Islet Amyloid Polypeptide Response to Glucose, Insulin, and Somatostatin Analogue Administration

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We determined islet amyloid polypeptide (IAPP) response in plasma to oral and intravenous glucose administration and intravenous insulin injection in nondiabetic subjects. Moreover, we studied the effect of somatostatin analogue SMS 201-995 on glucose-induced IAPP secretion in nondiabetic subjects. Plasma IAPP concentration was determined by radioimmunoassay. Oral administration of 75 g glucose (n = 8) significantly increased plasma IAPP levels from 4.5 ± 0.7 to 14.0 ± 1.7 pM ($P < 0.01$) 60 min after administration. Intravenous administration of 10 g glucose (n = 7) also caused a significant increase in plasma IAPP from 5.0 ± 0.4 to 11.6 ± 0.9 pM ($P < 0.01$) 5 min after injection. Plasma IAPP significantly decreased from 5.1 ± 0.4 to 2.9 ± 0.4 pM ($P < 0.01$) 60 min after intravenous insulin injection (n = 8). Pretreatment with SMS 201-995 completely abolished IAPP and insulin secretion to intravenous glucose injection. A significant correlation was found between plasma IAPP and insulin levels in oral and intravenous glucose administration and between plasma IAPP and C-peptide levels during insulin-induced hypoglycemia. These results suggest that IAPP is cosecreted with insulin in response to a glucose load and secretion of IAPP is inhibited by hypoglycemia and somatostatin. IAPP may serve as a novel pancreatic hormone to control carbohydrate metabolism. *Diabetes* 39:639–42, 1990

Islet amyloid polypeptide (IAPP; 1), also called amylin (2), is a 37–amino acid peptide that was isolated from pancreatic islet amyloid of non-insulin-dependent (type II) diabetic and insulinoma patients. Its amino acid sequence is 46% identical to human calcitonin gene–related peptide. IAPP is colocalized with insulin in human β-cell secretory granules (3). IAPP was shown to suppress insulin secretion from the islets of rats (4) and to be a potent inhibitor of both basal and insulin-stimulated glycogen synthesis in rat muscle (5). These findings suggest that IAPP may be a hormone related to β-cell function. We developed a sensitive and specific radioimmunoassay (RIA) for human IAPP and showed that IAPP is present in the pancreas, with a small amount of IAPP immunoreactivity detected in plasma and the upper intestinal tract (6).

To elucidate the role of IAPP in the control of carbohydrate metabolism, we determined plasma IAPP response to oral and intravenous glucose administration in nondiabetic subjects by RIA and analyzed the correlation between plasma IAPP and insulin. IAPP response to intravenous insulin injection and the effect of the somatostatin analogue SMS 201-995 on glucose-induced IAPP secretion were also studied.

RESEARCH DESIGN AND METHODS

Twenty-nine healthy volunteers with no personal or family history of diabetes mellitus who were within 10% of ideal body weight gave informed consent to participate in this study. They were subjected to one of four experiments. To study oral glucose administration, eight subjects (4 men, 4 women, aged 24–37 yr) were given 75 g glucose orally. Blood samples were collected before and 15, 30, 60, 120, and 180 min after glucose administration. For intravenous glucose injection, seven subjects (5 men, 2 women, aged 24–31 yr) were given 10 g glucose intravenously for 2 min. Blood samples were collected 30 min before and 0, 3, 5, 10, 15, 30, and 60 min after the start of glucose injection. To study the effect of intravenous insulin, eight subjects (all men, aged 23–26 yr) were given an intravenous bolus injection of insulin (0.1 U/kg). Blood samples were collected before and 15, 30, 45, 60, and 90 min after insulin injection. To determine the effect of SMS 201-995 on plasma IAPP response to intravenous glucose injection, six subjects (all men, aged 26–33 yr) were given 10 g of glucose intravenously for 2 min with pretreatment of subcutaneous injection of 50 μg SMS 201-995 (Sandoz, Basel) 30 min before glu-

