Impaired pancreatic polypeptide response to a meal in type 1 diabetic patients: vagal neuropathy or islet cell dysfunction?

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The pancreatic polypeptide (PP) response to a mixed meal was investigated in seven insulin-dependent diabetics without measurable signs of diabetic autonomic neuropathy, and in seven healthy subjects. Since acute changes in metabolic regulation might influence the meal-induced PP response, the insulin-dependent diabetic patients were studied during normo- and hyperglycemic experimental conditions at blood glucose levels of 5 and 15 mmol/l, respectively. The PP response was identical on the two occasions, the response being significantly smaller than in the healthy subjects. Thus, PP response is independent of short-term changes in metabolic control. Since the response was attenuated in the insulin-dependent diabetic patients, who had no otherwise measurable signs of neuropathy, the PP response to a meal could be a sensitive indicator of dysfunction of the reflex arc controlling PP secretion in insulin-dependent diabetic patients. Alternatively, the reduction in PP secretion in these patients reflects dysfunction of the PP secreting cells of the pancreas. IV injection of cholecystokinin-8 elicited a small but significant increase in PP concentrations, while IV secretin did not increase PP concentrations at all in healthy subjects. These stimuli are therefore less suitable in the assessment of vagal neuropathy.

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Autonomic neuropathy is a more frequent complication of diabetes mellitus than previously thought (1, 2). Generally, cardiovascular function tests are used in the diagnosis of autonomic neuropathy. There are certain limitations in the diagnostic potential of these tests however (3). Other diagnostic measures are therefore warranted. In insulin-dependent diabetic patients, both the meal-induced increase in pancreatic polypeptide (PP) concentrations (4–9) and the PP response to insulin-induced hypoglycaemia (10–12) are blunted. The decrease in PP response to both stimuli has been considered as being due to vagal neuropathy in diabetic patients, since atropine and truncal vagotomy prevent these responses in healthy subjects (13). However, since it has been shown that hyperglycaemia (14, 15) as well as hyperlipemia (16) decrease PP secretion, metabolic dysregulation might possibly per se influence the PP response to a meal. Indeed, the diabetic metabolic control has not always been optimal in previous studies (4–9). We have therefore studied the effect of acute changes in metabolic control on the PP response to a mixed meal in insulin-dependent diabetic patients and in healthy subjects. Furthermore, since it is well known that IV injection of several peptides elicits an increase in plasma concentrations of PP (13, 17–19), another aim of the study was to compare the magnitude of PP responses with IV injection of various peptides (secretin, cholecystokinin (CCK)-8 and insulin) with meal-induced PP increments in healthy man.

Subjects and methods

Subjects

Meal ingestion experiment. Seven non-obese Type 1 insulin-dependent diabetic patients and seven healthy subjects volunteered for the study after giving informed consent (Table 1). The patients had no signs of diabetic autonomic neuropathy (normal beat-to-beat variation in heart rate (20) and normal thresholds for sense of vibration (21)). They had no clinical signs or symptoms of other long-term diabetic complications.

IV injection of secretin, and CCK-8. Sixteen healthy subjects of both sexes volunteered for these experiments after giving informed consent. Age was 29 ± 5 years (mean ± SEM).

IV injection of insulin. Two newly diagnosed insulin-dependent diabetic patients (aged 35 and 38, respectively) were studied. The diagnosis was established within the week prior to the study. Both patients were non-ketotic during the experiment.

No patients or healthy subjects had signs or symptoms of other endocrine, metabolic or cardiovascular diseases,
nor did they take any drugs apart from insulin. The study was approved by the local Ethics Committee.

Protocols

Meal ingestion experiments. The insulin-dependent diabetic patients were admitted to the metabolic ward on two occasions. The patients arrived in the metabolic ward at 22.00 the night before the experiments. A cannula was inserted into a cubital vein in both arms, and an overnight iv infusion of soluble insulin (Insulin Actrapid Human, Novo Industry) dissolved in isotonic saline (500 IU/l) was started. The infusion rate was adjusted according to frequent blood glucose measurements. Blood glucose concentrations were kept between 4 and 7 mmol/l throughout the night on one occasion and on the other occasion between 15 and 19 mmol/l by administering isotonic glucose. The healthy subjects met in the laboratory at 08.00 after an overnight fast and at least 12 h abstinence from tobacco and alcohol. The PP response to a standard mixed meal was measured at 08.00 in all subjects. The meal consisted of egg, bread, butter, ham, cheese, jam, juice and coffee or tea, and contained 23 g of protein, 17 g of fat, and 50 g of carbohydrate, with a total of 2100 KJ. The meal was ingested in 20 min. Blood samples for determining PP and glucose were drawn 15 min before the start of the meal, at the start of the meal, and every 5 min during the next hour.

Iv injection of secretin and Ceruletide. a. Iv secretin. Eight healthy subjects met fasting in the laboratory on two occasions with an interval of at least two weeks at 08.00. They had a cannula inserted into a cubital vein, and were placed in the supine position on a couch. After 15 min rest, 1 mg secretin (Secretolin Diagnostik, Hoechst, Germany) dissolved in 10 ml isotonic saline was injected slowly iv over a 2 min period on the first occasion.

