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GLUCAGON LEVELS IN STZ DIABETIC RATS AFTER INJECTIONS OF SOMATOSTATIN ANTISERA

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INTRODUCTION

It is well known that somatostatin is a potent inhibitor of the secretion of GH and TSH and of the pancreatic hormones insulin and glucagon.[1,2,3] Although increases of plasma GH and TSH were found after injections of somatostatin antiserum into the circulation systems of normal rats, dogs and baboons, increases of plasma pancreatic hormones, insulin and glucagon levels were not observed in these mammals,[4,5,6] in spite of the finding that the addition of antisomatostatin antiserum to isolated Langerhans' islets stimulated glucagon and insulin release.[7] These studies, therefore, suggested that circulating somatostatin influences the release of GH and TSH via the circulation while it is not involved in mediating the inhibition of pancreatic hormones insulin and glucagon. Thus, it has been confirmed that the neutralization of circulating somatostatin did not have any influence on the release of pancreatic hormones in the normal mammals. However, it remains unclear whether or not the neutralization of circulating somatostatin with somatostatin antiserum influences the secretion of pancreatic hormones insulin and glucagon in a diabetic state.

To answer this question, the present study was designed to determine the effects of neutralizing the circulating somatostatin with somatostatin antiserum on the levels of plasma insulin, glucagon and glucose in streptozotocin (STZ) injected diabetic rats.

MATERIALS AND METHODS

Preparation of somatostatin antiserum: Five milligrams of synthetic somatostatin 15-28 (Protein Research Foundation, Osaka Japan) and 5 mg of BSA was dissolved into

2 ml of 0.1 M phosphate buffer pH 8.0. To this mixture, 0.1 ml of 1% glutaraldehyde was added and allowed to react at room temperature for 2 hrs and dialysed against saline. The solution containing somatostatin-BSA and BSA was emulsified with 2 ml of complete Freund's adjuvant (Difco Laboratories) and injected into five rabbits. Booster injections were given every 30 days after the initial primary injection. The antisera used in the present study were obtained on the 10th day after the third booster. Before use, antisera were incubated with BSA at 4℃ for 2 days to remove any contamination of BSA antisera in rabbit serum. Then, the antisera were purified to obtain rabbit globulin by using DEAE-cellulose column chromatography in which 0.01 M phosphate buffer pH 8.0 was used as the elution buffer.

Characterization of antisera: The titers of antisomatostatin rabbit globulin from five rabbits were determined with radioimmunoassay (RIA) for somatostatin using dextran-charcoal. Labelled N-tyrosyl-somatostatin was purchased from Otsuka Laboratory (Japan). The titers of five antisera were 1: 500, 1: 700, 1: 50000, 1: 64000, 1: 125000. The crossreactivity of the last three antisera to the other peptides including pancreatic polypeptide (PP), glucagon, vasoactive intestinal peptide (VIP), insulin, neurotensin etc were also checked. As shown in Fig. 1, negligible crossreactivities of three antisera to the other peptides were found. Antisera No. 2 were used as infusion materials and No. 3 were used as antisera in RIA. The affinity constant of No. 2 antisera is 1.37×10^{-10} liters/mol, maximum binding sites are 4.95×10^{-6}mol/liters, and 1 ml antiserum may bind 8.32 μg somatostatin.

