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Factors influencing the intestinal phase of pancreatic exocrine secretion in the turkey

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Abstract. The present study was done to investigate the factors regulating the intestinal phase of exocrine pancreatic secretion in the turkey. The intestine of turkeys equipped with pancreatic fistulas was perfused with peptone solution, fat emulsion and hydrochloric acid (HCl), and pancreatic flow and protein output were measured. Neither peptone solution nor fat emulsion had any effects on pancreatic secretion. HCl enhanced the flow rate of pancreatic juice but not protein output. To clarify the neural mechanism of this phenomenon, the vagal postganglionic blocker atropine was continuously infused and pancreatic secretion in response to intestinal HCl was measured. Atropine completely suppressed both pancreatic flow and protein output. It is suggested that the avian intestinal phase of pancreatic secretion is mainly controlled by cholinergic action though HCl stimulation.
Key words. Exocrine pancreatic secretion; intestinal perfusion; atropine; turkey.

Pancreatic secretion can be considered as the net result of four phases of secretion, the cephalic, gastric, intestinal and humoral phases. In the cephalic phase, sham-feeding significantly stimulates chicken pancreatic secreation and the effect is abolished by vagotomy[1]. In the gastric phase, distension of the proventriculus with peptone solution significantly elevates pancreatic protein secretion in the turkey; this is due to the hormonal control of gastrin releasing peptide (GRP) from endocrine cells of the proventriculus[2]. The intestinal phase is considered to be the most important, because the intestine is the source of two major gastrointestinal hormones, cholecystokinin (CCK) and secretin. In birds, however, this phase has not been well demonstrated. In the chicken, dietary supplementation by amino acids[3,4] or soybean trypsin inhibitor[5] elevates the plasma concentration of CCK. Thus the chicken has been considered to have both mechanisms regulating CCK release, i.e. luminal feedback regulation as in the rat[6] and direct stimulation by certain amino acids as observed in dog[7]. However, in our previous study using dispersed chicken pancreatic acini, physiological concentrations of CCK did not seem to regulate pancreatic secretion because the ED_{50} of CCK-8 was 1000 times higher than that observed in rat[8]. This fact was also confirmed with the CCK analogue, caerulein[9].
The present study was conducted first to determine the intraluminal factor that stimulates pancreatic secretion, and second to elucidate the importance of vagal innervation in anaesthetised turkeys.

Methods

Broad breasted bronze turkeys (1.8–2.3 kg; approximately 4 months old) were fasted overnight but allowed free access to water. They were anaesthetised with urethane (1.5 g/kg b. wt; i.p.) and placed in a heated room maintained at 37 °C. Figure 1 illustrates the surgical method used in the present study. The left ventral side of the abdominal cavity was opened and the isthmus (the gizzard-proventriculus junction) was ligated to prevent the entry of gastric acid into the duodenum. Then the right ventral side of the abdominal cavity was

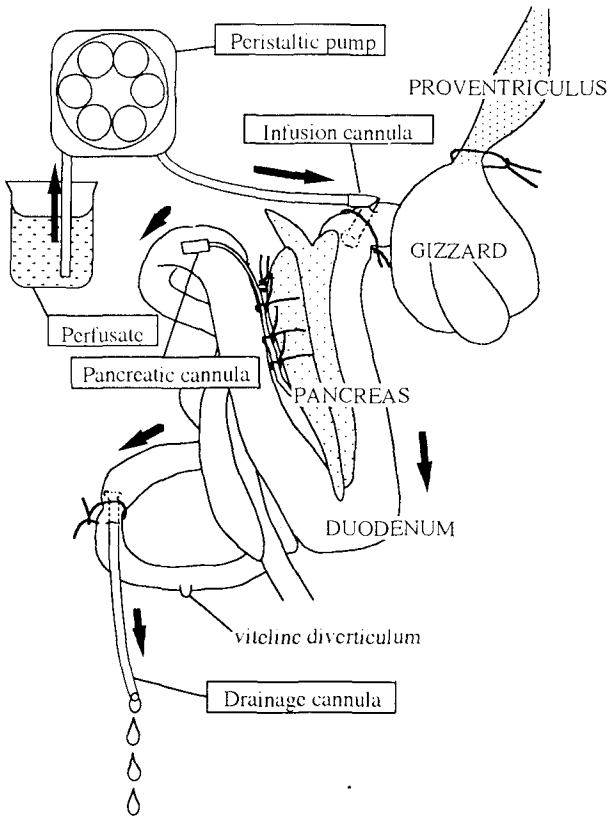

Figure 1. Intestinal perfusion and collection of pancreatic juice in turkey in vivo.

