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Colonic Neuroendocrine Peptide Levels in Patients with Chronic Idiopathic Slow Transit Constipation

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ABSTRACT

The motility disorders in patients with slow-transit constipation have been attributed to a disturbance in the peptidergic innervation of the colonic enteric nervous system. The nature of this disturbance is, however, controversial. In the present study 7 patients with long-standing severe slow- transit constipation were included, and normal tissues from the colon of 6 patients, which had undergone colonectomy because of polyp, chronic diverticulitis, prolapsis and volvulus were used as controls. The concentrations of several neuroendocrine peptides were measured in tissue extracts by radioimmuno-assays. The level of pancreatic polypeptide was high in 2 patients and low in one patient. Peptide YY level was high in 3 patients and low in one patient, and that of neuropeptide Y was high in 4 patients. Somatostatin and vasoactive intestinal polypeptide levels were high in 3 patients and substance P concentration was low in 3 patients. Neurotensin level was high in one patient and low in another patient. Galanin concentration was low in 2 patients and high in one patient. Gastrin-releasing peptide level was high in one patient and that of enkephalin was high in 2 patients. All patients had altered concentrations of several neuroendocrine peptides except one, who had only a low level of galanin. It is concluded that patients with slow-transit constipation have disturbed neuroendocrine peptides in common, though the nature of this disturbance varies between patients and in most patients several neuroendocrine peptides were affected. This may explain the controversial results obtained in previous studies.

INTRODUCTION

Idiopathic Slow-Transit Constipation (STC) is characterized by a chronic severe constipation, which is not alleviated by bulking agents, prokinetic drugs or other laxative treatment. These patients need the aid of an enema for defecation

(3). Routine light microscopy fails to identify any persistent abnormality in the colon of these patients (3). Slow colonic transit (4,14) and motility disorders of the colon and rectum (5-8) have been found in these patients. The motility disorders have been attributed to an abnormal peptidergic innervation of the colonic enteric nervous system (9,10,12,13,16), but the nature of this disturbance varied, in different studies. Thus, whereas vasoactive intestinal polypeptide (VIP) was found to decrease in patients with STC (9,12), it has been reported to be unchanged in other studies (10,13,16). Similarly, substance P has been found to be decreased (16) or unchanged (10,13). In yet another investigation the only neuropeptides found to be affected were calcitonin gene-related peptide (CGRP), and motilin (10).

The aim of the present study was to investigate the nature of the disturbance in colonic neuroendocrine peptide levels in patients with severe STC.

MATERIAL AND METHODS

Patients

Seven patients with long-standing severe constipation (5 females, 2 males; mean age 53 years; range 21-77 years) were included in this study. Their clinical data are given in Table 1. On barium enema, the diameter of colon and rectum was normal in all the cases, thus excluding megacolon or megarectum as the cause of the constipation. Non of the patients had any systemic disease, or medication that might have affected gut motility, except prokinetics. All the patients were long-term users of various laxatives and prokinetics (piscosulfate, lactulose and cisaprid). All had undergone colectomy and ileorectal anastomosis, except one who had a sigmoid stoma. The colon of these patients appeared macro- and microscopically normal. Tissue samples from different segments of the colon from 6 patients and from sigmoid colon of the seventh were studied in this investigation.

For comparison, macroscopically and histologically normal tissue samples from the colon of 6 patients (4 females, 2 males; mean age 66 years, range 34-80 years) were used as controls. These patients had undergone colonectomy because of polyp (2 patients), chronic diverticulitis (2 patients), prolapsis (1 patient) and volvulus (1 patient). All samples were taken from the sigmoid colon, except one which was from the ascending colon.

Table 1. Clinical data of the patients investigated

Patient no.	Sex	Age (years)	Oro-anal transit time (hours)	Defecation frequency (per week)	Duration (years)
1	M	62	128	1	25
2	F	77	108	<1	30
3	F	48	144	2	>5
4	F	39	132	1	>5
5	F	65	156	1	20
6	F	50	87	<1	25
7	M	64	240	<1	20

Tissue specimens were frozen immediately in liquid nitrogen and stored at -70°C. They were allowed to thaw and then weighed. The peptides were extracted by boiling the tissue in 0.5 M acetic acid, followed by homogenization and centrifugation for 20 min at 4000 rpm. The supernatant was neutralised with 2 M sodium hydroxide and stored at -70°C until the time for assays.