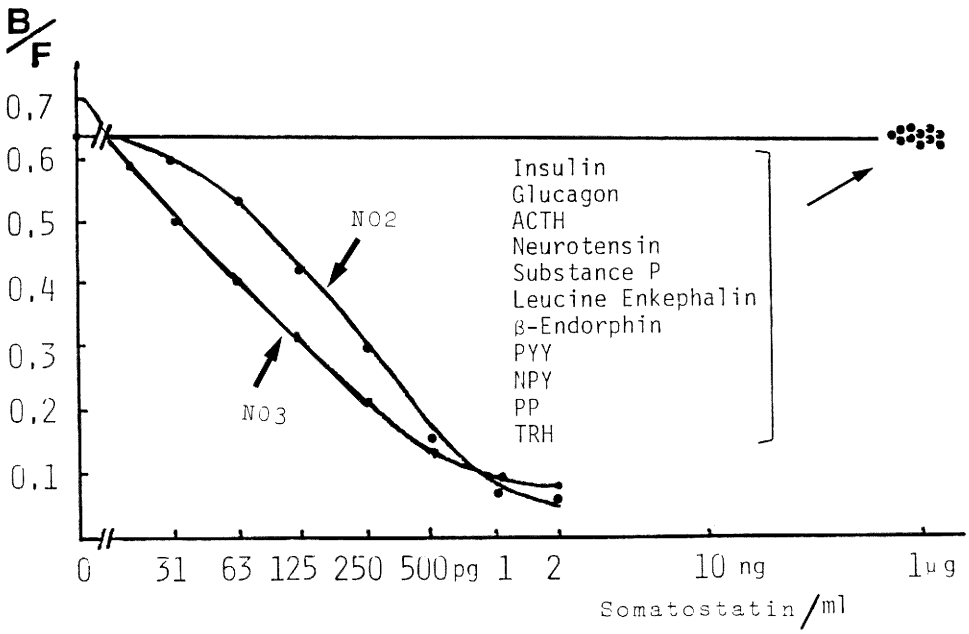


Fig. 1. Typical standard curves of somatostatin using antisera No. 2 and No. 3. These two kinds of antisera did not have any crossreactivities to other peptides examined.

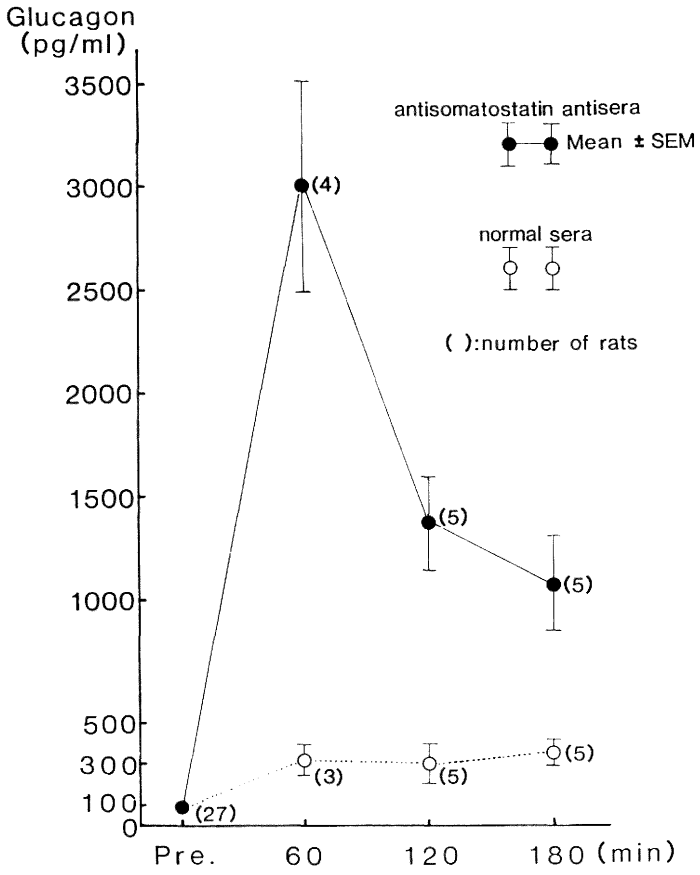
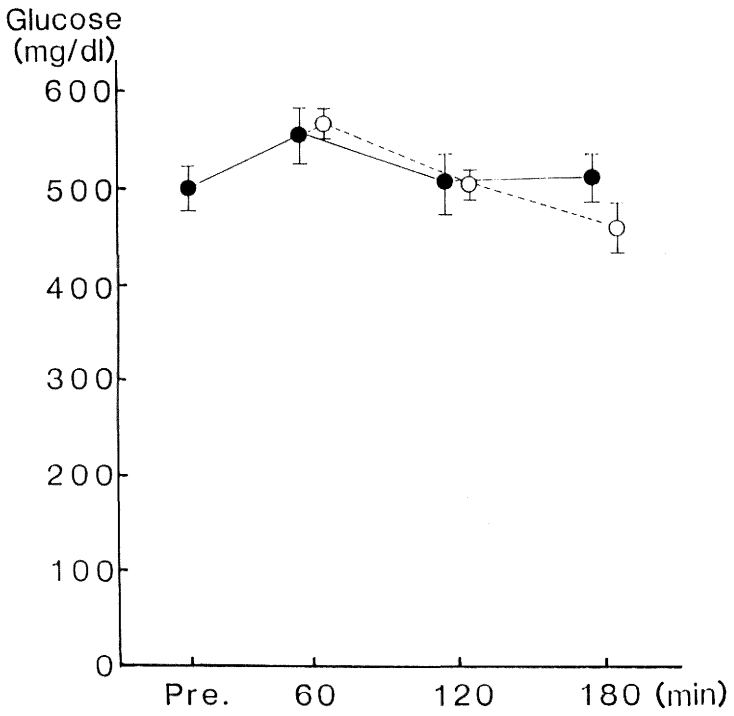


