Shown are pentagastrin with vagal block (VB + G), pentagastrin without block (G) and CCK with vagal block (VB + CCK). Two infusion rates of pentagastrin were used, 1.5 and 6 μg $kg^{-1} min^{-1}$. CCK was infused at 100 ng $kg^{-1} min^{-1}$. Five dogs were used.

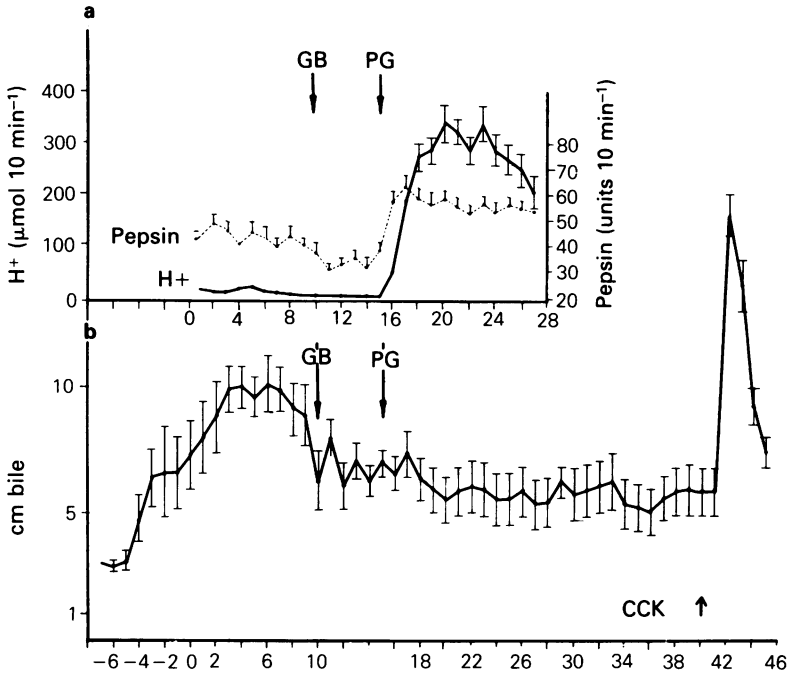

Figure 7 The mean effect of ganglionic blockade (GB) (pentolinium tartrate 1 mg kg^{-1}, subcutaneously) on pentagastrin (PG)-stimulated pouch acid and pepsin secretion (a). In (b) concomitant biliary pressure is shown. The ganglionic block was given just after the spontaneous peak (CCK 0.4 Ivy units). In (a) 9 dogs were used; in (b) 5 dogs.

blockade (Figure 7). Both of these abolished intrinsic periodic gallbladder contraction but did not prevent the action of CCK (Figure 7).

Gallbladder contraction following food was not prevented by intragastric $NaHCO_3$ (Figure 8). In the feeding experiments, intragastric $NaHCO_3$ raised the 100 min pouch acid secretion and depressed pepsin secretion. This was not significant in the pentagastrin experiments. By 100 min following feeding, most of the food had already left the stomach.

Mechanism of fade

In a series of experiments balloon distension of the main stomach was used during intravenous infusion of methacholine or pentagastrin (Figure 4, Table 1). Balloon distension significantly raised the basal Heidenhain pouch acid secretion and it significantly raised the 10 min mean pouch acid secretion over 100 min to a comparable extent with both methacholine and pentagastrin. In the former case, however, fade was as prominent as ever (Table 1) while in the latter there was no significant decline. The balloon, of course, significantly augmented all secretory parameters from the fistula. There was no significant difference between the slopes of the methacholine fade regression lines obtained with or without the balloon.

Discussion

The variable latent periods before methacholine-induced activity and the synchrony between maximal secretory activity and gallbladder contraction raises the possibility that methacholine acts via the intrinsic interdigestive periodic mechanism and that the decline or fade in activity with time represents the down phase or perigee while the peak represents the spontaneous apogee. This interpretation is supported by earlier findings that the action of this purely muscarinic drug on gastric secretion is, curiously, highly susceptible to ganglionic blocking agents (Odori & Magee, 1969; Etzel *et al.*, 1982). All the manifestations of interdigestive periodicity studied by us were suppressed by ganglionic blockade (Magee & Naruse, 1983). Against this idea are the possibilities that methacholine, itself, contracts the gallbladder or that acid entering the duodenum releases CCK which in its turn contracts the gallbladder. There is no convincing evidence for cholinergic regulation of gallbladder activity and the waxing and waning at regular 100 min intervals in response to a constant methacholine injection is not explicable in this way; the same argument can be applied to CCK. Our experiments, in which bicarbonate was used to neutralize gastric acid, refute these ideas since the initial peaks of gallbladder contraction

