MOTILIN ENHANCES GALLBLADDER PRESSURE IN THE PIG

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ABSTRACT

Intraluminal gallbladder pressure, measured by radio-telemetry and bile output were monitored during infusion of porcine motilin at doses of 1.8, 7.1, 23.8 and 95.7 pmol kg⁻¹h⁻¹ in healthy ambulatory pigs.

Gallbladder pressure rose in a dose-related manner during the motilin infusion by 2.7 ± 2.0 , 4.9 ± 1.4 , 7.5 ± 1.0 and 12.0 ± 2.0 mmHg above basal respectively. In a separate series of studies in cholecystectomised pigs, motilin had no effect on liver output of bile.

These results suggest that the regulatory peptide motilin, may influence gallbladder motor function in the pig but probably has no effect on hepatic bile secretion.

In 1928 Ivy demonstrated that an extract of the duodenum caused contraction of the gallbladder. The active agent causing this effect was named cholecystokinin (CCK), and was the first demonstration of a possible hormonal influence on the biliary system (11). This initial work has since been confirmed, and has encouraged the search for hormones or related peptides which may influence the physiological process of gallbladder emptying. In addition to the cholecystokinetic effect of CCK, studies have demonstrated that glucagon, pancreatic polypeptide and vasoactive intestinal polypeptide either decrease motility or pressure generated within the gallbladder (5, 15, 21).

Motilin, a peptide of 22 amino acid residues, was isolated from the upper gastrointestinal tract of the small intestine of the hog (4). Increased contraction of smooth muscle of the stomach, jejunum and taenia coli from various species has been demonstrated in response to synthetic 13-norleucine motilin (23). In addition, motilin has been shown to increase the rate of gastric emptying following a solid meal (7) and to stimulate colonic activity (19). However, an effect on

the pig gallbladder *in vivo* has not so far been reported. Motilin circulates in plasma, and concentrations rise in response to the ingestion of a fatty meal (6). With the development of a conscious ambulatory model in which changes in gallbladder pressure and bile output could be continuously monitored (17), the opportunity was taken to study the effect of motilin.

Previous studies have shown that choleresis is stimulated in response to secretin, CCK, gastrin and vasoactive intestinal polypeptide (12-14, 16). Because motilin might also induce choleresis, thereby affecting gallbladder pressure, the hepatic bile output was measured during motilin infusions in pigs after cholecystectomy.

MATERIALS AND METHODS

The model used in these studies to determine changes of intraluminal gallbladder pressure during an infusion of the peptide hormone motilin, has previously been described in detail (17). Pressure changes were monitored by a radio-telemetry pressure capsule in the fundus of the gallbladder. Alteration in bile output was measured by a silicone choledochal diversionary tube leading from the proximal common bile duct to the pig's back. A three-way tap connected this tube to a further silicone tube

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which led back to the distal common bile duct and duodenum. Bile could therefore be collected from the three-way tap during experiments but was always returned to the animal both between experiments and after individual studies. Arterial sampling and venous infusions were carried out via iliac cannulae, also exteriorised on the pig's back.

Two male and four female Large White pigs, weighing 35–45 kg, were used. Each experimental animal was tested as previously described with a bolus injection of intravenous caerulein (100 ng/kg) to confirm that the normal response could be observed (17).

Experimental Protocol

Prior to the experiment, at least 5 days post-operatively, the pig was placed in the restraining cage, a ferrite rod aerial was strapped to the right flank and the three-way tap in the choledochal diversionary tube was closed distally so that bile output could be monitored in a graduated volumetric cylinder.

The motilin infusate was prepared in 0.15 M saline containing 10% by volume of fasting plasma taken from the pig being infused, to minimise adsorption to plastic surfaces. Pure porcine motilin was infused via a syringe ram pump (Harvard Apparatus, Millis, MA) in 4 consecutive doses of four-fold increments (4, 16, 64, 240 pmol/kg/h), each dose lasting 30 min. The motilin infusion was preceded by a 30 min basal and followed by a similar period of recovery, during which 0.15 M saline was infused. The concentration of motilin in the infusate was monitored by collecting a sample from the infusion apparatus at the end of each infusion dose. Arterial blood samples were taken at 10 min intervals throughout the basal, motilin infusion and recovery periods. Intraluminal gallbladder pressures were continuously

monitored and the mean pressure recorded at 5 min intervals. The bile expelled into the graduated cylinder was measured at 30 min intervals. On completion of a study, bile was pooled and returned to the pig via the distal choledochal diversionary tube into the duodenum.

In order to determine whether motilin has any choleretic properties, 6 other pigs underwent cholecystectomy; biliary and vascular cannulae were inserted as previously described. The motilin infusions were carried out in the cholecystectomised animals as described above.

Blood samples were taken throughout the studies with aprotinin (Trasylol, 400 KIU/ml), immediately centrifuged and the plasma decanted and stored at -20°C . Motilin radioimmunoassay was performed using porcine label and standards (3). Statistical analysis was carried out using the Student's *t*-test for paired data.

RESULTS

The basal plasma motilin concentration in the conscious pig was 29 ± 4 pmol/l. During infusion of porcine motilin, plasma concentrations rose as expected in a dose-dependent manner (Table 1). The metabolic clearance rate of porcine motilin was 6.6 ± 0.8 ml $\text{kg}^{-1} \text{min}^{-1}$.

