Decreased Substance P Content in the Rectal Mucosa of Diabetics With Diarrhea and Constipation

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Substance P (SP), vasoactive intestinal polypeptide (VIP), and somatostatin content in rectal mucosa were determined by radioimmunoassay (RIA) in 38 diabetic patients (12 with normal bowel function, 13 with diabetic diarrhea, and 13 with constipation) and in 10 nondiabetic controls with normal bowel function. SP content (picograms per milligram) in the rectal mucosa of diabetics with normal bowel function was significantly higher than that of nondiabetic controls ($P < .05$). SP content in the rectal mucosa of diabetics with diabetic diarrhea and constipation was significantly lower than in diabetics with normal bowel habits and nondiabetic controls ($P < .05$). No differences were found in the rectal mucosa content of VIP and somatostatin between the different groups of diabetics and controls. Diabetic diarrhea is a condition with an intermittent nature and frequently alternates with constipation. Our findings showing low levels of rectal mucosa SP in both conditions suggest a possible common role of SP in the pathogenesis of diabetic diarrhea and constipation.

THE PREVALENCE OF DIABETIC diarrhea has been reported as varying from 8% to 22% in diabetic patients.[1,2] The pathogenesis of this condition is unclear. Abnormal intestinal motility and secretion,[3] anorectal dysfunction,[4] enteral bacterial overgrowth,[5] bile acid catharsis,[6] exocrine pancreatic insufficiency,[7] and even celiac disease[8] were proposed to explain this diabetic complication. However, the concept of intestinal dysmotility as the cause of human diabetic diarrhea remains the most acceptable explanation.

Small bowel and colonic motility is the result of a well-balanced and complicated mechanism including smooth muscle cell electrical properties, the intrinsic nervous system, extrinsic neural pathways, peptidergic transmitters, and hormonal mediators.[9]

Substance P (SP) stimulates small and large bowel motility. In diseases associated with diarrhea such as celiac disease and ulcerative colitis, SP content in the small intestine and colon, respectively, was found to be increased.[10-11] In a previous study, we showed that diabetics with constipation have less SP in the rectal mucosa than diabetics with normal bowel function; however, both diabetic groups had more SP than the respective nondiabetic controls.[12]

Vasoactive intestinal polypeptide (VIP) has been reported to have an active role in physiologic and pathologic processes in bowel motility and intestinal blood flow.[13] Somatostatin is also present in human gut, but little is known concerning its physiologic role in bowel motility.

Patients with diabetic diarrhea and diabetic constipation share several common clinical characteristics. Diabetic diarrhea frequently alternates with constipation, and both diabetic diarrhea and constipation are associated with chronic disease and a high incidence of autonomic neuropathy.[14]

The aim of the present study was to determine the content of SP, VIP, and somatostatin in the rectal mucosa of both motility disorders in diabetes: diarrhea and constipation. We also evaluated and compared the content of these polypeptides in the rectal mucosa of diabetics and controls with normal bowel habits.

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SUBJECTS AND METHODS

Thirty-eight diabetic patients attending the gastrointestinal outpatient clinic of Hadassah University Hospital and referred from two outpatient diabetic clinics and 10 nondiabetic subjects were included in the study.

Control Subjects

Twelve diabetic and 10 nondiabetics, all with a normal frequency of bowel movements (four to 10/week), served as control groups.

Diabetics With Diarrhea

Thirteen patients with diabetic diarrhea were included. Chronic diarrhea was defined as abnormal soft stools at least three times per day for at least 3 months. However, diabetics with the typical appearance of bouts of diarrhea with interposed constipation lasting more than 3 months were also included. Diabetic diarrhea was also defined as unexplained diarrhea after intensive evaluation and exclusion of other possible causes for chronic diarrhea. Diabetic patients with fat malabsorption or bacterial overgrowth were excluded from the study group. As is typical in diabetic diarrhea, the majority of patients did not have abdominal pain, although some reported mild abdominal discomfort. Autonomic neuropathy was determined by clinical evidence of autonomic insufficiency manifested by one of the following features: orthostatic hypotension, impotence, neurogenic bladder, or abnormal heart rate response to the Valsalva maneuver.

Diabetics With Constipation

Thirteen diabetic patients with chronic severe constipation were studied. Chronic constipation was defined as the absence of spontaneous bowel movements for a period of 5 days or more, lasting at least 12 months.

Protocol

All subjects participating in the study were subjected to colonoscopy or sigmoidoscopy. Subjects with normal bowel function underwent colonoscopy because of rectal bleeding, which was finally diagnosed as internal hemorrhoids.

None of the patients or controls examined in the study had mechanical intestinal obstruction as assessed by colonoscopy, any systemic disease (except diabetes), and any medication known to affect

gastrointestinal function and transit, with the exception of bulk-forming agents.