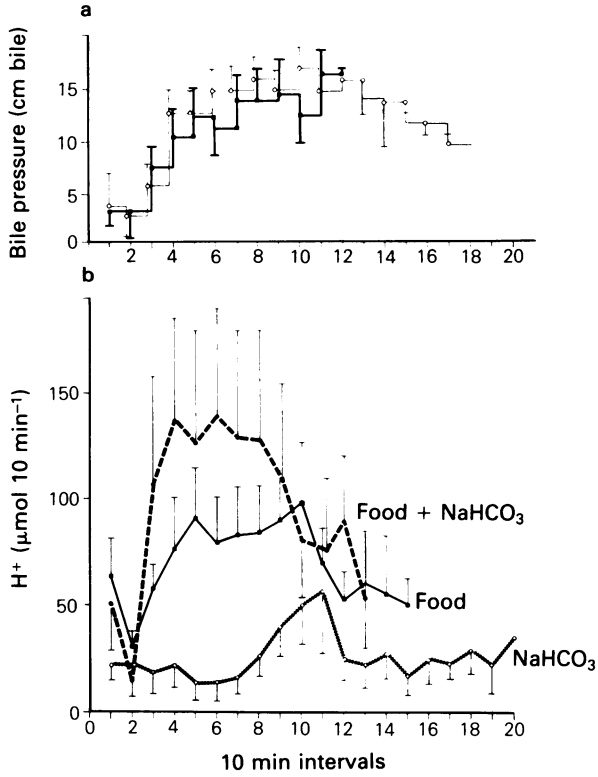

Figure 8 The mean effect of food, food and $NaHCO_3$ and $NaHCO_3$ alone on Heidenhain pouch acid secretion (b). In (a) the concomitant effect of food with and without alkalinization on gallbladder pressure. Six dogs were used.

and acid secretion still occur as usual and wane in unison. This means that methacholine simply accentuates the ordinary interdigestive activity of the stomach. In contrast, although the peak output of pepsin coincides with the acid, pepsin secretion does not fall off as rapidly.

If the above hypothesis were correct, a second peak should be seen at 100 min after the first and a third after 200 min in synchrony with the gallbladder peaks. The third attenuated peak was seen (Figure 2). The second was seen only in a few animals. We have no explanation for the suppression of the second peak.

In contrast to the action of methacholine, pentagastrin invariably produced copious gastric secretion and strong gallbladder contraction within 40 min of the start of the intravenous infusion, but acid secretion from the pouch declined with time. In these experiments neutralization of gastric acid, bilateral vagal block in the neck and pentolinium prevented the gallbladder contraction which follows pentagastrin injection. This makes it clear that acid and, therefore, CCK is responsible for the post-pentagastrin gallbladder activity. Both vagal nerve block and ganglionic blockade have been shown previously to prevent gallbladder contraction and pancreatic secretion in response to intraduodenal stimulants (Hong *et al.*, 1956; Hong & Magee, 1970). That there are factors other than acid causing CCK release is evident from the finding that alkalinization of a mixed meal does not abolish gallbladder contraction.

Following pentagastrin without neutralization of the stomach and food, whether neutralized or not, periodic gallbladder contraction is replaced by a continuous elevation in biliary pressure.

It seems, therefore, that pentagastrin itself interrupts the gastric secretory phase of periodic interdigestive activity and the gallbladder periodicity is interrupted by the entry of gastric chyme into the duodenum with consequent CCK release. Decreased secretin secretion could explain the higher pouch acid and lower pouch pepsin secretion in the methacholine and feeding experiments in which the main stomach was kept alkaline. This was expected also in the pentagastrin experiments, but did not reach significance. Ganglionic blockade does not diminish the action of pentagastrin on the Heidenhain pouch

(Figure 7 and Odori & Magee, 1969). This supports the idea that the initiation of the action of pentagastrin, unlike methacholine, does not depend on the interdigestive periodic activity, which is abolished by ganglionic blockade (Magee & Naruse, 1982).

It is evident from Figures 4 and 8 that the pentagastrin-stimulated acid secretion obtained from Heidenhain pouches, even with an infusion rate of 1.5 $\mu g\ kg^{-1}\ h^{-1}$ greatly exceeds that which follows feeding and that absence of fade in the feeding experiments indicates that pentagastrin is not a complete secretory stimulus. The obvious missing factor is gastric distension. This produced, as expected, clear augmentation of the fistula acid and pepsin secretion but unexpectedly, it also clearly augmented the pouch secretion, whether stimulated or not. It may be argued that the balloon causes endogenous gastrin release but gastrin causes fade and balloon distension abolished fade after pentagastrin; in the methacholine experiments, by contrast, distension did not abolish fade.

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