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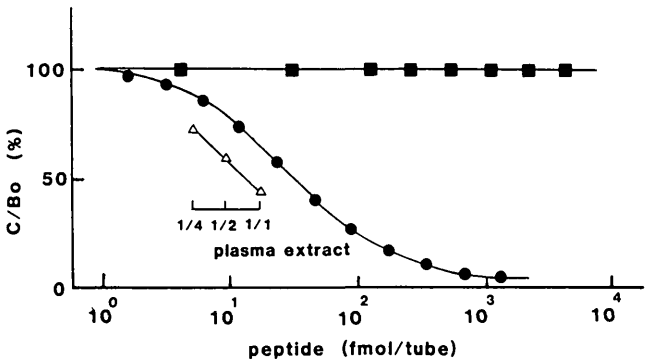

FIG. 1. Standard radioimmunoassay curve for human islet amyloid polypeptide (IAPP) and cross-reactivity of antiserum. Inhibition of radiolabeled human IAPP binding by serial dilutions of human IAPP(1–37) (●), human plasma extracts (△), and human calcitonin gene–related peptide, insulin, pancreatic polypeptide, glucagon, and somatostatin (■). C/B_0, count/bound at 0 pg of standard peptide.

cose injection. Blood samples were collected 30 min before and 0, 3, 5, 10, 15, 30, and 60 min after the start of glucose injection.

All experiments were performed after an overnight fast and at least 30 min of bed rest. Blood was drawn into chilled tubes containing EDTA-2Na (1 mg/ml) and Trasylol (500 U/ml). Plasma glucose was measured by the glucose oxidase method. Plasma insulin was determined by RIA (7), and C-peptide was determined by RIA with a commercially available kit (Shionogi, Tokyo).

Plasma IAPP was measured by an RIA developed in our laboratory (6). In brief, IAPP antiserum raised in rabbits against human IAPP(24–37) (corresponding to the subsequence 24–37 of human IAPP) coupled with bovine thyroglobulin, specifically recognized human IAPP and showed no cross-reactivity with human CGRP, insulin, pancreatic polypeptide, glucagon, or somatostatin (Fig. 1). The antiserum did not cross-react with up to 50 μg insulin. Human IAPP(24–37) was radioiodinated by the chloramine-T method, and ^{125}I-labeled peptide was purified by reverse-phase high-performance liquid chromatography. Human IAPP(1–37) was used as standard. Inter- and intra-assay variations were <13 and <8%, respectively. The limit of detection of this assay was 2 fmol/tube of human IAPP(1–37).

IAPP was extracted from 5 ml of plasma by adsorption onto a Sep-Pac C-18 cartridge, which was preequilibrated with 0.9% saline. The cartridge was washed first with saline and then 0.1% trifluoroacetic acid (TFA) solution, and IAPP was eluted with 60% CH_3CN solution containing 0.1% TFA. The eluate was evaporated, reconstituted with RIA buffer, and submitted to RIA. A serial dilution curve of a plasma sample before RIA was parallel to a standard curve for human IAPP (Fig. 1). Recovery of IAPP(1–37) added to the plasma was 82.7 ± 4.0% (n = 6).

Results are expressed as means ± SE. Statistical analysis was done with Student's t test. Correlation coefficients were calculated by linear regression analysis.

RESULTS

Figure 2 shows changes in plasma glucose, insulin, and IAPP concentrations after oral glucose administration. The basal level of IAPP was 4.5 ± 0.7 pM. Plasma IAPP level progressively increased after the glucose load, reaching a peak value of 14.0 ± 1.7 pM 60 min after the glucose load then gradually declining. Plasma IAPP level at 180 min remained elevated and was significantly higher ($P < 0.01$) than basal level. Normal responses of plasma insulin and glucose were observed. The basal level of insulin and a peak level at 30 min were 59.4 ± 12.0 and 303.0 ± 43.8 pM, respectively. A significant positive correlation was observed between plasma levels of IAPP and insulin (r = 0.496, $P < 0.01$) and between IAPP and glucose (r = 0.544, $P < 0.01$).