During colonoscopy (Olympus CF20L, Hamburg, Germany), three or four rectal biopsy specimens were obtained for polypeptide determinations from an area of normal mucosa 10 to 15 cm above the anal verge. From all subjects included in the study, specimens were also obtained for histologic examination.

The study was approved by the human studies committee of Hadassah University Hospital, and patients provided written informed consent.

Determination of Polypeptides

SP. The tissue content of SP-like immunoreactivity was determined by radioimmunoassay (RIA). SP was extracted from biopsy specimens as follows. The specimens were weighed and homogenized in acetic acid 2N (1:10 wt/vol) containing 10 mmol/L mercaptoethanol and phenylmethylsulfonyl fluoride (PMSF) (1 μg/mL). The homogenate was centrifuged at 3,000 rpm (4°C), the supernatant transferred to another tube, and the pellet extracted again and centrifuged as above. The supernatant was collected, added to that obtained after the first extraction, and lyophilized until used for RIA. The protein was resuspended in 1 mL 50-mmol/L phosphate buffer, pH 7.4, containing 145 mmol/L NaCl, 0.1% gelatin, and 0.02% NaN_3. SP immunoreactivity was analyzed using a commercial kit (Incstar, Stillwater, MN). The RIA uses simultaneous addition of 200 μL sample, 100 μL rabbit anti-SP antibody diluted in bovine serum albumin (BSA)-peptone buffer containing merthiolate, and 100 μL ^{125}I-SP. Synthetic human (Tyr 8) SP is labeled with iodine 125 diluted in BSA-peptone-EDTA buffer containing merthiolate. The tubes are incubated overnight at 4°C. Phase separation is accomplished by the addition of 100 μL rabbit gamma globulin diluted in BSA-borate buffer with merthiolate, followed by addition of 500 μL saturated ammonium sulfate. The tubes are vortexed vigorously until suspension is homogeneous, incubated for 25 minutes at 20°C, and centrifuged (1,600 rpm at 20°C). The tubes are immediately decanted, and the precipitate of each tube is counted in a gamma scintillation counter. The detection limit of the lowest standard was 39 pg/mL. In recovery studies, the percentage recovery was 91.8% ± 4.7% (mean ± SE, n = 9). Maximum binding of zero standard is 45% to 55%. The within-assay coefficient of variation is 1.9% to 4.0% and between-assay variation 7% to 9.6%.

The cross-reactivity of SP antibody was compared with that of the following peptides: SP, 100%; methionine enkephalin, less than 0.002%; leucine enkephalin, less than 0.002%; eledoisin, less than 0.002%; and physalaemin, less than 0.002%. There was no cross-reactivity with neurokinin A. Cross-reactivity with SP methyl ester was 70%.

VIP. VIP immunoreactivity was determined by a RIA kit (no. 39125; Incstar). The assay combines the use of a high–specific-activity VIP tracer, an antiserum specific for VIP (cross-reactivity 100%), and a VIP standard (range, 30 to 541 pg/mL). The specific binding of the tracer is 54%. Fifty percent B/Bo displacement is obtained with 135 pg/mL, and 82% B/Bo displacement with 30 pg/mL.

Somatostatin. Somatostatin immunoreactivity was measured by a RIA kit (no. 20100; Incstar). The antiserum is specific for somatostatin (cross-reactivity 100%). The range of the somatostatin standard is 16.5 to 265 pg/mL. The specific binding of the tracer is 53%. Fifty percent B/Bo displacement is obtained with 66 pg/mL, and 88% B/Bo displacement with 16.5 pg/mL.

Materials

The following materials were used: SP; neurokinin A; SP methyl ester; PMSF (Sigma, Petah Tikva, Israel); mercaptoethanol (Fluka, Buchs, Switzerland); gelatin (BDH Chemicals, Poole, England); sodium azide (Merck, Darmstadt, Germany); and SP, VIP, and somatostatin RIA kits (Incstar).

Statistical Methods

Statistical differences between the various groups studies were evaluated according to student test for unpaired data and by the non parametric Mann-Whitney test. P values less than 0.05 were considered significant. Results are expressed as the mean ± standard error (pg/mg wet weight).

RESULTS

Clinical characteristics of all subjects are listed in Tables 1 and 2. The age and female to male ratio were similar in all groups. Type I diabetes and autonomic neuropathy were more frequent in diabetic patients with diarrhea. The mean duration of diabetes and period before gastrointestinal symptoms appeared in the group with diabetic diarrhea were longer than in the other diabetic groups. Autonomic neuropathy was more frequent in absolute terms in diabetics with constipation than in those with normal bowel function, but this trend did not reach statistical significance. On the other hand, autonomic neuropathy was more frequent in diabetics with diarrhea compared with the group with normal bowel movements ($P < .05$).

