Human Pancreatic Polypeptide Secretion in Conditions of Exogenous and Endogenous Hyperglycaemia

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Summary. The effects of exogenous and endogenous hyperglycaemia on human pancreatic polypeptide secretion have been studied. In normal subjects elevation of plasma glucose concentration by glucose infusion both depressed the basal levels of circulating human pancreatic polypeptide (by 40–50%) and consistently reduced the human pancreatic polypeptide response to the ingestion of a protein-rich meal (areas above pre-meal value: 19.5 ± 4.1 (mean $\pm$ SEM) vs. 9.6 ± 2.1 , $p < 0.01$) as well as to caerulein infusion (areas above pre-caerulein value: 8.8 ± 2.2 vs. 4.6 ± 1.4 , $p < 0.01$). In diabetic subjects treated with sulphonylureas or diet (fasting plasma glucose: 166 ± 11 mg/dl, $n = 24$), human pancreatic polypeptide secretion evoked by food was similar to that of 24 healthy individuals (areas above basal value: 46.6 ± 9.9 and 33.6 ± 3.6 , respectively). In insulin dependent diabetics (fasting plasma glucose: 231 ± 19 mg/dl, $n = 21$) the human pancreatic polypeptide response to the meal (area above basal value: 78.2 ± 13.7) was significantly greater than that of the controls as well as that of the noninsulin-dependent group ($p < 0.05$). Since the administration of pancreatic polypeptide to man has been shown to decrease pancreatic exocrine output, postprandial human pancreatic polypeptide hypersecretion may contribute to the decreased exocrine function of the pancreas often found in insulin-dependent diabetics.

Key words: Human pancreatic polypeptide, glucose, protein meal, caerulein, normal subjects, noninsulin-dependent diabetics, insulin-dependent diabetics.

In the course of insulin purification from chicken pancreas, Kimmel and associates found a new polypeptide consisting of 36 amino acid residues [1, 2].

They have termed this molecule avian pancreatic polypeptide (aPP). Subsequently, an immunologically related substance was demonstrated in pancreatic extracts of several other species [3]. Chance et al., independently, isolated and characterized a homologous peptide from extracts of human, porcine, ovine and bovine pancrea [4]. Larsson et al. [5], have identified the storage site of pancreatic polypeptide in islet cells distinct from A, B and D cells.

In the chick, administration of aPP provokes depletion of liver glycogen and a reduction of plasma glycerol [6], but pancreatic polypeptide has not been shown to display metabolic effects in mammals [7, 8]. Floyd et al. [9] have described an elevation of basal pancreatic polypeptide plasma levels in patients with diabetes mellitus. Furthermore, in normal subjects circulating human pancreatic polypeptide (hPP) concentrations are inversely affected by experimental fluctuations in glycaemia [9, 10] thus suggesting an effect on hPP secretion by glucose.

The present work was designed to evaluate hPP secretion in conditions of exogenous and endogenous hyperglycaemia.

Materials and Methods

Subjects and Procedures

The effect of exogenous hyperglycaemia on hPP secretion was examined in healthy, non-obese volunteers (8 males and 4 females; age range 19 to 25 years, mean 21.3). Glucose was administered IV as a bolus (0.33 g/kg body weight) followed by a constant infusion (0.6 or 0.9 g/min as 10 g/100 ml or 15 g/100 ml solutions, respectively) for 60 or 90 min. In control experiments, an identical volume of 0.154 mol/l saline was given.

To provoke hPP secretion two different stimuli were employed: 1) A protein-rich meal (70 g smoked pork loin, one boiled egg, and 15 g white bread) consumed in 10 min; 2) Caerulein (Montedison Farmaceutica, 50 ng/kg body weight) dissolved in 10 ml of 0.154 mol/l saline and infused IV over a 10-minute

Fig. 1. Effect of IV glucose on the hPP response to a protein-rich meal in 12 normal subjects (mean $\pm$ SEM). The solid and broken lines correspond to the control and glucose experiments, respectively. The hPP values from 5 to 30 min as well as the integrated hPP response (area above pre-meal value) are significantly lower in the glucose than in the control experiments

period. These stimuli were applied 20 or 30 min after initiating glucose administration. Each subject served as his own control and the order of the glucose and saline tests was randomised.

In a second series of experiments the hPP response to a protein-rich meal was investigated in normal and diabetic subjects. In these tests the standard breakfast comprised 70 g smoked pork loin and 15 g white bread. The control group consisted of 24 healthy individuals (13 males and 11 females, age range 24 to 59 years, mean 42), whose body weight ranged -10% to 11% (mean 2%) of the ideal according to the Metropolitan Life Insurance Company tables. None were taking any medication. Two groups of diabetics were studied. The first group consisted of 21 insulin-dependent patients (13 males and 8 females, age range 16 to 60 years, mean 36) in whom the disease had been diagnosed from 0.2 to 11 years previously (mean 5). Their body weight fell between -10% and 7% of the ideal (mean -1%). Daily insulin requirements ranged from 10 to 64 U (mean 32).