Motilin induced a dose-related increase in gallbladder pressure (Fig. 1). The pressure increment was significantly increased above basal during the 3 higher doses of motilin (Table 1). Following termination of the highest infusion dose, plasma motilin levels and pressure fell promptly and these were not significantly different from basal levels 30 min after the end of the infusion.

Bile volumes during the motilin infusion are shown in Table 2. There was no significant change in bile output in the pigs either before or after cholecystectomy.

Table 1 Measured Infusate Motilin Concentrations, Plasma Motilin Increment and Gallbladder Pressure Increase during Step-Wise Motilin Infusion in 6 Healthy Conscious Pigs

Dose	1	2	3	4
Measured infusate dose (pmol $\text{kg}^{-1} \text{h}^{-1}$, mean $\pm$ SEM)	1.8 ± 0.2	7.1 ± 0.9	23.8 ± 2.7	95.7 ± 9.0
Incremental plasma motilin concentration (pmol/l, mean $\pm$ SEM)	4.0 ± 1.0	18.0 ± 3.0	62.0 ± 5.0	242.0 ± 12.0
Gallbladder pressure increment (mm Hg, mean $\pm$ SEM)	2.7 ± 2.0	4.9 ± 1.4	7.5 ± 1.0	12.0 ± 2.0
<i>P</i> <	NS	0.02	0.001	0.005

NS: not significant

Fig. 1 Gallbladder pressure rise during infusion of porcine motilin in 6 healthy conscious pigs. Bottom: motilin infusion in 4 consecutive doses of four-fold increments, each dose lasting 30 min. Dose scale is logarithmic.

Table 2 *Bile Output during Porcine Motilin Infusion (ml/30 min Periods) in 6 Pigs with Gallbladders and in 6 Cholecystectomised Animals*

Doses	Basal	1	2	3	4	Recovery	Recovery
Animals with gallbladders	31 ± 3	30 ± 4	28 ± 3	32 ± 3	31 ± 3	29 ± 3	29 ± 3
Cholecystectomised animals	32 ± 3	34 ± 3	32 ± 4	30 ± 6	31 ± 5	31 ± 5	32 ± 4

DISCUSSION

Motilin, discovered by Brown in 1966, has the ability to cause smooth muscle of the gut to contract in various species (23). It has been suggested that it may be responsible for regulation of the interdigestive myoelectric complexes (9) and these electrical changes in the gut are associated with intraluminal pressure changes (8).

Studies on guinea pig gallbladder muscle strips failed to show a contractile effect by motilin (23). However, recent studies by Itoh demonstrated that this peptide induced gallbladder contractions during the interdigestive period (10). Large rises in plasma motilin concentration have also been noted to accompany the formation of migratory motor complexes in the pig (2). This study in the conscious pig has shown that infused porcine motilin causes a rise of pressure within the lumen of the gallbladder.

The motilin-induced rise in gallbladder pressure appeared to be dose-dependent, but because the doses were administered consecutively, a possible summation effect should be considered. However, 30 min after cessation of the highest infused dose of motilin, intraluminal pressures had returned to basal values. This time period accords with the known short half-life of the peptide (18).

Dose 3 of motilin (24 pmol kg⁻¹h⁻¹) produced a measured plasma increment of 62 pmol/l, and the rise in gallbladder pressure of 7.5 mm Hg above basal was approximately 50% of the pressure rise attained in a previously reported study, in which a maximal dose of caerulein was given as a bolus injection (17). The highest motilin dose (96 pmol kg⁻¹h⁻¹) caused an increase in gallbladder pressure of 12 mm Hg, which is similar in magnitude to the change seen after food in this animal model (17). Infusion

of cholecystokinin octapeptide (CCK-8) at a dose of $6 \text{ pmol kg}^{-1} \text{ h}^{-1}$, also produced a similar rise in gallbladder pressure ($11.9 \pm 3.0 \text{ mm Hg}$) in this pig model (1).

The rise in intraluminal gallbladder pressure with motilin did not result in a significant increase in the output of bile. Previous studies with CCK suggest that the gallbladder does not expel its entire contents during contraction (22). It is estimated that the volume of these pigs' gallbladders, when distended, varied between 15–20 ml (unpublished data). With the large continuous output of bile in this model, 45 ml/h (17), any increased output from the gallbladder due to a sustained rise in pressure, would therefore be expected to produce only a small increment.

Previous workers have shown that production of hepatic bile may be influenced by the regulatory peptides (12, 13). Ryan and others have suggested that gallbladder motor function and pressure change may depend not only on the interaction of hormones but also on the intraluminal volume of bile (20). To exclude an increased output of bile effected by the peptide motilin, cholecystectomy was carried out and the infusion studies repeated. The results showed that motilin had no choleric property, precluding change of volume being responsible for the observed increase in intraluminal pressure.

The absence of any post-prandial increase in plasma motilin concentration suggests that its effect may be more important during the interdigestive period.

Evidence that humoral mechanisms are largely responsible for gallbladder emptying, are based on studies which have shown that CCK and less strongly gastrin, contract the gallbladder. This study shows a rise in gallbladder pressure during an infusion of the peptide motilin. Hormonal control of gallbladder motor function may thus be more complicated than hitherto realised.

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