Motilin release in the pig¹

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SUMMARY Motilin is found in the upper intestine of pig and man and in man is released by acid. A similar release by acid has now been found in the pig and is associated with markedly reduced immunostainable motilin in the upper small intestine. Clamping the arteries and veins to the stimulated segment immediately reversed the rise of plasma motilin, indicating the motilin release to be an entirely local phenomenon. The apparent half life of endogenous motilin was 3.9 minutes. No release of motilin was seen after a meal and the possible physiological role of motilin thus remains speculative.

Motilin, a 22 amino acid oligopeptide, has been demonstrated to be produced by enterochromaffin cells of the duodenum and jejunum of many species including pig and man (Pearse *et al.*, 1974). The physiological mechanisms controlling the release of motilin are not known. It was recently reported that in the dog, instillation of alkali into the duodenum gave rise to an increase in serum motilin (Dryburgh and Brown, 1975). In man, however, motilin was released after duodenal acidification but not by alkalinisation (Mitznegg *et al.*, 1976). As motilin has so far been isolated only from the pig and porcine motilin forms the basis of all motilin radioimmunoassays, we felt that a study of the release mechanisms in the pig would clarify problems of species difference. Moreover, the conflicting preliminary data concerning the release of motilin in humans and dogs need to be clarified before the role of this new hormonal polypeptide can be assessed.

Methods

Six pigs anaesthetised with nitrous oxide and oxygen were catheterised with portal venous and peripheral arterial cannulae and 30 cm of distal duodenal and proximal small intestine were perfused for 15 minutes with normal sodium chloride and thereafter for 70 minutes with 0.1 molar hydrochloric acid solution (Fig. 1). After 30 minutes of perfusion with the acid,

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Fig. 1 Pig preparation. Infusion of the upper small intestine with 0.1 M HCl at 1 ml per minute. Simultaneous extraction of pancreatic and biliary secretion.

the vascular pedicle of the perfused intestine was clamped for 20 minutes and then released. The proximal portion of the isolated bowel was also cannulated and drained to prevent accumulation of bile and pancreatic juice. Distension was avoided in the perfused gut by drainage catheters.

In a further group of six pigs, a soft plastic cannula was introduced into the jugular vein under anaesthesia, run under the skin and exteriorised in the middle of the back. After a 48 hour period of recovery the pigs were fed a standard meal of 300 g super pig meal—composition in percentages: oil 2.4, crude protein 16.8, crude fibre 4.7, lysine 0.78, methionine 0.26, calcium 0.8, phosphorus 0.6, salt 0.5; to this were added numerous vitamins and minerals in trace amounts—and blood samples drawn at five minute intervals for 15 minutes before and 75 minutes after the meal.

During each experiment, simultaneous 4.5 ml blood samples were withdrawn from the vascular cannulae into chilled heparinised tubes with 1000 Kallikrein inactivator units of aprotinin (Novo Ltd) and then rapidly centrifuged and the plasma frozen. Motilin concentrations were measured by a highly specific radioimmunoassay sensitive to 5 pmol/l plasma (95% confidence) as described in detail previously (Bloom *et al.*, 1976). Results are expressed as mean $\pm$ SEM and were analysed by Student's *t* test for paired data. The calculations of pharmacokinetic data were based on the plateau principle of Goldstein *et al.* (1974). Motilin half life ($t_{1/2}$) was determined by plotting against time, the log percentage of plateau values after subtraction of basal motilin concentration.

Results

In the 15 minutes of perfusion of the bowel with normal sodium chloride solution, the levels of motilin remained steady at 62 ± 10.6 pmol/l in the portal vein and 54 ± 9.7 pmol/l in the peripheral blood. Following acid perfusion (0.1 molar hydrochloric acid at 1 ml per minute) the concentration of plasma motilin increased to a peak at 15 minutes of 111.2 ± 9.4 pmol/l in the portal blood and 98 ± 8.6 pmol/l in the peripheral blood (Fig. 2). These values were then maintained until the vascular pedicle of the perfused bowel segment was clamped. At this stage, in spite of continued perfusion with acid, the mean portal level fell to 41 ± 4.8 pmol/l and the peripheral to 38 ± 5.0 pmol/l, which were below previous basal values. The release of the vascular clamp on the isolated segment, after 20 minutes, produced a rapid rise in motilin to a peak after 10 minutes of 191 ± 23 pmol/l in the portal blood and 180 ± 20 pmol/l in the peripheral blood.

The intraluminal pH changed from 8.0 to 3.5 after acid perfusion.

Calculation of the disappearance half-time after clamping gave a value of 3.9 ± 0.2 minutes, with a good straight line fit (r 0.992) (Fig. 3).

The fasting basal systemic plasma levels of pig motilin were 45 pmol/l ± 6 . Following ingestion of the 300 g meal there was no significant alteration of these values in the 75 minute sampling period after the meal (Fig. 4).

Discussion

Human motilin has not yet been isolated and porcine motilin has therefore been used to develop assays for measurement of human plasma motilin. It is thus important to check the validity of human data by parallel porcine experiments. In addition, as in the dog it has been suggested that duodenal alkalisation caused motilin release, while in man it is released by acid, the findings in a third species are of interest. Our results indicate that acidification of the distal duodenum and proximal small intestine produces a considerable increase in plasma motilin of similar magnitude to that previously reported in man (Mitznegg *et al.*, 1976). Motilin was also found to have a half life of 3.9 minutes, very closely comparable to that of 4.4 minutes found in man (Mitznegg *et al.*, 1977). Thus, the pig and man appear to be similar in this motilin response to upper small intestinal acidification. That they both differ markedly from the previously described situation in the dog implies a gross difference between species and that data on porcine motilin may be more relevant to human physiology. In man, eating a normal meal does not greatly alter the levels of motilin and the healthy conscious pig shows a similar absence of significant change after food (Bloom *et al.*, 1976).

The constantly higher values for motilin in the portal blood, as compared to peripheral blood, may perhaps be due partly to hepatic degradation, but more to dilution of the polypeptide on reaching the peripheral circulation. The relevance of this to humans is not clear as the plasma values of motilin in human subjects with severe liver disease have not been shown to be significantly different from those of the normal population.

The mechanism of motilin release by acid has not been previously investigated. Several possibilities have to be considered. The acid might act locally on the motilin cell. Alternatively, it might stimulate *via* a central nervous system reflex acting on the motilin cell, or perhaps even *via* the systemic release of another hormone. The finding that the increment of motilin produced by acidification of a particular

Fig. 2 Upper small intestinal acid perfusion in six pigs. Motilin rise in both portal and peripheral plasma following acidification of upper small intestine. The open circles and open triangles represent points which are statistically significant against the baseline values. Clamping of the local vascular segment is shown by the bar.

Fig. 4 Plasma motilin response after a standard pig meal. No alteration of plasma motilin values achieved statistical significance.

Fig. 3 Motilin half life, plot of log percentage of plateau values after subtraction of basal motilin concentration against time.

segment of bowel could be very rapidly and totally prevented by clamping the local blood vessels, leaving the majority of the motilin cells with an unaffected blood supply, suggests that the release of motilin is probably a purely local phenomenon. Both intraduodenal acid and high motilin levels can retard gastric emptying (Ruppin *et al.*, 1975). The release of motilin seen after acidification may thus play a physiological role in mediating the motor response to lowered duodenal pH.

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