($\varepsilon=3.5\times10^4$) is higher than that of the reaction products in the other enzymic methods.

In the proposed method, we added sodium azide to the substrate mixture to inhibit catalase present in biological materials. The concentrations of hypoxanthine and xanthine are very low in serum (1—2 μg/ml serum) and these compounds seem to have no effect on the proposed method in view of the good recovery.

Therefore it was concluded that the proposed method is precise and convenient.

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Calcitonin increases Serum Glucose Concentration independently of Insulin Secretion in Rats

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The effect of calcitonin (CT) on serum glucose and insulin secretion was investigated in rats. The subcutaneous administration of CT (80 MRC mU/100 g body weight) produced a significant increase in serum glucose, while it did not significantly alter serum insulin in fed rats. In addition, a marked elevation of insulin secretion after a single intraperitoneal administration of glucose (0.1 g/100 g) in fasted rats was not significantly altered by the treatment with CT. When both CT and somatostatin (250 μg/100 g) were subcutaneously administered simultaneously, the serum glucose level increased significantly compared with that of rats given somatostatin alone. The progressive increase in serum glucose caused by CT was significantly inhibited by the subcutaneous administration of insulin (0.1 U/100 g). These results indicate that CT increases serum glucose concentration independently of insulin secretion in rats.

Keywords——calcitonin; insulin; somatostatin; serum glucose; hyperglycemic effect of calcitonin

It is reported that calcitonin (CT) inhibits glucose uptake stimulated by insulin in the diaphragm muscle of rats, and that this effect of CT is not mediated by hypocalcemia.[1,2] Ziegler *et al.*[3] found that CT provoked a significant impairment of glucose assimilation and insulin output in man. Also, Passeri *et al.*[4] reported that the stimulation of insulin secretion by an intravenous glucose load in man was inhibited by intravenous infusion of CT. On the other hand, it is reported that CT impair glucose tolerance, failing to produce any significant

inhibition of insulin release following the glucose stimulus in man.[5] Thus, the effect of CT on insulin secretion has not been resolved fully.

Recently, it was found that CT increases serum glucose in fed and fasted rats.[6,7] The present study was therefore undertaken to clarify whether CT increases serum glucose independently of insulin secretion. This report describes the effect of CT on serum glucose and insulin secretion in rats.

Methods

Male Wistar rats, weighing approximately 100—120 g, were used in this experiment. They were obtained commercially (Nippon Bio. Supp. Center Co., Ltd., Tokyo). The animals were fed on laboratory chow containing 57.4% carbohydrate, 1.1% Ca and 1.1% P (Oriental Test Diet Co., Ltd., Tokyo) and tap water freely.

Calcitonin (lyophilized porcine calcitonin, 68 MRC U/mg protein, Armour Pharmaceutical Company, Kankakee, Ill., U.S.A.), somatostatin (Peptide Institute, Protein Research Foundation, Osaka, Japan), and insulin (crystalline bovine, 24.3 I.U./mg; Sigma Chemical Company, St. Louis, U.S.A.) were each dissolved in cold distilled water.

Calcitonin (CT, 80 MRC mU/100 g body weight) and somatostain (250 μg/100 g), or CT (80 MRC mU/100 g) and insulin (0.1 U/100 g) were subcutaneously administered simultaneously. The rats were killed at various times after administration. The control rats received distilled water.

Glucose was dissolved in distilled water. This solution (0.1 g/100 g) was intraperitoneally administered to rats fasted for 17 hr. CT (80 MRC mU/100 g) was subcutaneously administered immediately after a glucose load. The rats were bled at various times after CT administration.

Blood was obtained by cardiac puncture; 30 min later, it was centrifuged and the serum was separated immediately. The amount of glucose in the serum was determined with the Glytel *o*-toluidine reagent.[8] The serum insulin level was assayed by a double antibody radioimmunoassay method using the RI KIT (Dainabot RI Institute).[9]

The data were subjected to an analysis of variance, and the standard error (SE) was calculated from the residual error term. Statistical significance is expressed as p values from Student's t-test.

