[Chem. Pharm. Bull. 36(3)1210—1213(1988)]

Effects of Streptozotocin and of Glucose Loading on Activities of Lysosomal Thiol Proteinases in Rat Pancreatic Tissue

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(Received August 14, 1987)

Changes in the activities of lysosomal thiol proteinases and their endogenous inhibitors were examined in the rat pancreatic tissue. Imposed stress such as fasting affected the activities of cathepsins B and H but not those of their endogenous inhibitors. However, changes in the activities of cathepsins B and H are closely related to changes in the activities of their inhibitors. During the phase of hypoglycemia and hyperinsulinemia induced by streptozotocin, there were increments in the activities of cathepsins B and H and their endogenous inhibitors. These changes were not related to increases in the levels of serum insulin but rather to pathological changes in the pancreas.

Keywords——cathepsin B; cathepsin H; thiol proteinase inhibitor; streptozotocin; glucose loading; serum immunoreactive insulin; pancreas

Lysosomal enzymes apparently play a decisive role in the pathogenesis of acute pancreatitis, based on experimental evidence that digestive enzyme activation during the early stage of acute pancreatitis (prior to the appearance of signs of cell injury of inflammation) may result from an admixture of zymogens with lysosomal hydrolases capable of activating digestive enzymes.[1] Among the lysosomal enzymes, thiol proteinases such as cathepsin B are regarded as important enzymes responsible for triggering activation of zymogens.[2] The localization of cathepsin B-related proteases in the insulin secretory granule has been noted and such proteases may be involved in the conversion of proinsulin to insulin.[3]

The author wished to examine whether or not alterations in the activities of lysosomal thiol proteinases such as cathepsins B and H may be related to functions of the pancreas. In this study, the effects of streptozotocin, a diabetogenic agent, and of glucose loading on the activities of lysosomal thiol proteinases and their endogenous inhibitors in pancreatic tissue were examined.

Experimental

Animals——Male Wistar rats, weighing 170—230 g, were used.

Enzyme Substrates——Carbobenzoxy-L-arginyl-L-arginine 4-methylcoumaryl-7-amide (Z-arg-arg-MCA), L-arginine 4-methylcoumaryl-7-amide (arg-MCA) and 7-amino-4-methylcoumarin (AMC) were obtained from the Peptide Institute, Inc., Osaka.

Streptozotocin Experiment——Rats were fasted for 16 h prior to the injection of streptozotocin (Wako Chemicals Industries, Osaka). After this injection, the animals to be killed after 2 and 10 h were left fasting, while others were allowed access to food up to 16 h prior to decapitation. Streptozotocin, 55 mg/kg body weight, was dissolved in acidified 0.9% saline (pH 4.5) at a concentration of 27.5 mg/ml, immediately before use and was injected into the caudal vein, following anesthetization of the rats with ether. Control animals, injected with acidified saline alone, were included in each experiment and killed at the same time intervals.

Glucose Loading Test——Rats were fasted for 16 h before the test. Glucose, 750 mg/kg body weight, was dissolved in 0.9% saline at a concentration of 375 mg/ml and the solution was injected into the caudal vein, following anesthetization with ether. Animals injected with 0.9% saline alone were used as zero time controls in the experiment. The animals were decapitated at 5, 15, 30, 60 and 120 min after glucose loading.

Serum Glucose Assay and Serum Immunoreactive Insulin Assay——Blood collected from the neck and serum was separated by centrifugation. Serum was kept at −20 °C until assay. Serum glucose was measured by the glucose oxidase method using a commercially available kit (Glucose C-test; Wako Chemical Industries, Osaka). Serum immunoreactive insulin (IRI) was assayed using a kit (Insulotec Mochida; Mochida Pharmaceutical Co., Tokyo). Human insulin was used as a standard.

Samples for the Assays of Cathepsins B and H and of Their Inhibitors——The excised pancreas (kept at −70 °C) was homogenized with 4 volumes of distilled water containing 0.1 mM ethylenediaminetetra acetic acid (EDTA). The homogenate was centrifuged at 12000 × *g* for 20 min at 4 °C, and the supernatant fraction was used as an enzyme sample. Extract for the cathepsin B or H inhibitor assay, based on the method of Lenney *et al.*,[4] was prepared from the supernatant fraction. The supernatant fraction was adjusted to pH 2.5, using HCl, and was heated at 85°C for 20 min. After cooling, the pH was adjusted to 5.4, and the fraction was centrifuged at 30000 × *g* for 20 min. The resulting extract was used for the inhibitor assay.

