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Endocrine and exocrine pancreatic function after camostate-induced growth of the organ

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Abstract. It is well known that oral administration of camostate induces hyperplasia and hypertrophy of the rat pancreas. It is not clear, however, whether pancreatic hormone and enzyme secretion are affected by camostate treatment.

In rats, daily administration of 200 mg camostate/kg b. wt for 14 days significantly increased pancreatic weight and pancreatic content of DNA, protein, amylase, lipase, trypsin and chymotrypsin, as well as the amount of insulin, glucagon and somatostatin. In the intact animal, blood glucose levels and serum concentrations of insulin and glucagon in response to an oral glucose load were not impaired after camostate treatment. In the isolated perfused pancreas, however, insulin and glucagon secretions were reduced, whereas somatostatin release was not affected. The volume of pancreatic juice produced by the unstimulated isolated perfused organ, as well as protein and enzyme secretion, were increased after camostate treatment. Likewise, the isolated perfused pancreas from camostate-treated rats secreted a larger volume of pancreatic juice and more protein in response to cholecystokinin (CCK), while enzyme secretion was affected in a non-parallel manner: amylase release was markedly reduced, lipase release was unchanged, and release of trypsin and chymotrypsin was increased.
Key words. Camostate; endocrine and exocrine pancreas.

Numerous studies have consistently shown that cholecystokinin (CCK) potently stimulates pancreatic growth. This is true for exogenously-administered CCK, as well as endogenous CCK released by oral treatment with protease inhibitors. Thus both endogenous and exogenous CCK have been shown to increase pancreatic weight and content of nucleic acids, protein and enzymes[1–11]. Some studies have also investigated the effect of CCK on hormone content, but the results are inconsistent[3,4,6,10–14]. Endocrine and exocrine functions of the pancreas, however, have not been studied systematically after growth of the organ had been stimulated.

Thus there is no doubt that oral administration of camostate, a potent protease inhibitor, causes hyperplasia and hypertrophy of the pancreas through release of endogenous CCK[1,9]. Yet little is known about endocrine and exocrine pancreatic secretion after camostate treatment. Therefore this study was designed to investigate whether endocrine and exocrine pancreatic functions are intact after camostate-induced growth of the organ.

Methods

Animals. Male Wistar rats (Versuchstieranstalt Lippe, Lippe, Germany), weighing between 250 and 350 g, were used in this study. Before the start of the actual experiment, the animals were adapted to the housing conditions over a period of seven days. Animals were kept in wire bottomed cages in a light-controlled room at a temperature of 21 °C. Rats were pair-fed on a standard diet (Altromin, Altrogge, Germany) with free access to water, as described previously[11]. Thus each pair of animals differed only in whether or not they had been treated with camostate. Animals and food intake were weighed daily.

Over a period of 14 days, rats were treated with either camostate (Sanol Schwarz, Monheim, Germany) or isotonic saline. 200 mg camostate/kg b. wt, or the same volume of saline, were administered into the stomach once daily. At the end of the treatment period, animals were fasted for 24 h, but they still had free access to water. Rats were then anesthetized with pentobarbital (i.p. application of 60 mg Nembutal/kg b. wt; Ceva, Paris).

Pancreatic content of protein and hormones. In one group of animals, the pancreas was removed, rinsed with cold NaCl and weighed. Then the organ was divided and homogenized as described previously[11]. From one half of the organ, DNA, protein and enzyme content were determined according to standard laboratory methods[15–20]. From the other half of the organ, the content of immunoreactive insulin, glucagon and somatostatin were determined as previously described[21].

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Hormone secretion in vivo. A second group of rats underwent an oral glucose tolerance test with 1 ml of a solution containing 0.4 g of glucose, administered via an intragastric feeding tube. From a catheter in the V. jugularis, 200 µl blood samples were drawn at 0, 30, 60, 90 and 120 min. After each blood sample, 200 µl isotonic saline were injected into the catheter. Blood glucose and serum concentrations of immunoreactive insulin and glucagon were determined as described previously[21].

