Chronic Effects of Camostate on Growth and Endocrine Function of the Pancreas in Streptozotocin-Induced Diabetic Rats (43653)

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Abstract. **Chronic feeding of normal rats with camostate, a synthetic trypsin inhibitor, stimulates the growth of the pancreas chiefly by increasing cholecystokinin release. We examined the effects of camostate on the growth and the endocrine function of the pancreas in streptozotocin-induced diabetic rats. The pancreatic weight of the diabetic rats given camostate (200 mg/kg/day) by oral gavage for 14 days was significantly elevated by 120% over that of diabetic rats not given camostate, and was comparable to that in the nondiabetic rats given camostate. The total pancreatic contents of DNA, RNA, and protein in diabetic rats given camostate were also significantly higher than those in diabetic rats not given camostate, and did not differ from those observed in nondiabetic rats given camostate. The pancreatic growth seen in diabetic rats treated with camostate was associated with moderate hyperplasia and pronounced hypertrophy. By contrast, treatment with camostate did not improve hyperglycemia, hypoinsulinemia, or low pancreatic content of insulin seen in diabetic rats. These findings demonstrate a marked growth of the pancreas in diabetic rats stimulated by camostate, and suggest that camostate-induced pancreatic growth is not affected by the reduced level of the endogenous insulin. The present study also indicates that camostate has no beneficial effects on the function of residual B cells, failing to improve diabetes.**

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Chronic administration of camostate, a synthetic trypsin inhibitor, stimulates the growth of the normal rat pancreas (1–5). Camostate enhances the release of cholecystokinin (CCK) (2), which is known to stimulate growth and secretion of the exocrine pancreas (6). A specific CCK antagonist, L-364,718, reverses the stimulatory effects of camostate on pancreatic growth (4). These findings indicate that endogenous CCK plays a major role in the trophic action of camostate. Besides CCK, Logsdon (7) demonstrated that insulin, at supraphysiologic doses, increased DNA synthesis in pancreatic acinar cells *in vitro*. In his report, a combination of cerulein (a decapeptide analog of CCK) and insulin caused the additive stimulation of DNA synthesis in acinar cells, suggesting that the subcellular mechanisms of the mitogenic response to each agent were distinct. However, the role of endogenous insulin in the trophic action of camostate is poorly documented. With regard to the endocrine pancreas, specific receptors for CCK exist on pancreatic B cells as well as acinar cells (8). We have shown previously an exaggerated release of insulin in response to glucose from the isolated perfused pancreas of rats either fed cholestyramine, a bile salt sequestrant that enhances the release of endogenous CCK, or given exogenous CCK for 2 weeks, suggesting that CCK has a stimulatory effect on B cell function (9). The purpose of the present study was (i) to clarify the effects of camostate on the growth of the pancreas in streptozotocin-induced diabetic rats, which are characterized by selective destruction of pancreatic B cells with insulin depletion (10), and (ii) to examine whether camostate affected the function of residual B cells that had escaped from streptozotocin toxicity.

Materials and Methods

Male Wistar rats (180–200 g) from Japan SLC (Hamamatsu, Japan) were used. After an overnight fast,

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rats were rendered diabetic by injecting 30 mg/kg streptozotocin (Sigma Chemical Co., St.Louis, MO) freshly dissolved in 0.01 *M* sodium citrate buffer (pH 4.5) via the penile vein under light ether anesthesia. Another group of rats (nondiabetic rats) received the citrate buffer alone. All rats were then fed a standard pellet diet *ad libitum* until they were sacrificed. After 7 days, the rats were randomized into the following treatment groups (n = 10 rats/group): (i) diabetic animals treated with camostate (FOY-305; a generous gift of Ono Pharmaceutical Co., Osaka, Japan); (ii) diabetic animals treated with water instead of camostate; and (iii) nondiabetic animals treated with camostate. Camostate (200 mg/kg) mixed with 0.5 ml of water or water alone was administered daily (3:00 PM) by oral gavage for 14 days.