SP content in the rectal mucosa of diabetics with normal bowel function was significantly higher than that of nondiabetics (88.1 ± 18.2 *v* 53.2 ± 7.9 pg/mg, $P < .05$). Both diabetics with constipation and those with diarrhea had a significantly lower SP content in rectal mucosa (41.9 ± 4 and 39.6 ± 7 pg/mg, respectively) compared with diabetics with normal bowel function (Fig 1).

We plotted the duration of diabetes, hemoglobin A_{1C} level, and age against mucosal SP content, and found no correlation.

VIP content in rectal mucosa was similar in controls and in diabetics with normal bowel movements, constipation, or diarrhea (23.4 ± 2.3, 18.3 ± 3.5, 27.1 ± 6, and 17.8 ± 5 pg/mg, respectively). Also, no differences were found in somatostatin values for controls and diabetics with normal bowel movements, constipation, or diarrhea (44.7 ± 5.5, 48.3 ± 13, 53.7 ± 13, and 34.1 ± 12 pg/mg, respectively).

DISCUSSION

The pathophysiology of diabetic diarrhea is poorly understood. It appears to be related to the development of autonomic neuropathy, which may cause several abnormalities, including altered intestinal and colonic motility. Colonic dilatation has been reported in patients with nocturnal diarrhea associated with diabetes mellitus.[15]

Table 1. Sex and Age Distribution of the Patients and Controls

	Diabetic Subjects			
	Normal Bowel Function	Diarrhea	Constipation	Normal Subjects
No. of subjects	12	13	11	12
Age, yr (mean ± SE)	56.5 ± 2.2	49.8 ± 12.1	53.4 ± 2.4	40.3 ± 2.7
Female to male ratio	7:5	9:4	5:6	7:5

Table 2. Clinical Characteristics of the Diabetic Patients

Characteristic	Diabetic Group		
	Normal Bowel Function	Diarrhea	Constipation
Diabetes type (n)			
Type I	4	9	3
Type II	8	4	8
Duration, yr (mean ± SE)	11.3 ± 6	16.1 ± 7	10.8 ± 2
Duration before GI symptoms, yr (mean ± SE)		11.5 ± 6	6.3 ± 2
Hemoglobin A_{1c} (%)	9.8	10.1	10.4
Patients with autonomic neuropathy (n)	2	11	4

Abbreviation: GI, gastrointestinal.

Battle et al[16] demonstrated that the colonic motor response to eating is absent or extremely delayed in patients with diabetes and constipation in comparison to normal subjects. The role of colonic dysmotility in the pathogenesis of diabetic diarrhea is not well defined.

Recently, large concentrations of SP were reported to be present along the digestive tract from the mucosa to the muscle layer in animals[17] and man.[18-19] Sources of SP include intestinal mucosa, nerves of extrinsic and intrinsic origin, and the myenteric and submucosal plexuses.[17,19] SP seems to share several gastrointestinal effects, including inhibition of gastric secretion,[20] stimulation of the exocrine pancreas,[21] induction of intestinal water and electrolyte secretion,[22] and stimulation of small bowel and colonic motility.[17,23,24]

The role of SP was evaluated in several gastrointestinal disorders. Bioassays of rectal mucosal biopsies from patients with Chagas' disease and severe chronic constipation showed decreased SP content.[10,25] Reduced concentrations of SP were also shown in surgical colonic specimens from patients with Hirschsprung's disease.[26] In contrast, in diseases associated with diarrhea such as celiac disease and ulcerative colitis, SP content in the small intestine and rectal mucosa, respectively, was found to be increased.[10,11] Studies in isolated intestinal segments from streptozotosin-induced diabetic rats have shown reduced contractility of intestinal smooth muscle in response to SP,[27] suggesting low sensitivity of intestinal muscle to this peptide in experimental diabetes. In a previous study, we found a significantly lower concentration of SP in the rectal mucosa of diabetics with constipation and a higher content of SP in diabetics in general compared with nondiabetics.[12] VIP is produced by nerve endings in the intestinal tract and appears to have marked effects on gut motility and blood flow. VIP has been shown to be able to relax the colon smooth muscle and the internal anal sphincter.[13,28] Somatostatin is widely distributed through the human gastrointestinal tract. Little is known about the physiologic effects of somatostatin in the gastrointestinal tract. In contrast, the pharmacologic actions of natural synthetic somatostatin are well known. The somatostatin analog has an inhibitory action on gastric acid secretion, pancreatic enzyme and bicarbonate secretion, and bile flow. It is also able to inhibit stimulated intestinal secretion and the release of neuropeptides from the gut and the pancreas. It can also prolong orocecum transit time and prevent gallbladder contraction.[29] The somatostatin analog octreotide has been used successfully in diabetic diarrhea.[30]

