Gastric Acid and Pancreatic Polypeptide Responses to Sham Feeding Are Impaired in Diabetic Subjects with Autonomic Neuropathy

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SUMMARY

To assess the relationship between cardiac and extracardiac dysfunction in diabetic autonomic neuropathy, the gastric acid output and the pancreatic polypeptide (hPP) secretion in response to sham feeding were evaluated in diabetic patients with (group 1) and without (group 2) cardiac autonomic neuropathy (CAN), and in normal subjects (group 3). All patients assigned to the group with CAN exhibited an impaired beat-to-beat heart rate variation during deep breathing. The basal gastric acid output was comparable in the three groups (1.3 ± 0.5, 2.8 ± 1.5, and 3.9 ± 1.5 mmol/h, respectively). In contrast, the gastric acid output stimulated by sham feeding was significantly lower in patients with CAN (5.3 ± 1.3 mmol/h) than in diabetic subjects without CAN (14.0 ± 3.5 mmol/h; $P < 0.01$) and in controls (10.9 ± 3.1; $P < 0.05$). The maximal gastric acid secretion capacity, determined after pentagastrin injection, was similar in all patients. Mean basal hPP concentrations were comparable in the three groups (185 ± 53 pg/ml, 131 ± 29 pg/ml, and 116 ± 19 pg/ml). In the controls and diabetic subjects without CAN, a significant mean 60% increase of the hPP levels above basal values was observed during sham feeding. In contrast, no significant hPP response occurred in the group with CAN. These data suggest that diabetic CAN is associated with dysfunctions of the vagal pathways controlling the gastric acid output and the hPP secretion. Moreover, the results demonstrate a strong association between cardiac autonomic neuropathy and gastric vagal neuropathy ($P < 0.001$). DIABETES 1985; 34:1181–85.

Autonomic neuropathy is a common complication of long-standing diabetes. Its cardiovascular consequences are well documented, and are due to impaired parasympathetic as well as sympathetic reflexes.[1] Gastrointestinal symptoms such as a delayed gastric emptying[2] or impaired gastric acid secretion[3–6] have been reported to occur in diabetic patients and could also be attributed to autonomic neuropathy. However, no direct correlation between cardiovascular and gastrointestinal symptoms related to autonomic neuropathy have yet been described. In the present study, we investigated the gastric acid responses to a physiologic stimulus, namely a sham-feeding test, in diabetic patients with or without cardiovascular autonomic neuropathy (CAN). In addition, we determined the pancreatic polypeptide (hPP) response during the sham-feeding test to document a possible impairment of the vagal pathways that play a major role in the control of its secretion.[7] A possible involvement of these pathways has also been suggested by previous studies showing that the vagally mediated hPP response to hypoglycemia was impaired in diabetic autonomic neuropathy.[8,9]

MATERIALS AND METHODS

PATIENTS (TABLE 1)

Nineteen patients presenting with type I or type II diabetes and 10 normal, healthy volunteers of both sexes, comparable in age and in weight, were examined. All subjects gave informed consent and the protocol of the study was approved by the ethics committee of our university. After examination, three diabetic and two control subjects were excluded from the study for reasons described below. All patients had normal creatinine levels as well as normal liver function. None of these subjects had a history of alcoholism, chronic pancreatitis, digestive disease or surgery, or psychiatric disease. Retinopathy was assessed by fluoroangiography. Three patients in group 1 had proliferative retinopathy, and five other subjects in group 1 and two patients in group 2 had background retinopathy. The presence of peripheral neuropathy was determined by diminished deep tendon reflexes and by electromyography, which showed altered motor and sensory

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TABLE 1
Clinical characteristics

	Diabetic patients		
	With cardiac autonomic neuropathy (Group 1, N = 9)	Without cardiac autonomic neuropathy (Group 2, N = 7)	Control subjects (Group 3, N = 8)
Female/male	6/3	2/5	4/4
Age (yr)*	59 (36–74)	56 (45–70)	43 (20–73)
Type of diabetes			
I (N)	7	4	—
II (N)	2	3	—
Known duration of diabetes (yr)	18 (7–47)	6 (1–17)	—
Weight (% of ideal body weight)	123 ± 8	136 ± 11	113 ± 6
Complications			
Retinopathy (N)	8	2	—
Peripheral neuropathy (N)	9	4	—
Heart rate (beats/min)	88 ± 3‡	78 ± 4	72 ± 3
E/I ratio†	1.06 ± 0.01‡	1.22 ± 0.03	1.30 ± 0.04

*Results are expressed as mean and range, or as mean ± SEM.
†E/I ratio: ratio of the longest R-R interval (EKG) during expiration to the shortest R-R interval during inspiration.
‡$P < 0.01$ versus group 2 and group 3.

conduction velocities in all the patients of group 1 and in four patients of group 2.