Fig. 2. Changes of plasma glucagon levels after administration of antisomatostatin rabbit globulin (●——●) and of normal rabbit globulin (○·····○). Remarkable increases of plasma glucagon levels were found after adiministration of antisomatostatin rabbit globulin.

Measurement of plasma insulin, glucagon and glucose: Plasma glucagon levels were measured by a glucagon-RIA kit using specific antisera to C-terminal pancreatic glucagon.[8] Plasma insulin levels were determined by an insulin RIA kit purchased from Shionogi Co Ltd. Plasma glucose was measured by a Beckman autoanalyzer. The protein contents of antisomatostatin rabbit globulin and normal rabbit globulin were measured by the Lowry method.

Experiment: Streptozotocin 60 mg/kg was injected into the tail vein of Wistar male rats aged 6 weeks to induce diabetes mellitus 40 days before the experiment. These STZ diabetic rats were divided into two groups (Group 1 and 2), each group consisting of fifteen rats. Antisomatostatin rabbit globulin (7.5 mg/ml) was injected into group 1 and normal rabbit globulin (7.5 mg/ml) was administered to group 2. Each group was further divided into three subgroups each consisting of five rats (Subgroup A, B, C, D, E and F). By cardiac puncture, 1 ml of blood was taken from each rat under nembutal anesthesia (50 mg/kg) and placed into chilled tubes which contained 1000 units of aprotinin and 2.4