All patients selected for this study lacked circulating PP antibodies, as determined by previous screening. The presence of PP antibodies was investigated by examining the binding of radiolabelled PP to plasma samples in the absence of rabbit anti-PP serum. In our assay, the nonspecific binding of the solutions containing standard hPP has ranged from 4 to 9%. Those diabetics whose plasma bound a greater percentage of radiolabelled PP than that corresponding to the hPP standard samples, were not included in these experiments.

Fig. 2. Effect of IV glucose on the hPP response to IV caerulein in 11 normal subjects (mean $\pm$ SEM). The solid and broken lines correspond to the control and glucose experiments, respectively. The hPP values from 10 to 20 min as well as the integrated hPP response (area above pre-caerulein value) are significantly lower in the glucose than in the control experiments

The second group of diabetics consisted of 24 non-insulin-dependent subjects (12 males and 12 females, age range 37 to 66 years, mean 50, $p = 0.013$ vs. controls and $p = 0.002$ vs. insulin-dependent diabetics) in whom diabetes was diagnosed from 0.1 to 16 years previously (mean 5). Their body weight fell between -9% and 10% of the ideal (mean -1%). Eighteen patients were being treated with diet and sulphonylurea therapy and the remaining six were being controlled by diet alone. The diabetics of both groups suffered from no other apparent illness at the time of the study, and no antecedent pancreatitis had been recorded. None were taking drugs other than insulin or sulphonylurea. The proportion of males to females between the groups of control subjects, insulin-dependent patients and noninsulin-dependent diabetics was not significantly different, as analysed by the chi-square test. The mean ages of males and females were similar within each group. Informed consent was obtained.

Volunteers reported to the laboratory at 0900 h after an overnight fast. Blood specimens (7 ml) were drawn from an antecubital vein, placed in chilled tubes containing EDTA (final dilution 2.9 mM) and promptly centrifuged at 4°C . The plasma was stored at -20°C until the time of assay not more than four weeks later.

Analytical Methods

All samples were tested in duplicate. Plasma glucose was determined by means of a commercial glucose-oxidase preparation (Biochemica Test Combination, Boehringer Mannheim). Plasma hPP was assayed radioimmunologically [10] employing rabbit anti-hPP serum (lot # 615-1054B-248-19), hPP (lot #615-1054B-200) as standard, and bovine pancreatic polypeptide (lot #615-D63-188-9) for iodination. All of these substances were kindly donated

by Dr. Ronald E. Chance (Lilly Research Laboratories, Eli Lilly Co., Indianapolis, U. S. A.). In our assay, the minimal detectable concentration with 95% of confidence is 17 pg/ml. The slope of the logit dose response curve is -2.1 ± 0.16 (SD) and the intercept when B/T is $1/2$ of $(B/T)_0$ corresponds to 21 ± 2.6 (SD) pg in the incubation tube. The within-assay and between-assay coefficients of variation are 8.8% and 16.5%, respectively.

Data are presented as the mean $\pm$ SEM. Statistical significance of differences between plasma glucose values was calculated by Student's *t* test. The Wilcoxon rank sum test for paired observations and the Mann Whitney U test for unpaired observations were employed to calculate statistical significance of differences between plasma hPP levels.

Results

Effect of Intravenous Glucose on the hPP Response to a Protein-rich Meal in Normal Subjects (Fig. 1)

In the control experiments, mean fasting hPP level (average of three determinations) was 118 ± 19 pg/ml. The infusion of saline for 30 min had no effect. Immediately after the ingestion of the meal, an elevation of circulating hPP was found (435 ± 90 pg/ml at 40 min, $p < 0.01$) which persisted for the remainder of the experimental period, with values ranging from 608 ± 126 pg/ml ($p < 0.01$, at 60 min) to 427 ± 50 pg/ml ($p < 0.01$, at 160 min).

When glucose was infused, basal plasma hPP (127 ± 15 pg/ml) declined progressively (100 ± 15 pg/ml at 5 min, $p < 0.01$; 73 ± 9 pg/ml at 30 min, $p < 0.01$). The subsequent hPP rise observed after consumption of the meal (maximal value: 323 ± 64 pg/ml, at 40 min) was diminished with respect to the control series. The differences between the mean increments above the pre-meal value (range: 157–299 pg/ml) were statistically significant from 50 to 90 min. Furthermore, the hPP response, as expressed by the area above the pre-meal value (between 30 and 90 min), was smaller in the glucose (9.6 ± 2.1) than in the saline experiments (19.5 ± 4.1 , $p < 0.01$). Upon cessation of the glucose infusion, plasma hPP concentrations rose to levels markedly higher than those provoked by the meal. This rise coincided with a fall in plasma glucose to sub-baseline values.