Control subjects had normal fasting glucose levels. Two of the 10 controls were excluded from the study: one female subject was found to have achlorydia; another subject was excluded because macroscopic food debris were observed in the gastric aspiration juice during sham feeding (see below).

Diabetic subjects were classified into two groups according to the presence or absence of cardiac autonomic neuropathy (CAN). The latter was determined by the beat-to-beat variation in heart rate, monitored by electrocardiography, during deep breathing. The results are expressed as the E/I ratio, i.e., the longest R-R interval during expiration (E) to the shortest R-R interval during inspiration (I).[10] Values below the 95th percentile of the age-related, normal range were considered as affirming CAN (group 1). Patients with a ratio above the 80th percentile were considered as having no CAN (group 2). Three patients with a borderline E/I ratio (between the 80th and the 95th percentile) were excluded from the study. As shown in Table 1, the heart rate at rest was higher in diabetic patients with CAN than in control subjects or in diabetic subjects without autonomic neuropathy.

TEST PROCEDURE: SHAM FEEDING AND MEASUREMENT OF GASTRIC ACID SECRETION

Subjects were studied after a 12-h fast and abstinence from smoking. All drugs, except insulin, were discontinued at least 12 h before the study. Insulin-dependent diabetic patients received one-half of their usual dose of regular insulin subcutaneously (s.c.) at 7:30 a.m. The tests were started between 8 and 9 a.m. A naso-gastric tube was passed into the stomach and its position in the antrum checked by radioscopic control. Gastric secretions were continuously aspirated by hand suction and samples were collected every 15 min. The volume and titratable acidity of each sample were measured; acid output was expressed as millimoles per hour.[11]

Basal acid output (BAO) was first measured as the sum of four consecutive 15-min outputs (time −60–0 min). The subjects were then sham fed a steak, as described previously.[12] The meal was prepared in a separate room so that the patients could not see nor smell the food until the time of sham feeding. The subjects were instructed to chew but not to swallow the food during a 30-min period. No macroscopic food debris were observed in gastric juice at any time of the experiment, except in one female patient (see above).

Sham feeding-stimulated acid output (SAO) was then measured during four periods of 15 min (30 min during sham feeding and 30 min immediately after, i.e., time 0–60 min). To evaluate the maximal capacity of gastric acid secretion, 6 μg/kg of pentagastrin (Ayerst Labs, New York, New York) was injected s.c. Gastric juice was again collected during four periods of 15 min (time 60–120 min).

Peak acid output (PAO) corresponded to the two highest consecutive 15-min outputs after pentagastrin (multiplied by two to express results in millimoles per hour). The results of gastric acid secretion were also expressed as the SAO − BAO difference (basal output subtracted from the response to sham feeding), which evaluated the increase of secretion due to the vagal tone. The results were also calculated as the SAO − BAO/PAO ratio, which represented the basal output subtracted from the response to sham feeding relative to the maximal secretory capacity.

Finally, we determined the ratio SAO/PAO (ratio of the sham feeding-stimulated acid output to peak acid output), which is also considered a reliable marker of gastric vagal innervation.[13]

During the 3-h experiment, a forearm vein was kept patent for blood sampling. Glucose and hPP were measured under basal conditions, during sham feeding and pentagastrin stimulation, at −15, 0, 15, 30, 45, 60, 75, 90, 105, and 120 min.

In insulin-dependent diabetic patients, the blood glucose level was frequently monitored by Dextrostix reagents combined with a Glucometer (Ames Division, Miles Laboratories, Elkhart, Indiana) reading to avoid any hypoglycemic episodes. In these patients, a 5% glucose solution was infused intravenously (i.v.) and, if necessary, the infusion rate was adjusted so as to maintain blood glucose levels between 100 and 200 mg/dl.