After the 10-g glucose bolus injection, plasma IAPP level rose rapidly from a basal level of 5.0 ± 0.4 pM, reaching a peak value of 11.6 ± 0.9 pM 5 min after injection. Plasma IAPP level gradually declined; however, at 60 min, it remained significantly higher ($P < 0.05$) than the basal level (Fig. 3). Plasma insulin rose sharply from 73.8 ± 10.8 pM to a peak of 508.2 ± 72.6 pM at 3 min and then quickly declined. The plasma glucose curve showed a profile similar to that of insulin. Plasma IAPP level showed highly significant correlations to plasma levels of insulin (r = 0.769, $P < 0.01$) and glucose (r = 0.615, $P < 0.01$).

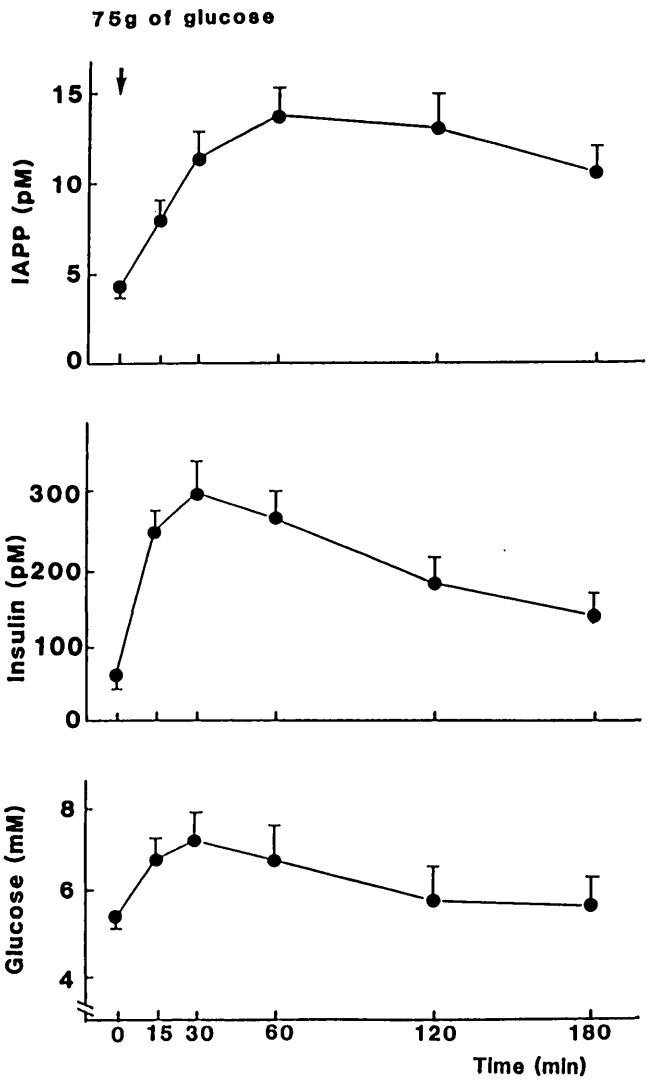

FIG. 2. Plasma islet amyloid polypeptide (IAPP), insulin, and glucose responses to oral administration of 75 g glucose in 8 nondiabetic subjects.

Plasma glucose substantially decreased after insulin injection, reaching a nadir of 2.3 ± 0.1 mM at 30 min. After insulin injection, plasma IAPP levels gradually decreased from the basal level of 5.1 ± 0.4 pM to a nadir of 2.9 ± 0.4 pM 60 min after injection (Fig. 4). Plasma IAPP levels at all points after insulin injection were significantly lower ($P < 0.01$) than the basal level. Plasma C-peptide levels reached a nadir of 0.16 ± 0.01 nM below the basal level of 0.38 ± 0.04 nM 45 min after injection. Significant correlation was observed between plasma IAPP and C-peptide levels ($r = 0.533$, $P < 0.01$), whereas no significant correlation was observed between plasma IAPP and glucose levels ($r = 0.197$).