Results

The effects of calcitonin (CT) on serum glucose concentration and serum insulin level in rats are shown in Fig. 1. The administration of CT (80 MRC mU/100 g) produced a progressive increase in serum glucose, while it did not significantly alter serum insulin.

The effect of CT on insulin secretion after a single intraperitoneal administration of glucose in fasted rats is shown in Fig. 2. The serum glucose was markedly elevated by the administration of glucose (0.1 g/100 g). This increase was not significantly influenced by the administration of CT (80 MRC mU/100 g). Meanwhile, the serum insulin level was markedly increased by a glucose load, and then decreased rapidly. Thus, insulin secretion was not significantly altered by the administration of CT.

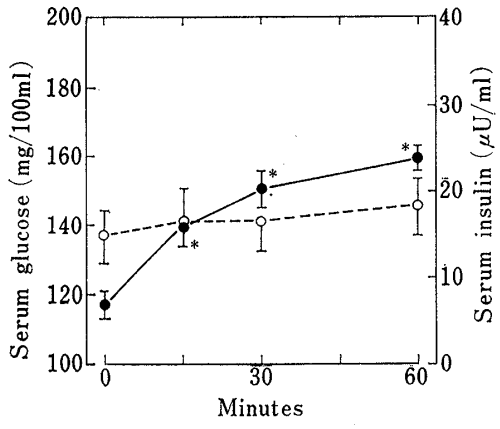

Fig. 1. Effects of Calcitonin on the Serum Glucose and Insulin Levels in Rats

Rats received a single subcutaneous administration of calcitonin (80 MRC mU/100 g). Each point represents the mean of 5 animals. Vertical lines represent the SE. *; $p<0.01$ as compared with control. —●—; glucose, --○--; insulin.

When both CT (80 MRC mU/100 g) and somatostatin (250 μg/100 g) were administered simultaneously, the serum glucose level significantly increased in comparison with that of somatostatin-treated rats (Fig. 3). The administration of somatostatin alone caused a significant elevation of serum glucose compared with that of nontreated rats.

The effect of insulin on the increase in serum glucose after CT administration is shown in Fig. 4. The administration of CT (80 MRC mU/100 g) caused a progressive increase in

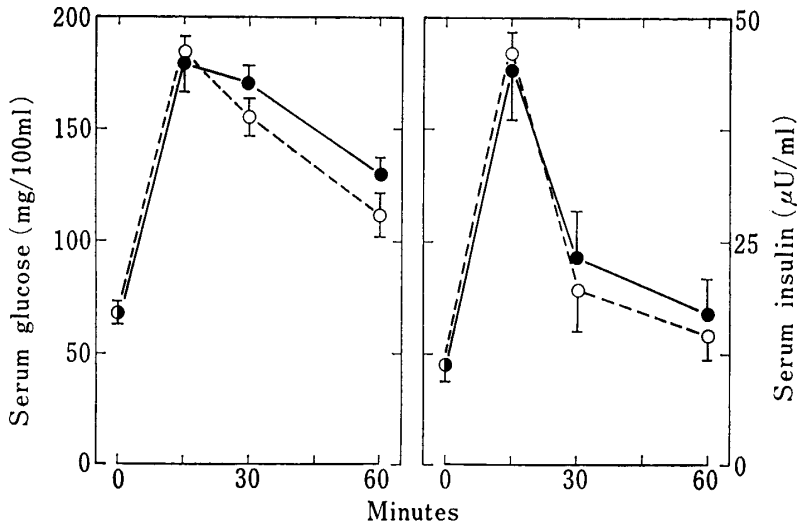

Fig. 2. Effect of Calcitonin on the Increases in Serum Glucose and Insulin after a Glucose Load in Fasted Rats

A single subcutaneous administration of calcitonin (80 MRC mU/100 g) was administered immediately after a single intraperitoneal injection of glucose (0.1 g/100 g) to rats fasted for 17 hr. Each point represents the mean of 5 animals. Vertical lines represent the SE. --○--; control, —●—; calcitonin.