Assays of Cathepsins B and H and of Their Inhibitors——Cathepsins B and H activities were determined with Z-arg-arg-MCA and arg-MCA, respectively, according to Barrett.[5] One milliunit of the cathepsin activity was defined as the quantity releasing one nmol of AMC per min. Cathepsins B and H used for the inhibitor assay, were purified from rat liver, according to Lenney *et al.*[4] The inhibitor was assayed under the conditions used for assay of cathepsins B and H, replacing some of the buffer with inhibitor solution. One unit of inhibitor was defined as the amount decreasing the cathepsin activity by one unit.

Results and Discussion

Changes in the levels of serum glucose and serum IRI and in the activities of cathepsins B and H, and their inhibitors in pancreatic tissue in the streptozotocin experiment and in the

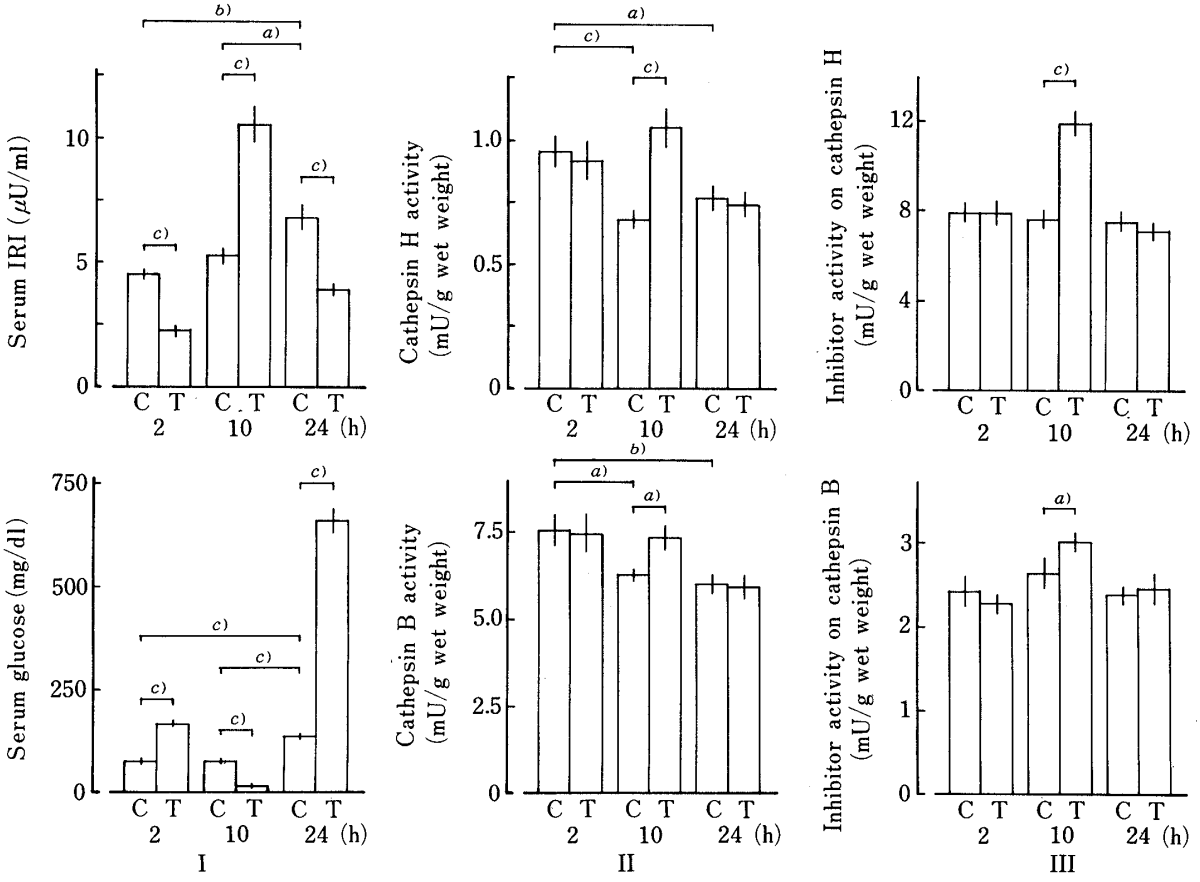

Fig. 1. Levels of Serum Glucose and Serum IRI and the Activities of Cathepsins B and H and Their Endogenous Inhibitors in Pancreatic Tissue

C, control group; T, streptozotocin (55 mg/kg, i.v.) treated group. The abscissa shows the time after injection of streptozotocin or acidified saline. Results show the mean from 10 rats ± S.E.M. The data were analyzed by using Student's *t*-test. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

glucose loading test are shown in Figs. 1 and 2, respectively.