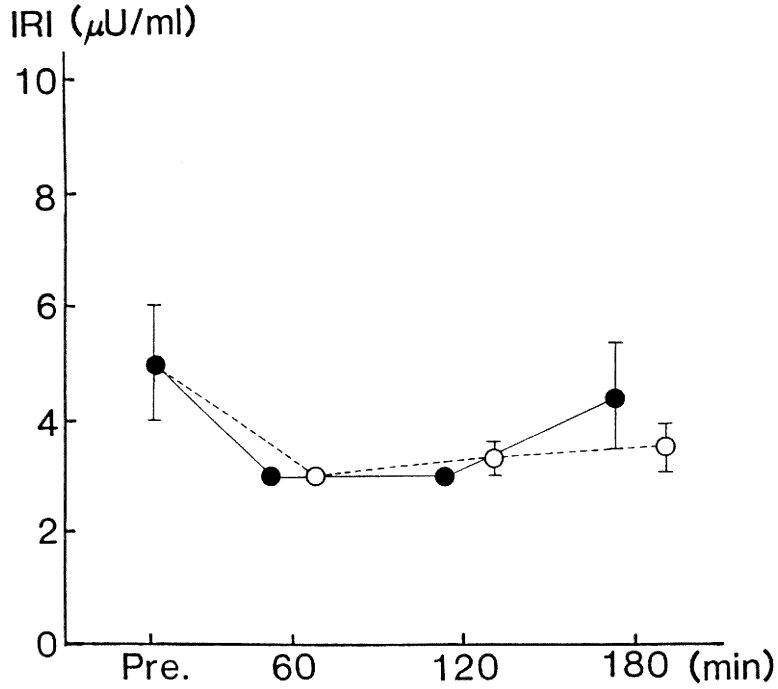


mg of EDTA-2Na. Then, 1 ml of antisomatostatin rabbit globulin or normal rabbit globulin was injected into the circulation from the tail vein of both groups. Two milliliters of blood were obtained by cardiac puncture at 60 min (Subgroup A and D), at 120 min (Subgroup B and E), at 180 min (Subgroup C and F) after the injection of antisomatostatin rabbit globulin or normal rabbit globulin. Blood was centrifuged at 4℃ for 30 min at 3000 rpm and plasma was stocked at −25℃ until assay. The analysis of date from the pancreatic hormones and glucose measured was carried out by the student T-test.

Antisomatostatin antibody titers in plasma of STZ diabetic rats after administration of antisomatostatin and normal rabbit globulin: Antibody titers in plasma of STZ diabetic rats obtained at 60, 120 and 180 min after injections of antisomatostatin rabbit globulin and normal globulin were checked by using labelled N-tyrosyl somatostatin. Plasma samples were diluted from 1: 50 to 1: 5000 in 0.2 M glycine-NaOH buffer pH 8.4 containing 0.25% BSA. Binding of these diluted samples to labelled somatostatin was determined using tubes containing 500 units of aprotinin.

RESULTS

As shown in Fig. 2. plasma levels of glucagon increased from 86±5 pg/ml to more than 1000 pg/ml at all examination times after the administration of antisomatostatin



Figs. 3 and 4. Changes of plasma insulin and glucose after administration of antisomatostatin rabbit globulin (●——●) and normal rabbit globulin (○·····○). No significant changes of plasma insulin and glucose were found in the both cases.

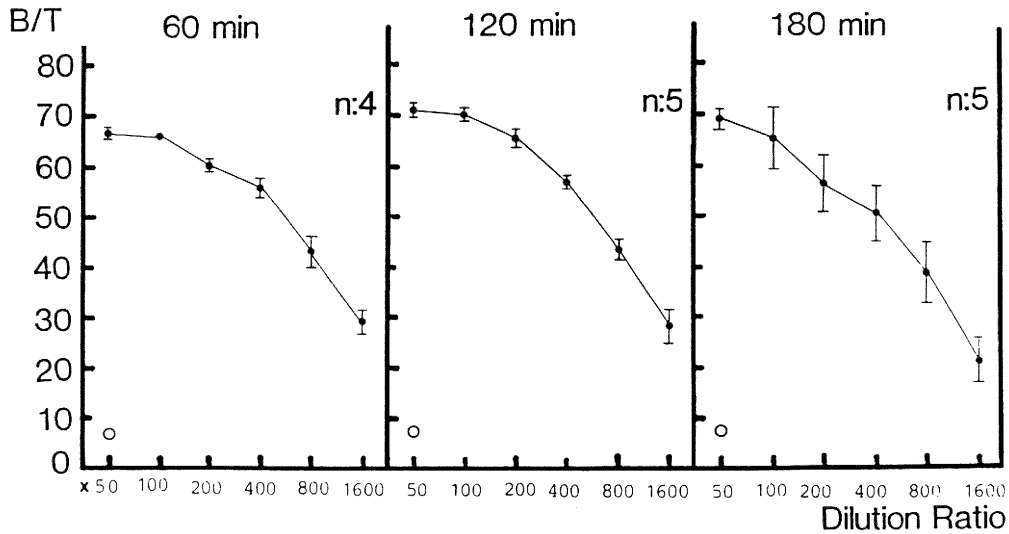


Fig. 5. Titers of antisomatostatin antisera in rat's plasma obtained at 60. 120 and 180 min after administration of antisomatostatin rabbit globulin and of normal rabbit administration of somatostatin antisera were 1: 600 (initial dilution) at 60 and 120 min and 1: 400 at 180 min respectively (● ——●) while those after adiministration of normal rabbit globulin were negligible (○).