TABLE 2
Gastric acid secretion in response to sham feeding and pentagastrin*

	Diabetic patients		
	With cardiac autonomic neuropathy (Group 1)	Without cardiac autonomic neuropathy (Group 2)	Control subjects (Group 3)
Basal acid output (BAO)†	1.3 ± 0.5	2.8 ± 1.5	3.9 ± 1.5
Sham feeding acid output (SAO)†	5.3 ± 1.3‡	14.0 ± 3.5	10.9 ± 3.1
(SAO − BAO)†	4.0 ± 0.9§	12.2 ± 2.3	7.0 ± 1.7
Peak acid output (PAO)†	25.3 ± 4.1	31.8 ± 6.0	21.9 ± 4.8
$\left(\frac{SAO - BAO}{PAO}\right)$	0.16 ± 0.03‖	0.38 ± 0.06	0.32 ± 0.03
SAO/PAO	0.20 ± 0.03‖	0.47 ± 0.04	0.50 ± 0.07

*Mean ± SEM.
†Expressed as mmol/h.
‡$P \leq 0.01$ group 1 versus group 2, and $P < 0.02$ versus controls.
§$P \leq 0.01$ group 1 versus group 2, and $P < 0.05$ versus controls.
‖$P \leq 0.01$ versus groups 2 and 3.

ASSAYS

Plasma glucose was measured by a glucose-oxidase method (Glucose Analyzer, Beckman Instruments, Fullerton, California). Plasma human PP levels were determined by radioimmunoassay, using an anti-PP antiserum (lot 615-1054 B-248-19) and highly purified hPP as standard (lot 615-1054 B-200-8), kindly provided by R. Chance (Eli Lilly and Company, Indianapolis, Indiana). ^{125}I-labeled porcine PP (specific radioactivity: 200 μCi/μg) was obtained from the Novo Research Institute Immunological Laboratory (Copenhagen, Denmark). Incubations were performed in a 0.05 M phosphate buffer (pH 7.5), 0.075 M in NaCl, containing 2.5 g/L of human serum albumin (Behring) and 100 mg/L of merthiolate.

The total incubation volume was 500 μl, containing 100 μl of the plasma samples, or of the standard hPP, ~50 pg of ^{125}I-hPP, and the anti-hPP antiserum at a final dilution of 1/150,000.

The incubations were performed for 72 h at 4°C. Antibody-bound radioactivity was separated by polyethylene-glycol precipitation. To this end, 200 μl of incubation buffer with 3 mg (standard curve) or 1 mg (plasma samples) of bovine gamma-globulin were added to each incubation tube; this was immediately followed by the addition of 400 μl of a 40% (wt/wt) solution of polyethylene-glycol 6000 in distilled water. Each tube was mixed (Vortex, 5 s) and centrifuged at 4°C (1500 × *g*, 30 min); the radioactivity of the pellet was determined in a gamma counter. The minimal detectable amount of hPP (95% confidence level) was 28 ± 4 pg/ml (mean ± SD; N = 16); the intra- and interassay coefficients of variation for duplicates were 5.2% and 9.0%, respectively (mean value: 221 pg/ml; N = 16).

The presence of free-circulating anti-PP antibodies was evaluated by incubating each plasma sample (100 μl) in quadruplicate with ^{125}I-porcine PP (~50 pg) for 72 h at 4°C in a total volume of 500 μl (assay buffer). The antibody-bound radioactivity was precipitated by PEG as described above. The precipitated radioactivity (B) is expressed in terms of a percentage of total radioactivity (T). In a group of 43 normal subjects, the B/T × 100 value was 7.1 ± 0.8% (SD) (non-specific binding, normal range ± 2 SD: 5.5–8.8). All diabetic subjects included in the present study fell within the normal range (CAN [−]: 7.3% ± 0.8; CAN [+]: 7.0% ± 0.5), with the exception of one patient (M.A., CAN [+]), who exhibited a B/T × 100 value of 12.4%. Such a value was considered to be only marginally elevated; moreover, the hPP response was normal and this patient had been treated with purified insulins for 1 yr. He was, therefore, included in the study. The validity of the method for circulating anti-hPP antibodies was established in another independent study of 22 type I diabetic patients receiving insulin therapy for at least 10 yr. Three patients exhibited clearly elevated B/T × 100 values (18.1%, 17.5%, and 56.0%), and two others had borderline values (11.5% and 13.4%). The rest of the group was strictly normal: 7.3 ± 0.98% (mean ± SD, N = 17).

STATISTICAL METHODS

Results are presented as means ± SEM. Since it is likely that the acid outputs and hPP values were not normally distributed, the significance of differences was examined using the nonparametric Wilcoxon signed-ranks test for two related variables. The r-coefficient was calculated to assess the correlation between two groups of variables. The correlations between patients with cardiac, gastric, and pancreatic autonomic neuropathy were analyzed with the chi-squared test and the Yates correction for continuity. P-values <0.05 were considered significant.

RESULTS

Gastric acid secretion. The results of gastric acid secretion tests are shown in Table 2. BAO was comparable in the three groups of patients.