Blood was drawn from the tail vein once a week during the treatment period, and blood glucose levels were determined using a glucose analyzer (Dri-Chem 2000; Fuji, Tokyo, Japan). At the end of the treatment period, animals were sacrificed by decapitation between 11:00 AM and 1:00 PM. Trunk blood was collected in chilled tubes containing 100 KIU of aprotinin (Bayer, Leverkusen, Germany) and 15 units of sodium heparin (Novo, Denmark)/ml of whole blood. Plasma was separated by centrifugation and stored at -20°C for subsequent radioimmunoassay for insulin. The pancreas was removed, weighed, and stored at -60°C until processed for biochemical measurements.

Homogenates of the pancreas were prepared in 5 vol of ice-cold distilled water using a Polytron homogenizer (Kinematica, Luzern, Switzerland). The protein content of the homogenate was determined according to the procedure of Bradford (11). DNA was determined by the Burton diphenylamine method, using calf thymus DNA as the standard (12). RNA was analyzed by the orcinol method, using yeast RNA as the standard (13). Insulin was extracted from the homogenate with 70% ethanol containing 0.15 *M* HCl (14), and assayed with an enzyme immunoassay kit (Sanyo, Kyoto, Japan). Plasma levels of insulin were determined with a radioimmunoassay kit (Incstar, Stillwater, MN) instead of the enzyme immunoassay, because plasma nonspecific interference hampered the enzyme immunoassay from detecting plasma insulin.

The results were expressed as the mean ± SE. Data were analyzed by a one-way classification analysis of variance; the separation between means was done by the least significant difference procedure. Differences were considered significant at a level of $P < 0.05$.

Results

The initial body weight of rats in all groups did not differ significantly from one another (Fig. 1A). While nondiabetic rats gained weight during the experimental period, diabetic rats lost body weight slightly. At the beginning of the treatment with camostate, nonfasting blood glucose levels in diabetic rats were much higher than those in nondiabetic rats, and remained elevated until the end of the treatment period (Fig. 1B). Camostate given to diabetic rats failed to reverse weight loss or to improve hyperglycemia (Fig. 1, A and B).

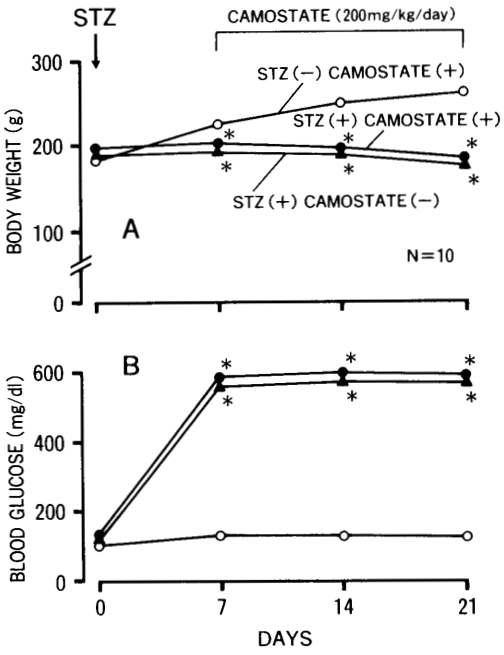

Figure 1. Changes in body weight (A) and nonfasting blood glucose levels (B) during the experimental period. Rats were divided into three treatment groups: (i) diabetic rats given camostate (●—●); (ii) diabetic rats given water instead of camostate (▲—▲); and (iii) nondiabetic rats given camostate (○—○). Streptozotocin (STZ) was injected intravenously to render rats diabetic, while nondiabetic rats received the vehicle alone. Camostate (200 mg/kg) mixed with water or water alone was given daily by oral gavage for 14 days beginning at 7 days after STZ injection. Data points are the mean values; SE does not exceed 4%. *$P < 0.05$ compared with the nondiabetic rats given camostate.