In the present study, SP, VIP, and somatostatin content were evaluated in healthy controls and three groups of diabetics, those with normal bowel function, constipation, or diabetic

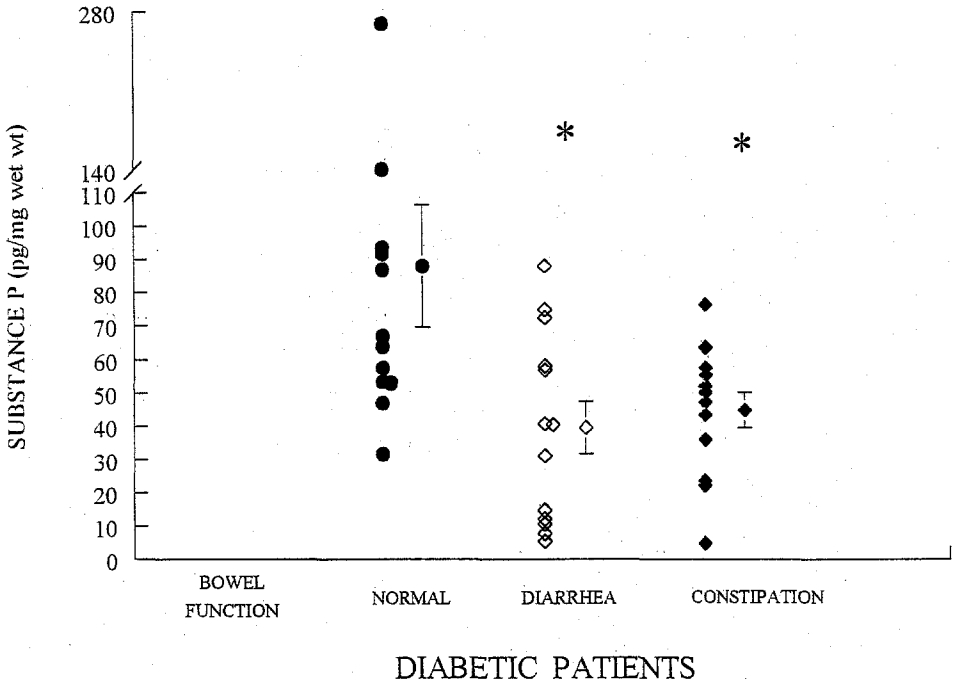

Fig 1. SP content in rectal mucosa of diabetic patients with normal bowel function, diarrhea, or constipation. Results are the mean ± SE. *$P < .05$ *v* normal.

diarrhea. Diabetic diarrhea was diagnosed after intensive evaluation and exclusion of other possible causes of chronic diarrhea in diabetic patients. Seventeen diabetic patients with diarrhea were excluded from the study because specific reasons for the diarrhea were found. The present results confirm our previous observations that diabetics with normal bowel function have higher rectal mucosa SP content than healthy controls. We also have confirmed that diabetics with constipation have lower rectal mucosa SP content compared with diabetics with normal bowel habits.[12] Now we have demonstrated that also diabetics with diabetic diarrhea have lower rectal mucosa SP content compared with diabetics with normal bowel function. On the other hand, we found no differences in the rectal mucosa content of VIP and somatostatin between the various groups of diabetics, and no differences were found between diabetics with normal bowel movements and healthy controls.

In diabetic patients, complications like constipation and diarrhea are associated with the presence of autonomic neuropathy, with both symptoms more common in patients with other symptoms of diabetic autonomic dysfunction and in patients with long-standing and poorly controlled diabetes.[13] The frequent intermittent nature of this diarrhea in some patients and its alternation with periods of constipation in others may suggest a common pathophysiology of both complications, at least in some patients. Fecal impaction and overflow diarrhea are an unlikely explanation for this condition. We found no evidence of fecal impaction in our patients. Gastrointestinal dysmotility, including colonic motility disorders, may explain the diarrhea and constipation changes in these patients. Indeed, nine of our patients had intermittent diarrhea, five of whom had alternating diarrhea and constipation.

In summary, abnormalities in the mucosal SP content of diabetics with diarrhea and constipation may be the result of degenerative changes in the submucosal plexus.[31,32] However, the association of both diabetic diarrhea and constipation states with autonomic neuropathy, the intermittent nature of diabetic diarrhea, which frequently alternates with constipation, and the decreased rectal mucosa SP concentration in both groups suggest a possible common pathway for constipation and diarrhea in some diabetic patients. SP, but not VIP and somatostatin, may play a role in the pathogenesis of both disorders in diabetic patients.

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