Hormone release and exocrine pancreatic secretion in vitro. In a third group of rats, the pancreas was dissected as previously described[21,22]. Briefly, the preparation consisted of pancreas with a small residue of duodenum. The proximal end of the bile duct was ligated and every 10 min a calibrated polyethylene tube was inserted into the distal end of the common duct to collect pancreatic juice. The preparation was perfused via the superior mesenteric artery and the celiac trunc at a constant rate of 4 ml/min without recirculation. The perfusate was a modified Krebs-Ringer bicarbonate solution with 0.2% bovine serum albumin (RIA grade) and 3% dextran (T70). Glucose concentration was between 2.8 and 25 mM, as given in the text or as indicated in the figures. The perfusate was gassed with 95% O_2 and 5% CO_2 to give a pH of 7.4.

After an equilibration period of 10 min, the actual experiments were performed in consecutive 10-min intervals. In some experiments, CCK-8 (Sigma) was infused via a side-arm injection using an infusion pump (Braun-Melsungen). CCK-8 was used at concentrations between 25 and 800 pg/ml. Hormone concentrations in the perfusate were increased stepwise every 10 min.

Protein content and enzyme activities in pancreatic juice were determined using standard laboratory methods[15-20]. Complete portal vein effluent was collected at 60-s intervals, aliquoted and stored at −20 °C prior to determination of insulin-, glucagon- and somatostatin-like immunoreactivities, as described previously[21].

Data analysis. All experiments were performed on 8 to 10 animals. Data are given as mean ± SEM. Statistical analysis was performed with Student's *t*-test for unpaired data. P values less than 0.05 were considered significant.

Results

Body weight, food intake, organ weight and pancreatic content of DNA, protein, enzymes and hormones. Neither body weight nor food intake differed significantly between animals treated with camostate and controls at any point during the 14-day treatment period (fig. 1). Camostate significantly increased the weight of the pancreas (3.9 ± 0.2 vs 1.5 ± 0.1 g), and pancreatic content of DNA (16 ± 1 vs 10 ± 1 mg), protein (562 ± 33 vs 165 ± 20 mg), amylase (37540 ± 2310 vs 30980 ± 1270 U), lipase (467 ± 35 vs 25 ± 1 U), trypsin (1073 ± 53 vs 487 ± 53 U) and chymotrypsin (309 ± 26 vs 155 ± 11 U). Camostate also increased pancreatic content of immunoreactive insulin (7.9 ± 0.2 vs 4.5 ± 0.2 U), glucagon (1019 ± 59 vs 108 ± 5 ng) and somatostatin (165 ± 6 vs 50 ± 8 pmol).

Homone secretion in vivo. Blood glucose levels and serum concentrations of immunoreactive insulin and glucagon after oral administration of 0.4 g glucose were not significantly different between camostate-treated rats and controls (table).

Hormone secretion in vitro. Glucose-induced insulin secretion was significantly lower from organs of camostate-fed rats than from controls (fig. 2). Glucagon secretion in the presence of increasing concentrations of glucose (2.8, 8.3, 16.7 and 25 mM at 10-min intervals) tended to be lower in camostate-treated rats than in controls, but the differences were not statistically significant (fig. 2). The difference in glucagon release between organs from camostate-treated rats and controls only became statistically significant when hormone secretion was integrated over 10-min periods. Somatostatin secre-