The pancreatic weight of diabetic rats given camostate was significantly elevated by 120% over that of diabetic rats not given camostate, being comparable to that in nondiabetic rats given camostate (Fig. 2A). When expressed as relative to body weight, pancreatic weight was significantly greater in diabetic rats given camostate than in nondiabetic rats given camostate (Fig. 2B). The total pancreatic contents of DNA, RNA, and protein showed the same pattern of change as pancreatic weight. These parameters in diabetic rats given camostate were significantly increased when compared with values in diabetic rats not given camostate, and did not differ from values observed in nondiabetic rats given camostate (Fig. 3). Because the DNA content, a measure of number of cells, in diabetic rats with camostate was significantly increased by 33% over that in diabetic rats without camostate (Fig. 3 and Table I), camostate was thought to produce moderate hyperplasia in diabetic rats. Table I shows the ratios of content

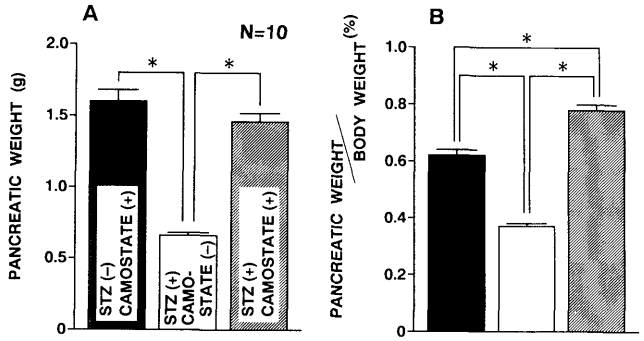

Figure 2. Effects of camostate on pancreatic weight. Streptozotocin (STZ)-induced diabetic rats were given camostate or water for 14 days. Nondiabetic rats were also given camostate for 14 days. Pancreatic weight is expressed as absolute value (A) or related to body weight (B). *$P < 0.05$.

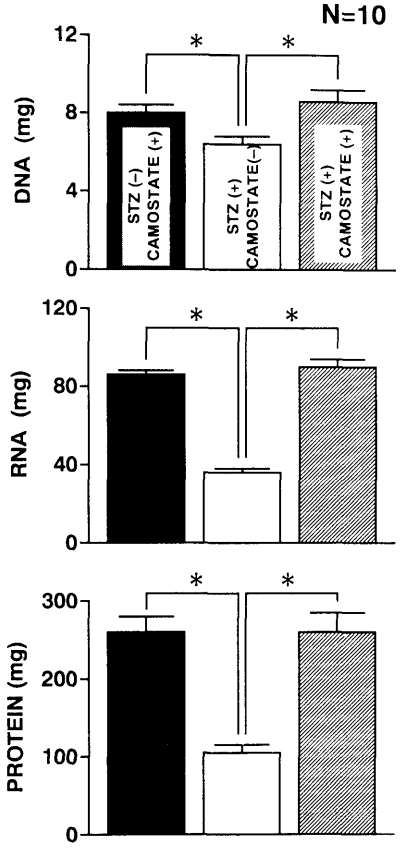

Figure 3. Effects of camostate on pancreatic contents of DNA, RNA, and protein. Streptozotocin (STZ)-induced diabetic rats were given camostate or water for 14 days. Nondiabetic rats were also given camostate for 14 days. *$P < 0.05$.

of other constituents (RNA, protein, and pancreatic weight) to content of DNA as indices of hypertrophy. For example, the ratio of RNA content to DNA content was significantly increased by 90% in diabetic rats given camostate compared with that in diabetic rats not given camostate, and did not differ between diabetic and nondiabetic rats, both of which had been treated with camostate. Thus, the treatment with camostate caused

Table I. Effects of Camostate on Indices of Pancreatic Hyperplasia and Hypertrophy[a]

	Nondiabetic rats	Diabetic rats	
	Camostate(+)	Camostate(−)	Camostate(+)
Index of hyperplasia			
DNA (mg)	8.01 ± 0.36[b]	6.56 ± 0.22	8.75 ± 0.42[b]
Index of hypertrophy			
RNA/DNA (mg/mg)	10.9 ± 0.5[b]	5.4 ± 0.2	10.3 ± 0.3[b]
Protein/DNA (mg/mg)	32.9 ± 2.9[b]	16.4 ± 0.8	30.1 ± 2.3[b]
Pancreatic wt/ DNA (mg/mg)	203 ± 14[b]	102 ± 4	168 ± 9[b,c]

[a] Data are expressed as mean ± SE of 10 rats in each group.
[b] $P < 0.05$ versus diabetic rats not given camostate.
[c] $P < 0.05$ versus nondiabetic rats given camostate.

