

Elevated motility-related transmucosal potential difference in the upper small intestine in the irritable bowel syndrome

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Abstract The pathophysiology of irritable bowel syndrome (IBS) is complex and incompletely known. Very little has been studied regarding the role of sub-mucous neuronal activity. We therefore measured small intestinal transmural potential difference (PD, reflecting mainly electrogenic chloride secretion), and its linkage with fasting motor activity [migrating motor complex (MMC)] in controls ($n = 16$) and patients with IBS [$n = 23$, 14 diarrhoea predominant (d-IBS) and nine constipation predominant (c-IBS)]. Transmural-PD and its relation to MMC phase III was measured by modified multilumen manometry for 3 h in the fasting state using one jejunal and one duodenal infusion line as flowing electrodes. The amplitude and duration of motor phase III was similar in controls and IBS patients, but the propagation speed of phase III was higher in IBS patients. In IBS patients, maximal PD during MMC phase III was significantly elevated in both the duodenum and jejunum ($P < 0.05$) and the PD decline after phase III was significantly prolonged in the jejunum ($P < 0.01$). The PD elevation was seen in both duodenum and jejunum in d-IBS patients, but only in the jejunum in the c-IBS patients. On the basis of previous modelling studies, we propose that the enhanced secretion may reflect disturbed enteric network behaviour in some patients with IBS.

Keywords celiac disease, irritable bowel syndrome, migrating motor complex, secretion, transmucosal potential difference.

Abbreviations AHP, Afterhyperpolarization potential; CFTR, Cystic fibrosis transmembrane conductance regulator; IBS, Irritable bowel syndrome; d-IBS, Diarrhoea-predominant IBS; c-IBS, Constipation-predominant IBS; IPAN, Intrinsic primary afferent neurons; MMC, Migrating motor complex; PD, Potential difference.

INTRODUCTION

Irritable bowel syndrome (IBS) is a common gut disorder, characterized by abdominal pain and/or discomfort associated with disturbed bowel habits.¹ The pathophysiological factors in IBS are still incompletely known despite numerous studies.^{2,3} So far, psychological factors, altered gut motility, visceral hypersensitivity and dysregulation of the brain–gut axis have been considered to be associated with the pathophysiology of IBS.^{2–8} The most established pathophysiological model is enhanced visceral sensitivity, but this is only seen in about 50% of a mixed IBS population.^{3,8,9} Enhanced sensitivity probably involves extrinsic sensory systems, but the gut is also supplied by a complex system of interconnected intrinsic primary afferent neurons (IPAN).¹⁰ Intrinsic primary afferent neurons are afterhyperpolarizing (AH)-type neurons characterized by their long-lasting AH potential (AHP) (for a review, see Furness¹¹). Afterhyperpolarizing neurons form interconnected networks which can be self-reinforcing due to excitatory transmission within the networks.¹² In two recent modelling studies, we have shown that the changes in the degree of suppression of

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the AHP would lead to severe disturbances in the overall behaviour of both myenteric and submucous networks.^{12,13}

The migrating motor complex (MMC)¹⁴ is a complex motor pattern that is activated in the fasting state. In our modelling study, we proposed that it may be generated by a tendency of myenteric AH neuron networks to go into uncontrolled firing due to recurrent positive feedback.¹⁵ The most characteristic feature of the MMC, motor phase III, is also associated with the activation of intestinal secretion measured as transmural potential difference (PD).^{16,17} This is most likely due to the fact that submucous neurons are directly or indirectly mechanosensitive, thus linking intestinal motor activity with intestinal secretion.¹⁸ Combined monitoring of fasting motor and secretory function in IBS may thus be a way to test the hypothesis of a dysfunction of IPAN networks in these patients. To our knowledge, there is only one report describing mucosal secretory function, measured as PD, in the small intestine of IBS patients. This study demonstrated that in the terminal ileum, infusion of bile acids induced an exaggerated PD response in patients with 'irritable colon'.¹⁹

The aim of the present study was to test the hypothesis of a disturbed linkage between intestinal motor activity and secretion in IBS, a phenomenon that may reflect enteric network dysfunction. Fasting motility and PD were recorded in the duodenum and proximal jejunum in healthy volunteers and in a group of patients with IBS as defined by Rome II criteria.¹ As we have previously described the upregulation of the phase III-associated PD changes in untreated celiac disease, six patients of this type were included to allow quantitative evaluation of possible effects in IBS patients.