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opened to expose the pancreas and duodenum. The major pancreatic duct was cannulated with polyethylene tubing (i.d. 1 mm) and the minor one was ligated. The duodenum 2 cm distal to antrum and the jejunum 10 cm proximal to the vitelline diverticulum were cannulated with rounded polyethylene tube (i.d. 3.5 mm) and ligated tightly. Then the intestine between the two cannulae was perfused with 0.85% physiological saline for at least 2 h using a peristaltic pump (1.5 ml/mm) until basal pancreatic flow became stable. Pancreatic juice was collected every 10 min in capillary tubes connected to the pancreatic fistula.

As an index of digestive enzyme secretion, the protein content of the pancreatic juice was determined from absorption of diluted samples at 280 nm and expressed relative to a standard of bovine serum albumin. Results were expressed as the precentage of basal rates, using the values at 10 min before each infusion as basal.

The perfusates were: HCl (100 mM), peptone solution[2] (4.5% peptone; Sigma, MO, USA) or fat emulsion[10] (corn oil : deionized water : sodium deoxycholate = 52:46:0.24 (wt/wt/wt); emulsified using a homogenizer). After three stable basal periods, each perfusate was given intestinally for 30 min in random order to the same bird (n = 5), and pancreatic juice was collected at times 0, 10, 20, 30, and 40 min after changing the perfusion from saline. Atropine solution[11] (4 μg/ml) or saline as control was infused via a wing vein at the flow rate of 2.5 ml/h in random order to the same bird (n = 7).

One way analysis of variance was applied to the pancreatic flow and protein output in response to each perfusate. Statistical significance in pancreatic flow responses to HCl was analysed using a paired *t*-test. Split plot analysis was done for the effects of atropine on the pancreatic flow and protein output enhanced by intestinal perfusion of HCl, considering bird as main plots and time as subplots. All of the data analyses were done using a commercially available statistical package[12].

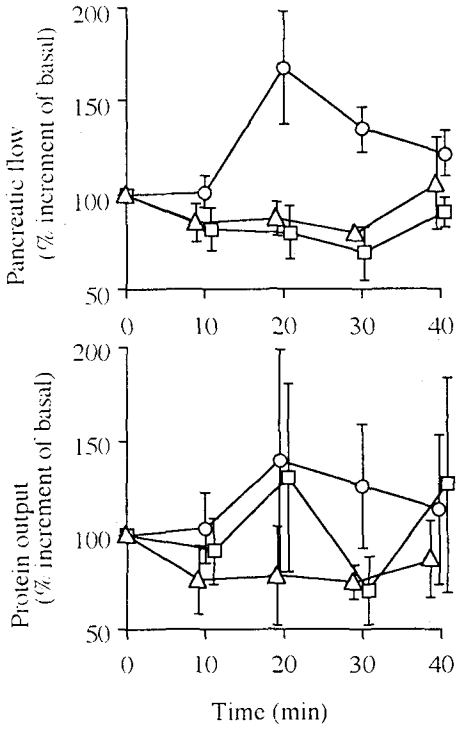

Figure 2. Effects of intestinal perfusion with HCl (circle), fat emulsion (square) and peptone solution (triangle) on pancreatic flow (upper pannel) and protein secretion (lower panel). Each perfusate was given intestinally for 30 min. Pancreatic juice was collected at times 0, 10, 20, 30, and 40 min after changing the perfusate from saline. Each point represents mean ± SE of five replicates. Pancreatic flow for intestinal perfusion with HCl at the time 20 and 30 min was significantly higher than control level at the time 0 min.

Results

The effects of intestinal perfusion of peptone solution, fat emulsion and HCl on pancreatic flow and protein output are shown in figure 2. Peptone and fat emulsion had no effect on pancreatic flow rate, but HCl significantly ($p < 0.05$) increased it at 20 and 30 min after infusion. HCl and fat emulsion slightly elevated the protein output but the responses were not significant. Peptone had no effect on protein output.

The effects of atropine on HCl-induced pancreatic flow and protein output are shown in figure 3. There were significant changes in both pancreatic flow and protein output ($P < 0.05$). Atropine abolished the HCl-induced pancreatic flow and depressed protein output below the basal level.