rabbit globulin, with the highest peak glucagon of 4000 pg/ml at 60 min. Although slight increases of plasma glucagon levels were also found after the injection of normal rabbit globulin, the increases were clearly smaller than those obtained after the injection of antisomatostatin rabbit globulin. Plasma insulin and glucose levels did not change during this experiment, as seen in Figs. 3 and 4. From this result, it was considered that the most effective time at which circulating somatostatin was neutralized with antisomatostatin rabbit globulin was 60 min among the course examined. Titers of somatostatin antisera which were calculated as 50% binding of labelled somatostatin were 1: 600 (initial dilution) in rat plasma obtained at 60 and 120 min and 1: 400 at 180 min, as indicated by Fig. 5.

Discussion

The present study showed that the administration of antisomatostatin rabbit globulin into the circulation system did induce a remarkable increase of basal plasma glucagon levels in STZ diabetic rats, although it did not cause any changes in plasma insulin and glucose levels. In previous reports, the injection of antisomatostatin antiserum into the circulation did not induce any changes in pancreatic hormones, insulin and glucagon, though it induced the secretion of pituitary hormones TSH and GH.[5,6] Thus, it was suggested that the effects of the administration of antisomatostatin antisera on the secretion of pancreatic hormones may differ between normal and diabetic mammals and that somatostatin may play an important role in the regulation of glucagon release in diabetes mellitus.

It is necessary to consider the reason for the different effects of the adiministration of antisomatostatin antisera on the secretion of glucagon between normal and diabetic mammals. As to the non-effects of administration of antisomatostatin antisera on the secretion of pancreatic hormones in normal mammals, Schusdziarra et al.[6] suggested that endogenous somatostatin influences the secretion of growth hormone (GH) and glucagon-like immunoreactivity (GLI) via a pathway readily accessible to intravenously injected antibodies, probably via the circulation, whereas pancreatic somatostatin may influence the secretion of insulin and glucagon via a pathway inaccessible to circulating antibodies, i. e., within the islets. Thus, they thought that somatostatin in the pancreas exerts a "paracrine" or local effect via the intercellular spaces. It is well known that the diabetogenic action of streptozotocin is due to its marked B-cytotoxic activity. Therefore, injection of streptozotocin destroys pancreatic B-cells[9] and this effect may lead to the loss of the intercellular connections of endocrine cells in the islets. As a result, somatostatin secreted from the pancreas of STZ diabetic rats may enter a circulation in which somatostatin is neutralized with antisomatostatin antisera. Thus, anatomical changes may induce different effects, after the injection of antisomatostatin antisera, on glucagon release between normal and diabetic mammals.

In spite of the remarkable increases of plasma glucagon levels, plasma glucose did

not change during the experiment. Although no exact explanation for this finding is offered, there seems to be two possibilities. One is that the remarkable increase of plasma glucagon may be due to the increase of GLI which does not induce the release of plasma glucose. However this seems unlikely, since the antiglucagon antisera used in the present study were prepared by using C-terminal fragment of glucagon and the antisera against C-terminal part of glucagon were found to have negligible crossreactivity to GLI.[8] Another possibility is that effects of glucagon in STZ diabetic rats possessing extremely increased plasma glucose levels may reach almost the maximum points, so that increases of plasma glucagon levels do not induce any changes in plasma glucose. In addition to these possiblities, unknown factors including anesthesia may also be present.

In contrast to the remarkable increase of plasma glucagon levels, plasma levels of insulin did not change during the experiment. This finding implies that the function of the pancreatic B-cells was almost completely destroyed by the administration of streptozotocin rather than that pancreatic somatostatin has no pathophysiological effects on insulin release in diabetes mellitus. Considering that pancreatic B-cells were absent in IDDM, at least in the late stage of IDDM, the present findings suggest that the main effects of pancreatic somatostatin in IDDM may be inhibitory effects on glucagon release rather than on insulin release.

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