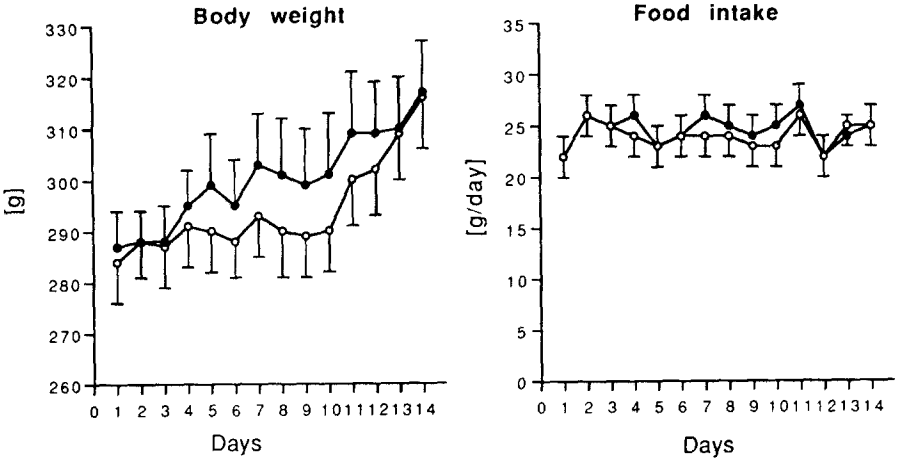

Figure 1. Body weight and daily food intake during camostate treatment. (Closed circles: camostate; open circles: controls.)

Table. Blood glucose levels and serum concentrations of immunoreactive insulin or glucagon after oral administration of 0.4 g glucose after a 14-day treatment with camostate.

Min	Glucose [mg/dl]		Insulin [μU/ml]		Glucagon [pg/ml]	
	control	camostate	control	camostate	control	camostate
0	47 ± 4	52 ± 4	63 ± 18	85 ± 7	333 ± 22	273 ± 58
30	79 ± 12	77 ± 8	117 ± 31	122 ± 31	274 ± 15	220 ± 72
60	104 ± 6	109 ± 4	130 ± 35	142 ± 28	259 ± 38	195 ± 32
90	97 ± 7	95 ± 3	141 ± 16	114 ± 18	141 ± 31	193 ± 30
120	83 ± 5	91 ± 4	104 ± 4	102 ± 5	98 ± 19	134 ± 21

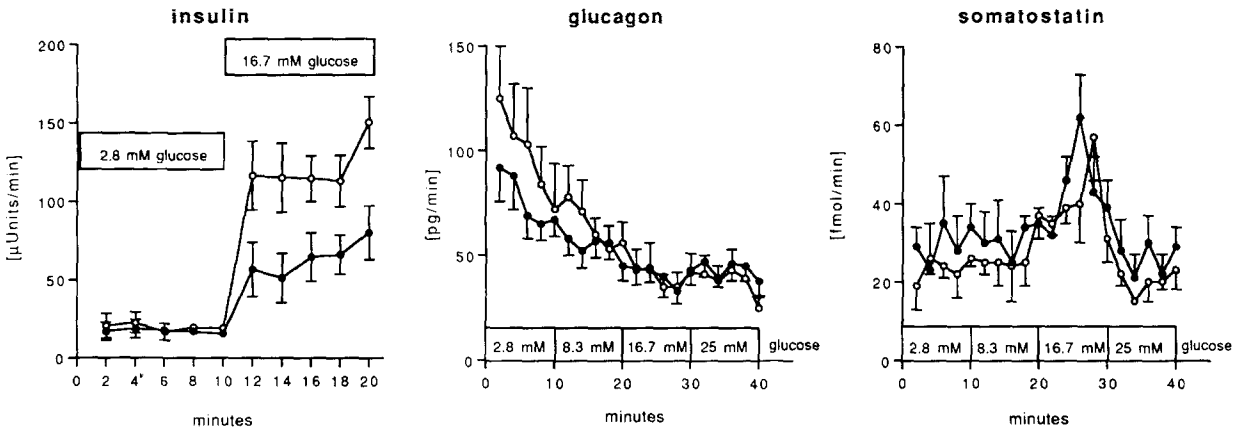

Figure 2. Insulin, glucagon and somatostatin release from the isolated perfused rat pancreas after camostate treatment. Glucose concentrations in the perfusate are given in the figures. (Closed circles: camostate; open circles: controls.)