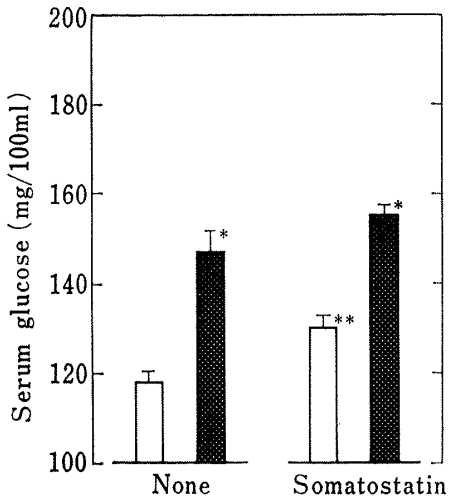

Fig. 3. Effect of Calcitonin on the Serum Glucose Concentration in Rats treated with Somatostatin

Rats received single subcutaneous administrations of calcitonin (80 MRC mU/100 g) and somatostatin (250 μg/100 g), and 30 min later they were bled. Each bar represents the mean of 5 animals. Vertical lines represent the SE. *; $p<0.01$ as compared with the control, **; $p<0.05$ as compared with "none" control. □; control, ▩; calcitonin.

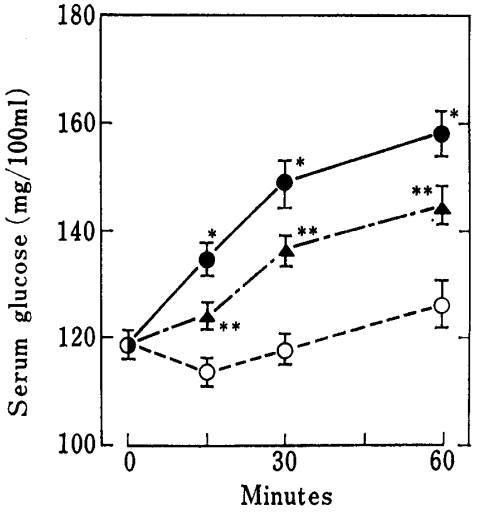

Fig. 4. Effects of Calcitonin and Insulin on the Serum Glucose Concentration in Rats

Rats received single subcutaneous administrations of calcitonin (80 MRC mU/100 g) and insulin (0.1 U/100 g). Each point represents the mean of 5 animals. Vertical lines represent the SE. *; $p<0.01$ as compared with control, **; $p<0.01$ as compared with calcitonin. --○--; insulin, —●—; calcitonin, —▲—; insulin and calcitonin.

serum glucose. This increase was significantly prevented by the administration of insulin (0.1 U/100 g) with CT (80 MRC mU/100 g). However, the serum glucose level observed after the administration of both CT and insulin was significantly higher than that of insulin-treated rats. Thus, CT increased serum glucose level in the presence of exogenous insulin and did not inhibit the lowering effect of insulin on serum glucose.

Discussion

Recently we have found that CT increases serum glucose concentration in fed and fasted rats.[6,7] This effect of CT does not result from epinephrine release from the adrenal glands in rats.[10] In the present work, it was examined whether CT could increase serum glucose independently of insulin secretion.

The administration of CT caused a significant increase in serum glucose, while it did not significantly alter serum insulin. Also, CT had no significant effect on serum glucose and insulin levels increased by a glucose load in fasted rats. These results suggest that CT does not inhibit insulin secretion in rats. In rats treated with somatostatin, furthermore, CT produced a significant increase of serum glucose. Since somatostatin inhibits glucagon and insulin releases in intact rats,[11] this result suggests that the hyperglycemic effect of CT is not related to insulin and glucagon releases.

On the other hand, the increase in serum glucose caused by CT administration was significantly inhibited by insulin treatment, indicating that CT does not prevent the action of insulin to decrease serum glucose in rats. This suggests that the hyperglycemic effect of CT does not result from an inhibition of insulin action on serum glucose in rats.

Thus, in the present investigation, it was demonstrated that CT increases serum glucose independently of insulin secretion in rats.

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The Primary Structure of Toxin C from the Venom of the Indian Cobra (*Naja naja*)

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The primary structure of toxin C, a neurotoxin isolated from Indian cobra (*Naja naja*) venom, was determined. Toxin C is a highly toxic polypeptide consisting of 71 amino acid