SAO and (SAO − BAO) were significantly decreased in diabetic subjects with CAN. On the other hand, no significant difference in PAO was recorded among the three groups. The ratio SAO − BAO/PAO was thus markedly lower in the patients with CAN than in the two other groups of patients.

The SAO/PAO ratio, considered as a reliable index of gastric vagal innervation, was also significantly lower in diabetic subjects with CAN than in the two other groups. Moreover, the presence of an abnormal SAO/PAO ratio, defined as a value <0.30 (the lowest value observed in controls) was strongly associated with the presence of cardiac neuropathy (Table 3). Gastric secretion volumes were not significantly

TABLE 3
Correlation between cardiac autonomic neuropathy and gastro-pancreatic vagal impairment

	Diabetic patients		
	With cardiac autonomic neuropathy (Group 1)	Without cardiac autonomic neuropathy (Group 2)	χ^2
Sham-feeding acid output*			
Responders	1	7	12.658 ($P < 0.001$)
Nonresponders	8	0	
Pancreatic polypeptide†			
Responders	4	6	2.438 (NS)
Nonresponders	5	1	

*Responders: SAO/PAO (as defined in Table 2) > 0.30.
†Responders: hPP rise above basal ≥ 113%.

different in the three groups (basal period: 41 ± 9, 53 ± 11, and 90 ± 20 ml; sham-feeding period: 111 ± 23, 177 ± 29, and 145 ± 29 ml in groups 1, 2, and 3, respectively).

Plasma glucose and pancreatic polypeptide levels. Mean plasma glucose concentrations were not significantly different during the test in diabetic subjects with CAN (131 ± 10 mg/dl) and those without CAN (153 ± 32 mg/dl). Plasma glucose in controls was 75 ± 5 mg/dl (Figure 1). Mean basal hPP concentrations were similar in diabetic subjects with CAN (185 ± 53 pg/ml), without CAN (131 ± 29 pg/ml), and in normal subjects (116 ± 19 pg/ml).

In normal controls, as well as in diabetic subjects without CAN, the mean plasma level of hPP exhibited a prompt 60% increase in response to sham feeding, which was maintained until the 60th min (Figure 1). In contrast, there was no significant increase of the hPP levels in diabetic subjects with CAN during the sham feeding. No correlation was found between the plasma glucose and hPP areas under the curves during sham feeding ($r = 0.22$). No individual association between an abnormal hPP rise during sham feeding, defined as an increase <113% of the basal value (the lowest rise in controls), and the presence of CAN or of an abnormal gastric acid output was found (Table 3). After pentagastrin injection, a similar increase in hPP was observed in the three groups (Figure 1).

When the hPP levels at time 60 min were considered as 100%, areas under the hPP curves between the 60th and 120th min (integrated responses to pentagastrin) were comparable in the three groups: 4116 ± 1323, 2463 ± 1251, and 2885 ± 1636 pg/ml/h^{-1} in groups 1, 2, and 3, respectively.

DISCUSSION

The present study shows that in a group of diabetic subjects with overt CAN, the gastric acid secretion response to sham feeding is significantly impaired. Moreover, this defect is closely correlated with the presence of CAN, since an abnormal SAO/PAO ratio was evidenced in eight of the nine patients with CAN, and since all diabetic subjects without CAN had a normal gastric response to sham feeding. The gastric acid response to sham feeding is thought to be mediated by the vagal cephalic pathway.[12] The data thus suggest that, in diabetic autonomic neuropathy, the cardiac and gastric vagal innervations are affected together. In nondiabetic patients who underwent a complete vagotomy for duodenal ulcus, Feldman et al.[13] reported SAO/PAO ratios equal to or lower than 0.10. The mean value observed in our group of diabetic subjects with CAN (0.20) appeared to be somewhat higher, but significantly lower than in normal controls. Diabetic autonomic neuropathy may thus lead to a state of incomplete functional auto-vagotomy affecting the pathways involved in the control of gastric acid secretion. The impaired