SMS 201-995 significantly suppressed basal plasma IAPP levels from 5.6 ± 0.7 pM at −30 min to 3.8 ± 0.4 pM at 0 min ($P < 0.01$) and completely abolished plasma IAPP response to intravenous glucose injection despite a rise of plasma glucose similar to that observed in response to glucose alone (Fig. 3). Plasma insulin levels were also suppressed and failed to rise in response to intravenous glucose injection.

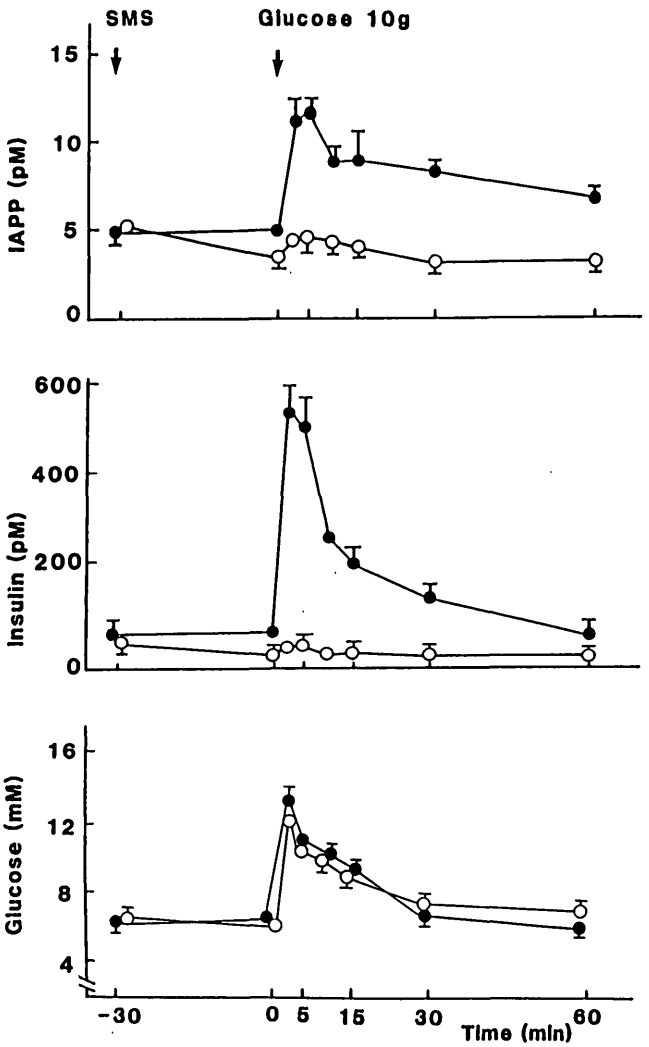

FIG. 3. Plasma islet amyloid polypeptide (IAPP), insulin, and glucose responses to intravenous glucose bolus injection (●) and intravenous glucose injection with pretreatment by somatostatin analogue SMS 201-995 (○).