In the streptozotocin experiment, the control group at 24 h had higher levels of serum glucose and serum IRI than those of the control group at 2 h or at 10 h (Fig. 1, I). These changes may be related to the conditions described in Experimental, *i.e.*, after injecting saline the former group was refed up to 16 h prior to death. Administration of streptozotocin resulted in a triphasic pattern of serum glucose fluctuation, as reported by Junod *et al.*[6]: an early hyperglycemia at 4 h, followed by severe hypoglycemia at 10 h, and then by marked hyperglycemia at 24 h. The serum IRI levels were in inverse relationship with the serum glucose levels (Fig. 1, I). Even in the marked hyperglycemia induced by streptozotocin at 24 h, the level of serum IRI was similar to that of the control group at 2 h. The same result was obtained by Junod *et al.*[6] and by Schein and Bates.[7] Thus, there may be an alteration of the biologic activity of the insulin, despite the retention of immunologic reactivity.[7]

In the streptozotocin experiments, pancreatic cathepsins B and H activities in the control group at 10 h or at 24 h were lower than those in the control group at 2 h (Fig. 1, II). The inhibitor activities in the control groups remained unchanged regardless of the difference in time (Fig. 1, III). These findings suggest that stress such as fasting or fasting-refeeding-fasting affects the activities of cathepsins B and H in the pancreatic tissue, but not those of their endogenous inhibitors.

After the injection of streptozotocin, higher activities of cathepsins B and H, and their inhibitors in the pancreatic tissue than those of the control groups were observed at 10 h, a time at which hyperinsulinemia was observed (Fig. 1, II, III). In the glucose loading test, no change was observed in the activities of cathepsins B and H and their inhibitors in the pancreatic tissue, regardless of changes in serum IRI levels (Fig. 2). These results suggest the absence of a relationship between the increase in the activities of cathepsins B and H and their inhibitors in the pancreatic tissue observed after streptozotocin injection and the incremental level of serum IRI. The following results confirmed this finding: in the streptozotocin experiment, the control group at 24 h had a higher level of serum IRI and a lower activity of cathepsins B and H than did the control group at 2 h (Fig. 1, I, II). It has been reported that hyperinsulinemia observed after streptozotocin treatment may result from the destruction of β-cells induced by the action of streptozotocin on β-cells.[8] The increase in the activities of

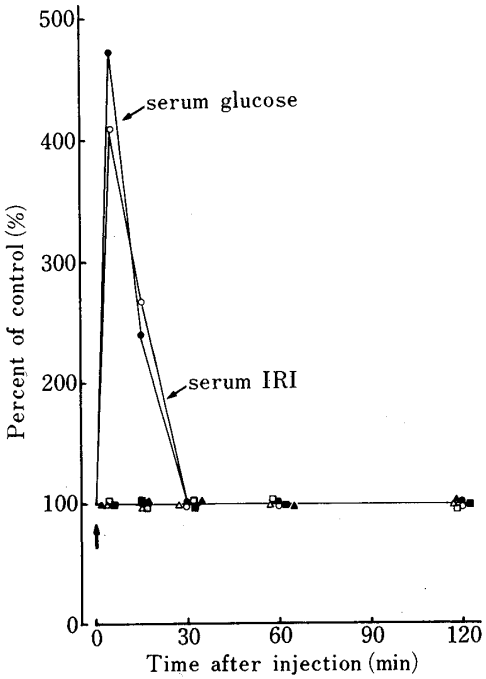

Fig. 2. Changes in the Levels of Serum Glucose and Serum IRI and in the Activities of Cathepsins B and H and Their Endogenous Inhibitors in Pancreatic Tissue after Glucose Loading

Glucose (750 mg/kg) was loaded intravenously, and the animals were killed at various times. Animals injected with 0.9% saline alone were used as zero time controls. The levels of serum glucose and serum IRI and the activities of cathepsins B and H and their endogenous inhibitors in pancreatic tissue at various times after glucose loading were divided by the corresponding zero time values, and are shown on the ordinate as percent of control. The results are the mean values from 4 rats. I_B, Inhibitor(s) of cathepsin B; I_H, inhibitor(s) of cathepsin H.
□—□, cathepsin B; △—△, cathepsin H; ■—■, I_B; ▲—▲, I_H.

cathepsins B and H and their inhibitors at 10 h after streptozotocin treatment may be related to the early stage of β-cell destruction but not with the degree of the destruction, since an increase in the activities as compared with those of the control group was not observed at 24 h, a time when the β-cells are irreversibly damaged.[6)]

When increments in the activities of cathepsins B and H appeared, increases in the activities of their inhibitors were also observed. When cathepsins B and H showed the same activities as in the control groups, the activities of their inhibitors were the same as those of the control groups. These findings suggest a close relationship between the activities of cathepsins B and H and those of their endogenous inhibitors, in pancreatic tissue.

The exact relationship between the incremental changes in the activities of cathepsins B and H and their inhibitors in the pancreatic tissue and the destruction of β-cells induced by streptozotocin requires further study.

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