Discussion

In the present study, intestinal administration of peptone did not stimulate pancreatic secretion. In dogs, intestinal administration of amino acids or oligopeptide stimulated exocrine pancreatic secretion through CCK release[13]. On the other hand, luminal protease activity regulates pancreatic secretion via CCK release and this occurs in response to the direct stimulation of pancreatic secretory trypsin inhibitor[14] or CCK releasing peptide[15] in rats. The chicken had both mechanisms for CCK release observed in dogs and rats. Although peptone is a crude enzymatic hydrolysate of protein and consists of a mixture of protein, oligopeptides and amino acids, no significant effects were observed in either protein output or flow in turkey. However, CCK-like immunoreactive peptides exists in the distal part of jejunum[16]. The intestine used in this study was duodenum and upper jejunum, just outside the area where CCK-like immunoreactive cells exists. Even if released, the concentration of CCK would be too low to stimulate amylase secretion from dispersed pancreatic acini in chickens[8].

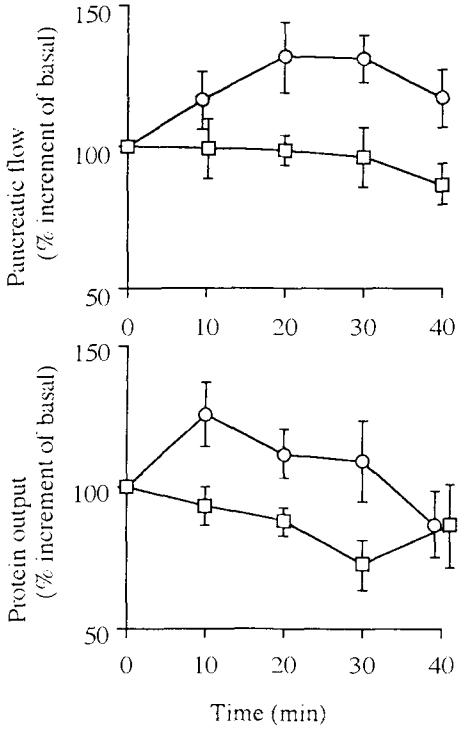

Figure 3. Effect of atropine on HCl-stimulated pancreatic flow (upper panel) and protein secretion (lower panel). HCl (100 mM) was perfused intestinally for 30 min. Pancreatic juice was collected at times 0, 10, 20, 30 and 40 min after changing the perfusate from saline to HCl. Atropine (square) or saline (circle) was administered intravenously during the experiments. Each point represent mean ± SE of seven replicates. There were significant effects between treatments in both pancreatic flow and protein output ($P < 0.05$).

Intraluminal fats are considered to be emulsified by vigorous contractions of the gizzard, and are then digested to fatty acids and other products. In this study, fat emulsion did not stimulate pancreatic secretion. In mammals, fatty acids in the intestine stimulate pancreatic secretion[17,18]. Since the fat used in this study was an emulsion of triglyceride, it would appear that fat has no effects before it begins to be digested at the earlier intestinal phase. Further study is needed to elucidate the effect of fatty acids.

The phenomenon observed in this study, HCl stimulation of the pancreatic flow but not protein output, might be due in part to secretin-related mechanisms. Secretin, a major gastrointestinal hormone, is a strong stimulant of pancreatic flow and bicarbonate secretion in mammals. In birds, however, the potential of chicken secretin in stimulating pancreatic flow and bicarbonate secretion was considerably weaker than in mammals[19] and it seems that another mechanism may be responsible. Atropine, a vagal postganglionic blocker, was used to investigate the participation of vagal nerve in the HCl-enhanced pancreatic flow. The pancreatic flow enhanced by HCl was completely suppressed to basal levels by atropine, suggesting that avian pancreatic flow is mainly regulated by the nervous system rather than by a humoral mediator such as secretin. The effect of atropine on protein output, was different from that on pancreatic flow. The slight elevation in pancreatic flow induced by HCl was further decreased below the basal secretion level by atropine. Thus the vagal nervous system may not only mediate HCl-enhanced pancreatic flow but may also mediate the basal protein output mechanism. According to Campbell et al.[2], GRP released from proventriculus stimulates pancreatic protein output in the turkey. Thus, the porventriculus seems to be an important organ regulating exocrine pancreatic secretion in avian species. Consequently, avian exocrine pancreatic secretion is mainly regulated by the gastric phase rather than the luminal phase, which differs from the situation in mammals.

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