Neuroendocrine peptide content was determined with commercially available RIA kits. Thus, pancreatic polypeptide (PP), somatostatin, substance P, VIP, and neurotensin were measured with RIA kits from Eurodiagnostica (Malmö, Sweden), and peptide YY (PYY), neuropeptide (NPY), galanin, gastrin-releasing peptide (GRP) and enkephalin with RIA kits from Peninsula Laboratories

(Belmont, Calif., USA). The assays were performed according to the manufacturers' instructions in duplicates of undiluted extracts and of 1:2 and 1:4 diluted extracts. Briefly, standards and samples were incubated with an excessive amount of respective antibodies and then incubated with the corresponding [^{125}I]-tracer. The antibody bound [^{125}I]-tracer was separated from the unbound fraction by using the double antibody-polyethylene glycol (PEG) precipitation technique. Thus, goat anti-rabbit IgG serum and PEG 6000 were added. After incubation, bound and free labels were separated by centrifugation at 4°C. The supernatant was removed by aspiration and the precipitate was counted in an automatic gamma counter.

Statistical analysis

A comparison between the patients and 95% confidence limits of the controls was performed and values outside these limits were considered significant.

RESULTS

The concentrations of different colonic neuroendocrine peptides of both patients and controls are given in Table 2. The PP concentration was high in 2 patients and low in one patient; PYY was high in 3 and low in one patient; NPY was high in 4 patients; somatostatin and VIP were high in 3; substance P was low in 3; neurotensin level was high in one patient and low in another patient; galanin was low in 2 and high in one patient; GRP was high in one patient and enkephalin was high in 2 patients. All patients had altered concentrations of several neuroendocrine peptides, except one who had only a low level of galanin.

DISCUSSION

The present findings of abnormal concentrations of neuroendocrine peptides in the colon of patients with STC agree with previous investigations (9,10,12,13,16). However, the present study showed that although these patients have abnormal

Table 2. The concentration of various neuroendocrine peptides (pg/mg wet tissue) in the colon of patients with slow-transit constipation and of controls

Patient no.	PP	PYY	NPY	Somatostatin	Substance P	VIP	Neurotensin	Galanin	GRP	Enkephalin
1	6.7*	141	31*	1.8	4.7	30.3*	4.3	8.1	9*	1.7*
2	7*	118	15	0.9	5.4	28*	3.4	5.7	7.2	0
3	6.3	117	15	1	5.3	23	1.4	4.7*	6.8	0
4	0*	18*	20	1.3	4.9	32*	0.02*	4.8*	6.8	0.02
5	5.7	193*	29*	3*	4*	7	8.2*	9	7.3	0
6	1.8	204*	32*	6*	3.2*	4.3	6.3	12.3*	8	1.5*
7	4.5	242*	36*	5*	3.8*	21	5.2	8.6	5.9	0
Controls mean (95% CI)	4.5 (2.4-6.5)	120 (97-144)	14.7 (6.4-23.1)	1.78 (1.01-2.35)	5.2 (4.6-5.9)	10.6 (0-27.6)	4.4 (2.1-6.7)	7.1 (5.1-9.1)	1.2 (2,9-8,3)	0.14 (0-0.46)

concentrations of colonic neuroendocrine peptides in common, the nature of this disturbance differs between patients. It also showed that, in most patients, several neuroendocrine peptides were affected. This may explain the controversial results obtained in previous studies on the neuroendocrine system of similar patients. It is tempting to speculate that the abnormality in the neuroendocrine system may be primary and contributory to the development of constipation, though the possibility that this disturbance is secondary to a long-standing state of constipation can not be ruled out.

The motility disorders of the colon and rectum in patients with STC have been attributed to altered autonomic function (7) and/or dysfunction of the colonic enteric nervous system (2). The neuroendocrine peptides play an important role in regulating colonic motility, absorption and secretion (1,11). The present observations, together with the previous findings (9,10,12,13,16), suggest the possibility that a disturbance of the colonic neuroendocrine system may be one cause of the colonic dysmotility observed in these patients. The findings of the present investigation showed that the disturbance in the neuroendocrine system is not a simple one affecting a single neuroendocrine peptide, rather, in most cases, several neuroendocrine peptides were affected. It is therefore conceivable that disorders in the neuroendocrine system in STC patients reflect a shift in the balance between the different neuroendocrine peptides, which in turn causes dysmotility in the large intestine.

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