Table II. Effects of Camostate on Plasma Levels and Pancreatic Content of Insulin[a]

	Nondiabetic rats	Diabetic rats	
	Camostate(+)	Camostate(−)	Camostate(+)
Insulin			
Plasma levels (μU/ml)	18.6 ± 4.2[b]	4.6 ± 0.2	4.5 ± 0.3
Content (mU/pancreas)	730 ± 33[b]	17 ± 6	12 ± 1

[a] Data are expressed as mean ± SE of 10 rats in each group.
[b] $P < 0.05$ versus diabetic rats.

pronounced hypertrophy of the pancreas in diabetic rats.

The plasma insulin levels in diabetic rats given camostate did not differ significantly from those in diabetic rats not given camostate, being one fourth of the levels in nondiabetic rats given camostate (Table II). Similarly, diabetic rats, irrespective of the treatment with camostate, showed significant reduction in pancreatic content of insulin compared with nondiabetic rats (Table II).

Discussion

The trophic effects of camostate on the normal pancreas are well documented (1–5). Peroral administration of camostate for 5 to 14 days at the same dose as used in the present study resulted in hyperplasia and hypertrophy of the normal rat pancreas (1–3). In the present study, camostate induced the growth of the pancreas in diabetic rats. The pattern of pancreatic growth (moderate hyperplasia and pronounced hypertrophy) in diabetic rats given camostate was similar to that reported in normal rats given this agent (1). Our data are consistent with a previous report (15) in which alloxan-induced diabetic rats treated with peroral administration of bovine lung trypsin inhibitor displayed an increased weight and protein content of the pancreas

compared with the untreated diabetic controls, although it was not clear in that report whether pancreatic growth was associated with hypertrophy or hyperplasia, or both.

The role of insulin in cell growth has been controversial. Insulin was once considered as a hepatotrophic factor acting on liver cells to cause liver regeneration (16); however, few reports have thereafter supported this concept. Rather, insulin is likely to play a permissive role for liver regeneration (17, 18). With regard to pancreatic growth, supraphysiologic doses of insulin increased DNA synthesis in pancreatic acinar cells *in vitro*, probably through the receptor for insulin-like growth factor (7). The present study clearly demonstrated that the endogenous insulin level has no influence on camostate-induced pancreatic growth, because the extent of pancreatic growth caused by camostate did not differ between diabetic rats and nondiabetic rats. Indeed, when expressed as relative to body weight, the pancreatic weight of diabetic rats given camostate was even greater than that of nondiabetic rats given camostate, indicating a preserved capacity of pancreatic growth in the face of poor nutritional condition.

We found no beneficial effects of camostate on the endocrine pancreas in diabetic rats in the present study. Camostate failed to improve hyperglycemia, hypoinsulinemia, or low pancreatic content of insulin seen in diabetic rats. By contrast, Ihse *et al.* (15) reported that chronic administration of bovine lung trypsin inhibitor to alloxan-induced diabetic rats resulted in an improvement of the diabetic condition of the animals. The difference in the extent of B cell destruction between the present study and that of Ihse *et al.* (15) may explain these conflicting findings. In their study, diabetic control rats showed a pancreatic insulin content of as much as 20% of that of normal control rats, and gained body weight, although much less than normal control rats did. On the other hand, in the present study, the pancreatic content of insulin in diabetic rats, which lost body weight slightly during the experimental period, was less than 5% of that seen in normal rats.

Chronic administration of camostate to normal rats exerted no effects on the release of insulin (19, 20). Our data on diabetic rats are in accordance with these results and put a clear contrast to our previous findings that chronic treatment of normal rats with exogenous CCK or oral cholestyramine, a bile salt sequestrant that stimulates the release of endogenous CCK, enhanced insulin release from the isolated perfused pancreas (9). Because camostate has a variety of properties besides stimulating CCK release (20, 21), some unknown factors related to treatment with camostate might interfere with the insulinotropic effects of circulating CCK on the endocrine pancreas. In addition, the present study does not exclude the possibility that camostate enhanced insulin release in diabetic rats transiently after its administration or during a short period after a meal.

In conclusion, the present study indicates a marked stimulation of the growth of the pancreas in diabetic rats by camostate, and suggests that camostate-induced pancreatic growth is not affected by the reduced level of the endogenous insulin. This study also shows that camostate has no beneficial effects on the function of residual B cells, failing to improve the disturbed function of the endocrine pancreas.

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