On the second occasion, the injection of secretin was accompanied by an iv injection of 1 mg atropine. On both occasions blood samples were drawn before the injections and every 10 min for 60 min after the injection.

b. Iv (CCK)-8. Eight healthy subjects met fasting in the laboratory on two occasions with an interval of at least two weeks at 08.00. The experimental procedures were identical to the above with the exception that 1 mg synthetic CCK-8 (Cambridge Research Biochemicals, Cambridge, UK) was injected iv instead of secretin.

c. Iv insulin. Soluble insulin (Insulin Actrapid Human, Novo Industry) was injected iv (0.15 IE iv/kg body weight). Blood samples were drawn according to protocol a and b.

Analytical procedures

Blood samples were cooled immediately and centrifuged. Plasma was kept at −20°C until analysis. Blood glucose was determined spectrophotometrically with the hexokinase method. Plasma concentrations of PP were determined with a previously described radioimmunoassay (22).

Statistical analysis

The data are expressed as mean ± SEM. The Friedmann test was used to determine variance within groups in normo- and hyperglycemic experimental conditions. The Wilcoxon signed-rank test and Mann-Whitney’s rank- sum and test were used for comparisons within and between groups. The level of statistical significance chosen was p less than 0.05.

Results

Blood glucose response to a standard mixed meal was significantly lower in the healthy subjects than in the diabetic groups (Fig. 1). The meal-induced PP response in the diabetic group (calculated as the net incremental area under the individual curves) was identical in normo- and hyperglycemic experimental conditions (Fig. 1). In the healthy subjects the meal-induced increases in PP concentrations were significantly greater (p < 0.05) than in the diabetics (Fig. 1). After iv administration of secretin no significant increase were found in PP concentrations (p > 0.15) in healthy subjects (Fig. 2). We found a significant (p < 0.05) increase in PP concentrations after CCK-8 iv injection (Fig. 2), the increase being smaller than the meal-induced increase in PP concentrations (p < 0.00001) in healthy subjects (Fig. 1). There were no significant differences in the PP responses to secretin or CCK-8 with or without atropine suppression.

Iv insulin injection lowering blood glucose in the two diabetic patients from 8.8 mmol/l to 5.3 and from 11.6 to 9.4, respectively, did not increase plasma PP concentrations at all.
Discussion

In the present study the pancreatic polypeptide (PP) response to different stimuli was investigated in healthy subjects. Neither purified secretin nor CCK-8 induced any major increments in PP concentrations. In contrast, a large PP response was found in response to a mixed meal. In diabetic patients without measurable signs of autonomic neuropathy, the PP response was significantly attenuated and, furthermore, the response was not dependent on acute changes in metabolic control.

The PP response to hypoglycemia and to a mixed meal has previously been investigated in diabetic patients without measurable signs of neuropathy (5, 6, 8). In these studies, the PP response was diminished compared with response in healthy subjects. The reduction in PP response was considered due to vagal neuropathy. These patients had a comparatively long duration of diabetes, making the existence of neuropathy possible.

We therefore chose to investigate well controlled diabetic patients, treated with multiple injection regimens, having a short duration of diabetes (mean duration four years), in whom the development of neuropathy would be considered to be unlikely. It is therefore surprising that these patients had a significantly lower PP response to a mixed meal than healthy subjects had. Two mechanisms may be operative: either the decrease in PP response was due to dysfunction of the neural arc controlling PP secretion, or the decrease in PP response was due to islet cell dysfunction, or both. The arguments against the existence of subclinical neuropathy affecting the neural arc (CNS components and vagal efferents (13)) have been given above; for the evidence of neuropathy speaks the fact that even partial vagal denervation (parietal cell vagotomy) is sufficient to induce a decrease in the meal-induced PP response (23). In favour of the latter explanation, it is established that the exocrine pancreatic function in diabetic patients is slightly but significantly reduced (24), and it is conceivable that the PP cell mass is reduced in newly diagnosed diabetic patients, although no histological studies so far are available to document this.

The reduction in meal-induced PP response in short-term diabetic patients is strikingly similar to the reduction in glucagon response to hypoglycemia in the same patient group. Both occur early in the course of Type 1
diabetes, and, for both peptides, both neural and non-neural stimuli seem to be active in the secretion of the peptides (25). The evidence for a neural component in the regulation of glucagon secretion is abundant (although not definitively established as regards glucagon response to hypoglycemia), and for PP, non-neural stimuli have now been documented to be active in the secretion of the peptide. This is so, since CCK antagonists significantly reduce (by more than 50%) the meal-stimulated PP release (26). The effect of hyper-insulinemia on glucagon and PP responses has not been fully established; in the present study, we have been able to exclude any major stimulatory effect of insulin on PP secretion, since in insulin-dependent diabetic patients no increment at all was found in plasma PP concentrations after iv insulin, lowering blood glucose from one hyperglycemic level to another still hyperglycemic level. Indeed, the natural history of the reduction in PP response to a meal could possibly give a clue to the cause of the reduction in glucagon to hypoglycemia.

Previous investigations have suggested that secretin (17) is a powerful stimulant of PP secretion. However, the recent results indicate that injection of the synthetic peptide results in no PP increase at all, suggesting that the increases previously reported could have been due to impurities of the preparations employed.

In conclusion, the PP response to a mixed meal is significantly reduced in patients with short duration IDDM, possibly indicating a dysfunction of the reflex arc controlling PP secretion. Alternatively, the reduction in PP secretion in these patients reflects dysfunction of the PP secreting cells of the pancreas. Short-term changes in metabolic control have no effect on PP secretion in these patients.

References

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