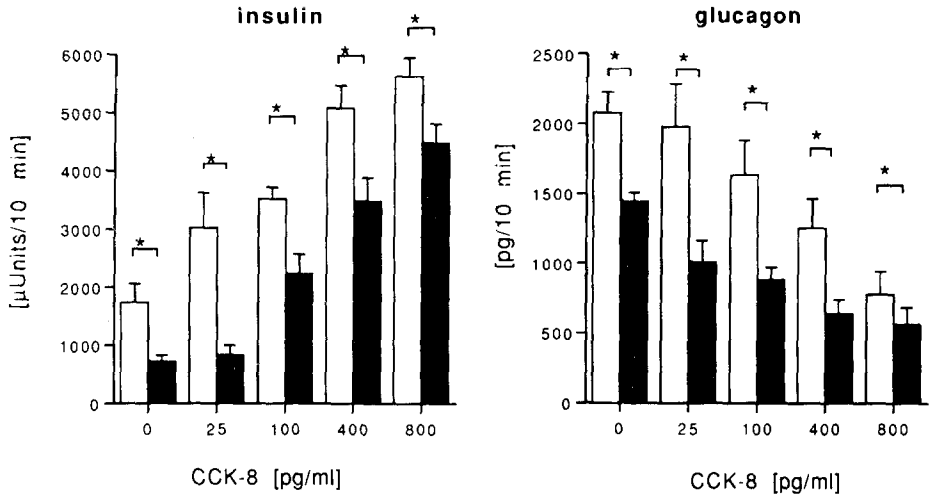

Figure 3. Insulin and glucagon output per 10 min from the isolated perfused rat pancreas after camostate treatment. Concentration of CCK in the perfusate was increased every 10 min. (Closed bars: camostate; open bars: controls; *$p < 0.05$.)

tion in the presence of increasing concentrations of glucose (2.8, 8.2, 16.7 and 25 mM in 10-min intervals) was not significantly different between organs from camostate-treated rats and controls (fig. 2).

When the concentration of CCK-8 in the perfusate was increased stepwise at 10-min intervals, while glucose concentration was kept constant at 8.3 mM, insulin and glucagon secretion (output per 10 min) were both significantly lower from organs of camostate-fed rats compared to controls (fig. 3).

Exocrine pancreatic secretion in vitro. Basal exocrine pancreatic secretion differed significantly between organs from camostate-treated animals and controls. Organs from camostate-treated animals secreted a significantly higher volume, more protein, more chymotrypsin and more trypsin, but less amylase. Lipase secretion was not significantly different (fig. 4).

In the presence of CCK, the pancreas from camostate-treated animals secreted higher volumes of exocrine juice (the difference being statistically insignificant