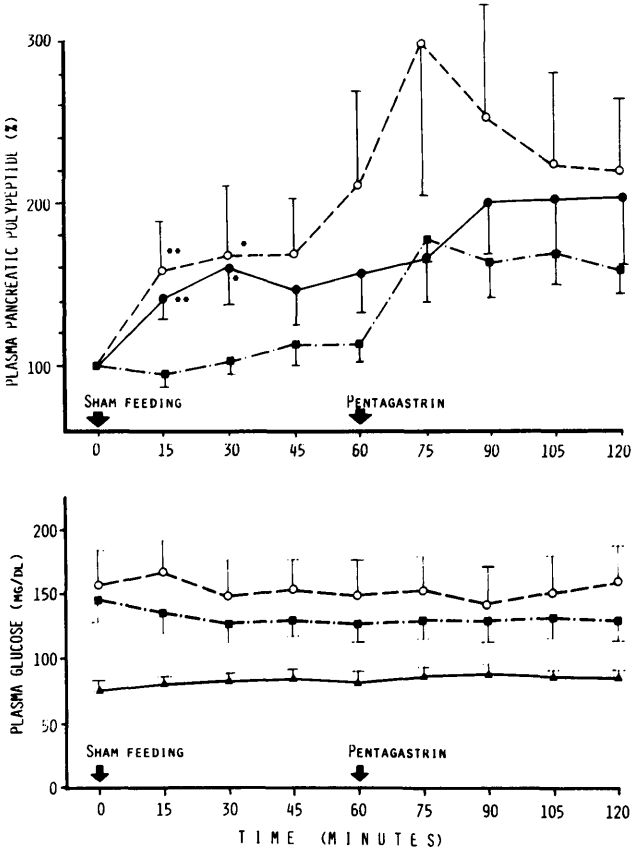

FIGURE 1. Mean plasma pancreatic polypeptide levels (expressed as percentages of the values recorded at time 0 considered as 100%) and mean plasma glucose levels during the sham-feeding test (starting at time 0) and after pentagastrin injection (time 60 min) in normal subjects (▲——▲) and in diabetic patients with (■···■) and without (○---○) cardiac autonomic neuropathy. Results are expressed as mean ± SEM. *P < 0.05, **P < 0.01 versus levels in patients with cardiac autonomic neuropathy.

acid secretion during sham feeding in diabetic subjects with CAN could in principle be due to a decreased total gastric parietal cell mass. However, this is unlikely, since the secretory responses to pentagastrin were comparable in the three groups of patients.

Taylor et al.[7] have shown that the hPP response to modified sham feeding is greatly reduced after truncal vagotomy. They concluded that this response is likely to be mediated by the vagal nerve. The hPP response of our normal patients and diabetic subjects without autonomic neuropathy to this modified sham-feeding procedure are equivalent to those previously reported by others.[7,14,15] Thus, it produced a prompt and significant increase of hPP, after which the level remained elevated for as long as 60 min. The modified sham-feeding procedure is recognized to be associated with some small but significant leakage of saliva and meal juice to the stomach.[14] Therefore, it cannot be excluded that the late increase of hPP observed after sham feeding (up to the 60th min) is to be partially attributed to extravagal cholinergic mechanisms involved in the prolonged (up to 180 min) hPP response induced by a protein-rich meal ingestion.[16] However, Schwartz et al. have reported data in which hPP remained elevated above basal levels for as long as 90 min after adequate sham feeding, a procedure in which the possibility of leakage is practically eliminated.[14] The present study shows that the mean hPP response to modified sham feeding was significantly impaired in diabetic subjects with CAN, suggesting an involvement of the vagal pancreatic innervation. However, a partial involvement of the extravagal cholinergic mechanisms as described above cannot be ruled out by our data. The results are in accordance with previous studies, showing an impairment of the hPP responses in patients with CAN during hypoglycemia, a stimulus known to be vagally mediated. We could not establish any significant correlation between an impaired hPP response and the presence or absence of CAN. The large individual variation of the hPP responses to sham feeding among normal and diabetic subjects may have hindered any possible relationship between the pancreatic and cardiovascular expressions of diabetic autonomic neuropathy. However, our data are supported by those of Levitt et al.[8] who also failed to demonstrate such a correlation between hPP responses to hypoglycemia and cardiac or gastric indices of autonomic neuropathy.

Blood glucose changes are known to affect hPP levels.[17] This could not explain the differences observed in our study, since no correlation was found between plasma glucose and hPP areas under the curve during the sham-feeding period. Moreover, there was no significant difference in plasma glucose between patients with and without CAN. Rahier et al.,[18] using stereologic and morphometric methods, showed the existence of an identical hPP cell mass in normal subjects and in diabetic patients. The present results, which demonstrate a comparable response of hPP to pentagastrin (a nonvagal stimulus of hPP secretion) in the three groups of subjects, show that the hPP cells are functional, even when autonomic neuropathy is present.

In conclusion, this study shows that both gastric and pancreatic vagal functions are often impaired in diabetic patients with CAN. Moreover, our data show that an impaired beat-to-beat variation is most likely to be associated with an abnormal gastric acid secretion. The assessment of gastric acid secretion together with the measurement of plasma hPP levels during sham feeding may thus complement the clinical evaluation of patients with suspected autonomic neuropathy.

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