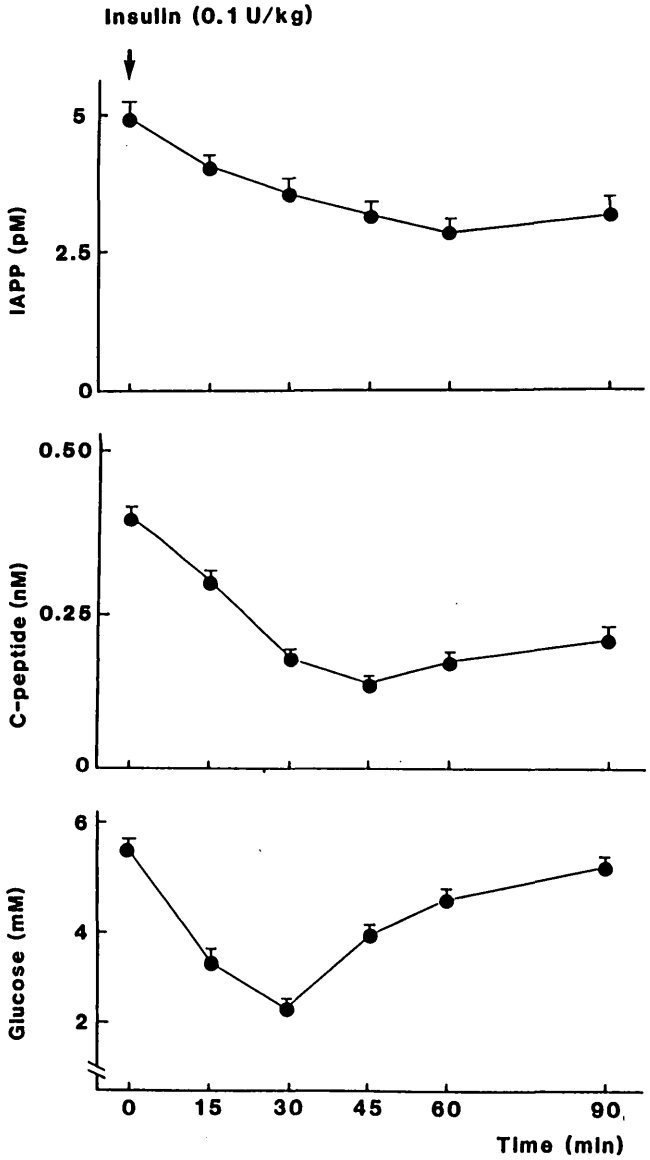

FIG. 4. Plasma islet amyloid polypeptide (IAPP), C-peptide, and glucose responses to intravenous insulin bolus injection in 8 nondiabetic subjects.

DISCUSSION

IAPP was first identified in insoluble islet amyloid in type II diabetes and insulinoma (1,2). We isolated IAPP from nondiabetic human pancreas, determined its amino acid sequence, and showed that IAPP is a normal constituent of human pancreas (unpublished observations). IAPP was demonstrated to be colocalized with insulin in β-cell secretory granules in nondiabetic individuals by immunologic and electron-microscopic studies (3). To prove that IAPP is a pancreatic hormone secreted into the bloodstream in response to physiological stimulation, we determined plasma IAPP response to glucose load with an RIA specific for human IAPP. This study showed that plasma IAPP level increased by 3.1- and 2.3-fold of the basal level in response

to oral and intravenous administration of glucose, respectively. On the other hand, plasma IAPP level decreased to 58% of the basal level in response to insulin-stimulated hypoglycemia. Both plasma IAPP and insulin responses to glucose administration were completely abolished by pretreatment with SMS 201-995. These results indicate the possibility that IAPP also serves as a pancreatic hormone functioning in the control of glucose metabolism. The plasma IAPP level was well correlated to that of endogenous insulin, suggesting that IAPP may be cosecreted with insulin in response to glucose load and that secretion of IAPP and insulin may be simultaneously inhibited by both hypoglycemia and somatostatin.

Amyloid deposits in the islets were observed in >90% of type II diabetic patients (8). A semiquantitative immunohistochemical study indicated that β-cell IAPP immunoreactivity and islet amyloid deposits were increased in cats with impaired glucose tolerance compared with nondiabetic cats (9). An increase in β-cell IAPP immunoreactivity might result from impaired catabolism of IAPP in β-cells and/or increased production of IAPP preceding development of type II diabetes, which may be associated with amyloid deposition in the islets of type II diabetic individuals. IAPP has been reported to inhibit both basal and insulin-stimulated rates of glycogen synthesis in rat soleus muscle in vitro (5). β-Cell dysfunction related to amyloid deposition and action of IAPP opposite to insulin might play an important role in development of type II diabetes. Determination of plasma IAPP response to glucose in nondiabetic subjects and type II diabetic patients promises to help elucidate the pathophysiological significance of IAPP.

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