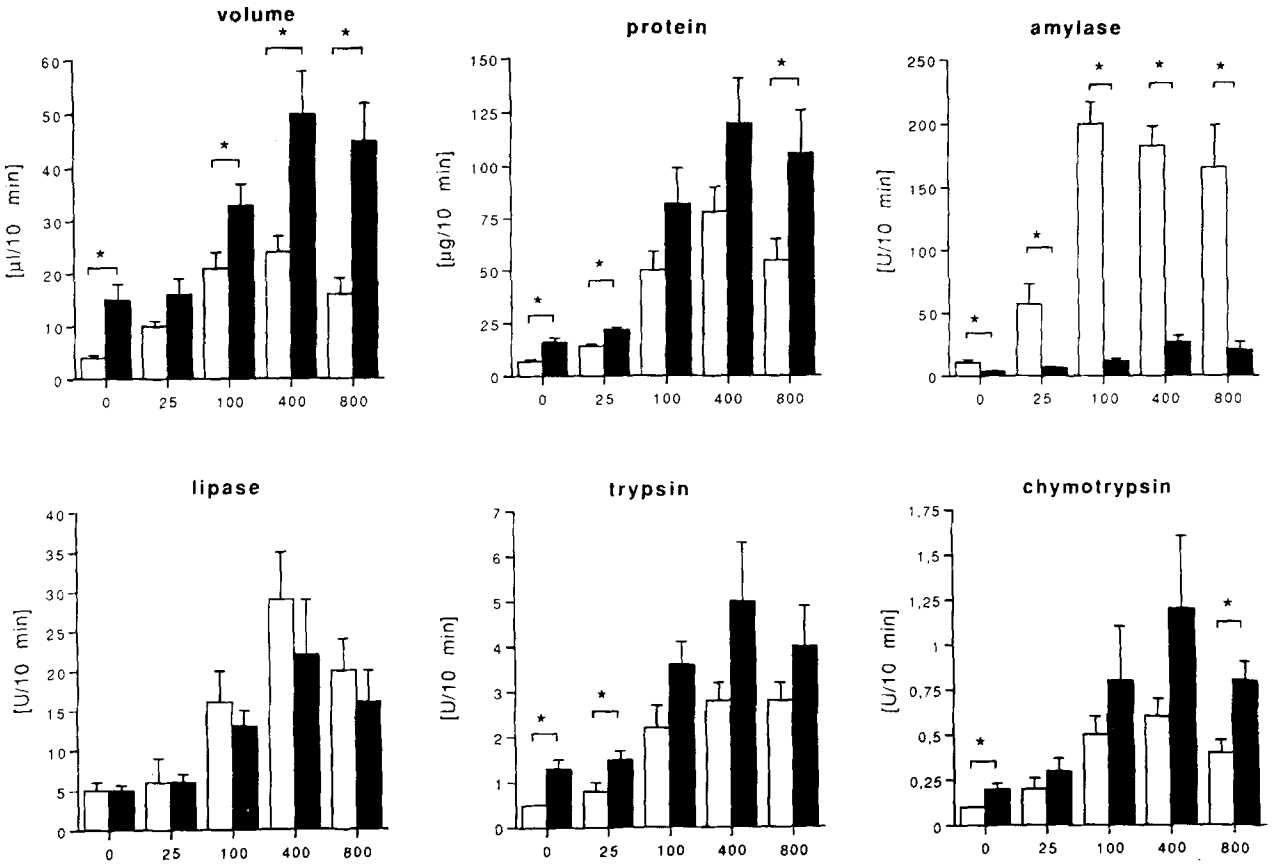

Figure 4. Exocrine pancreatic secretion from the isolated perfused rat pancreas after camostate treatment. Concentration of CCK in the perfusate was increased every 10 min. (Closed bars: camostate; open bars: controls; *$p < 0.05$.)

only for 25 pg/ml CCK-8) and more protein (the difference being statistically insignificant for 100 and 400 pg/ml CCK-8). Camostate had non-parallel effects on CCK-stimulated enzyme secretion. Amylase secretion was markedly reduced and lipase secretion was not significantly different. Secretion of trypsin and chymotrypsin, however, was higher, although this was not statistically significant for all concentrations of CCK-8 used (fig. 4).

Discussion

Camostate is a potent protease inhibitor. The compound causes a reversible hyperplasia and hypertrophy of the pancreas[1,9] probably through release of endogenous CCK. In agreement with previous work from different groups[1,4,5,7,9,11], we were able to demonstrate an increase in pancreatic weight and in pancreatic content of DNA, protein, amylase, trypsin, chymotrypsin and lipase. It is of interest that the increase in protein content was more pronounced than the increase in DNA content.

The few studies on the effect of intraluminal inhibition of proteases on the endocrine pancreas, however, have given inconsistent results. Some groups reported that insulin content in rat pancreas or isolated pancreatic islets was not affected by camostate[4,11]. In the present study, however, insulin content was significantly increased, confirming the results of a study by Temler et al.[12]. Likewise, there are contradictory reports on glucagon content after camostate treatment. Glucagon content has been reported to be unchanged, increased or insignificantly decreased by treatment with camostate or CCK[11-14]. Finally, the increase in pancreatic content of somatostatin after camostate treatment in our study is consistent with results previously reported by different groups[10,13,14].