Effect of Intravenous Glucose on the hPP Response to Caerulein in Normal Subjects (Fig. 2)

In the saline experiments, caerulein infusion (50 ng/kg body weight) elicited a clear rise in plasma hPP levels (basal value: 94 ± 15 pg/ml; peak: 590 ± 135 , $p < 0.01$). Intravenous administration of glucose depressed fasting hPP concentrations (basal value: 108 ± 22 pg/ml; 20-min value: 53 ± 12 pg/ml, $p < 0.02$)

Fig. 3. hPP response to a protein-rich meal in 24 control subjects (solid line), 24 non-insulin-dependent diabetics (broken line) and 21 insulin-dependent diabetics (dotted line) (mean $\pm$ SEM). The integrated hPP response (area above basal value) of the insulin-dependent group is significantly greater than both that of the control and non-insulin-dependent groups

and inhibited the hPP elevation evoked by caerulein (peak: 308 ± 80 pg/ml, $p < 0.01$). The differences between the mean increments above the pre-caerulein value were statistically significant from 30 to 45 min (range: 145–225 pg/ml). Moreover, during glucose administration, the hPP response, as calculated by the incremental area above the pre-caerulein value (between 20 and 60 min), was smaller than that of the control series (4.6 ± 1.4 vs. 8.8 ± 2.3 , $p < 0.01$).

hPP Response to a Protein-rich Meal in Normal and Diabetic Subjects (Fig. 3)

In normal subjects, the fasting plasma hPP level was 111 ± 13 pg/ml. Ingestion of the meal was followed by an elevation of circulating hPP which persisted throughout the experimental period (area above basal value: 33.6 ± 3.6). In the noninsulin-dependent diabetics (basal plasma glucose 166 ± 11 mg/dl) fasting hPP concentration (121 ± 24 pg/ml) as well as the hPP response to the meal (area above basal

value: 46.6 ± 9.9) were not statistically different from those of the controls. In the insulin-dependent patients (basal plasma glucose 231 ± 19 mg/dl, $p < 0.01$ vs. noninsulin-dependent diabetics), the fasting hPP level was 193 ± 47 pg/ml ($p = 0.4$ vs. controls) and the elevation of plasma hPP evoked by the meal (area above basal value: 78.2 ± 13.7) was greater than that of the control group ($p < 0.01$) as well as that of the noninsulin-dependent diabetics ($p < 0.05$).

Discussion

Our data confirm the depressor effect of hyperglycaemia, as induced by IV glucose administration, on basal plasma levels of hPP in normal subjects. They also show that under this circumstance, the hPP response to food ingestion as well as to the infusion of caerulein appears markedly reduced. It is of note that Adrian et al. [11], using the isolated canine pancreas, found no influence of glucose on pancreatic polypeptide release, thus suggesting that glucose does not directly affect the secretory activity of the PP-cell. Since cholinergic blockage inhibits PP output [12, 13, 14], it is conceivable that acute hyperglycaemia reduces hPP secretion through a vagally mediated mechanism. A similar conjecture has been suggested by MacGregor et al. [15] to explain the suppressant effect of rising blood sugar levels on gastrin and gastric acid secretion.

In contrast, the insulin-dependent diabetics, who were markedly hyperglycaemic, responded to the protein-rich meal with a marked hPP hypersecretion. On the other hand, in the non-insulin-dependent diabetics the hPP response to the meal was not different from that of the normal subjects and significantly smaller than that of the insulin-treated patients. These data are in agreement with the report of Floyd et al. [9], who found higher fasting hPP values in diabetics receiving insulin than in both control subjects and in those patients treated with diet or sulphonylureas. In our series of insulin-dependent diabetics the mean basal hPP level was higher than that of the healthy individuals and also that of the noninsulin-dependent patients. However, these differences were not statistically significant.

At present, it seems difficult to interpret the hypersecretion of hPP in insulin-dependent diabetics. It is known that basal hPP levels increase with age [9, 10, 16]. However, in our study the mean age of the insulin-treated patients was similar to that of the controls and significantly less than that of the noninsulin-dependent group. The non-insulin-dependent diabetics were significantly older than the normal

individuals. This may have accounted for their slightly greater, though not statistically significant, hPP response as compared to the controls. It has been shown that in healthy obese subjects there is a reduction of basal hPP concentrations as well as a diminished hPP response to a meal [17, 18]. In our series, none of the subjects were obese and the mean body weight of the control and diabetic individuals was similar. Track et al. [16] have reported that male subjects have higher fasting hPP concentrations than females. The proportion of males to females in the three groups studies was similar. Floyd et al. [9] have discussed the tendency of plasma hPP levels to rise with the clinical severity of diabetes, as determined by the degree of fasting hyperglycaemia. In our patients, there was no correlation between basal glycaemia and the integrated response to the meal. On the other hand, Gepts and De Mey [19] have presented histological evidence of an increased population of PP-cells in the pancreas of juvenile diabetics. According to them, the PP-cell hyperplasia represents atypical regeneration of the damaged islet and would be more prominent in longstanding cases of the disease. There was no correlation, however, in our series, between the duration of diabetes and the magnitude of the hPP secretion evoked by the meal.

It is uncertain whether elevated hPP plasma levels in insulin-dependent diabetics have any pathological significance. However, the administration of bovine pancreatic polypeptide to man [20] and dogs [7] has been shown to reduce pancreatic exocrine output. Thus, postprandial hPP hypersecretion could contribute to the decreased exocrine function of the pancreas, often found in these patients [21].

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