Compared to the number of studies on pancreatic hormone and enzyme content, there are only very few studies on endocrine and exocrine pancreatic function after camostate treatment. In spite of the marked increases in pancreatic hormone content, endocrine pancreatic secretion and blood glucose levels after an oral glucose load were not different between camostate-treated rats and controls in our study. Thus the hypertrophied pancreas was able to maintain an adequate hormone response to a metabolic stimulus in the intact animal. This is compatible with a report from Arnesjö et al. In their study, blood glucose levels after a glucose load were not different and insulin release was only impaired after three weeks' treatment with a naturally occurring trypsin inhibitor, but not after one week[23].

The same group, however, also reported that insulin response to intravenous glucose was impaired in rats treated with CCK[6].

It was only in our in vitro experiments that impairment of hormone secretion became obvious. Glucose- or CCK-stimulated insulin release was significantly lower in isolated perfused organs from camostate-treated animals. Likewise glucagon secretion was lower, although this was statistically significant only when the glucagon output was integrated over a 10-min period. Impairment of endocrine pancreatic secretion in vitro may have been due to extrinsic denervation of the organ; alternatively it could have been due to the different glucose concentrations in the in vitro experiments. Göke et al., however, reported that glucose-stimulated insulin release was not affected by camostate treatment[4]. The discrepancy may be due to the fact that they administered a higher daily dose of camostate, although we have previously also seen impairment of glucose-stimulated insulin release when rats were treated with 400 mg camostate per kg per day[11]. Camostate treatment also affected basal and CCK-stimulated exocrine pancreatic secretion from the isolated perfused organ. Organs from camostate-treated animals secreted a higher volume of exocrine juice and more protein. Enzyme secretion, however, was affected in a non-parallel manner. Although pancreatic content of all enzymes was increased by camostate, only chymotrypsin and trypsin were secreted at a higher rate. Secretion of lipase was unaffected, and secretion of amylase was markedly reduced. Similar non-parallel changes have been seen by our group after a higher daily dose of camostate[11]. The decrease in amylase secretion, as seen in our study, is also in agreement with findings from a previous in vitro study, in which isolated lobules from rats treated with camostate secreted less amylase in response to caerulein, when enzyme release was calculated in percentage of initial content[7]. This may differ from in vivo experiments, as enzyme secretion of both amylase and trypsin were increased in rats treated with CCK for 20 days[3].

It is unlikely that amylase secretion after camostate treatment was reduced as a consequence of lower insulin release[24], because in previous studies from our laboratory, insulin secretion had to be reduced more markedly before exocrine pancreatic secretion was affected[25,26]. Moreover it would be difficult to explain the non-parallel changes in enzyme secretion exclusively by inhibition of insulin release. Alternatively, a downregulation of pancreatic CCK receptors may have played a role in camostate-induced reduction of amylase secretion. Thus camostate releases endogenous CCK[1,9], and both endogenous and exogenous CCK have been shown to downregulate the CCK receptor on pancreatic acinar cells[27,28]. The maximal effect of CCK on exocrine pancreatic secretion in our study, however, was consistently seen with a concentration of 400 pg/ml both for the control group and the camostate group. This suggests that the sensitivity of acinar CCK receptors was in fact not impaired in our study.

We conclude that camostate treatment increased pancreatic content of hormones and enzymes. In vivo, pancreatic hormone secretion was not imparied in response to an oral glucose load. In vitro, however, insulin and glucagon secretion were reduced. The volume and the protein content of the fluid released from the hypertrophied pancreas were increased, while enzyme secretion was affected in a non-parallel manner. Thus, marked differences in endocrine and exocrine pancreatic function can be observed in vitro after camostate-induced growth of the organ.

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