MATERIALS AND METHODS

Controls and IBS patients

The study was performed in 16 healthy volunteers (mean age 39, range 21–61, 12 females and four males) and 23 patients with IBS (mean age 40, range 22–63, 19 females and four males). The IBS patients were characterized according to the ROME II criteria,¹ and organic gastrointestinal diseases were excluded using appropriate investigations based on the presenting symptoms. Fourteen of the IBS patients were defined as diarrhoea-predominant IBS patients (d-IBS – 11 females and three males), while nine were defined as constipation-predominant IBS patients (c-IBS – eight females and one male). Patients with diarrhoeal symptoms had been evaluated by both gastroscopy and colonoscopy, with

normal results. None of the patients had had previous abdominal surgery, with the exception of appendectomy. Symptomatic pharmacological treatments (e.g. laxatives, fibres, loperamide) were withdrawn for at least a week prior to the measurement day. None of the patients were on antidepressants.

Celiac disease patients

As we have previously demonstrated upregulation of motility-associated duodenal PD in untreated celiac disease,^{17,20} we also recruited six new patients of this type (mean age 49, range 34–78, five females and one male) to be able to compare the magnitude and shape of the motility-related PD curve with that in IBS. Another rationale for the inclusion of these patients is that we have previously shown that their PD increase is indeed associated with an increased net fluid secretion. We accepted a low patient number since the key observation of an upregulated motility–PD linkage in the duodenum has already been published, since these data were confirmatory. The jejunal data are, however, new. The patients with celiac disease were diagnosed on the basis of suspected malabsorption and/or anaemia, and in some cases, irregular bowel habits. Only one of them had moderate dominating diarrhoeal symptoms. At the time of PD and motility recording, five of them were on a normal, gluten-containing diet. The sixth patient (a male) had remaining villus atrophy despite a gluten-free diet. All the individuals gave informed consent and the study was approved by the ethics Committee at the University of Göteborg.

Experimental setup and recording

The general experimental setup and recording methods have been described previously.¹⁷ Briefly, after an overnight fast, the subjects were intubated transnasally with a multilumen polyvinyl tube (Arndorfer Inc., Greendale, WI, USA) containing eight separate channels, six of which were used in the experiment. The tip of the tube was placed in the proximal jejunum, under fluoroscopic guidance. In this position, the recording points were placed as follows: proximal jejunum, duodenojejunal junction, three channels (1.5-cm distance) in the mid duodenum in the papilla region, and one channel in the antrum. Each pressure-recording channel (except those used for the recording of transmural PD) was perfused with water at a rate of 30 mL h⁻¹ using a pneumohydraulic perfusion system. A steady flow was generated by a narrow capillary (No 6009; Triplus, Kungsbacka, Sweden) connected to the pressure transducer (23 DC; Statham Instruments,

Oxnard, CA, USA). At two sites (the middle recording point in the proximal duodenum and the jejunal recording point at the end of the tube), we also simultaneously recorded the transmural PD, using an infusion of isotonic saline (instead of water) as a flowing electrode. PD was measured between calomel half cells (Radiometer, Copenhagen, Denmark) and a common reference electrode connected to a subcutaneous infusion of saline. This mode of recording thus ensured a stable chemical environment (ion composition) at the site of recording, and enabled us to measure pressure and PD at exactly the same site. Intestinal motor activity and PD were recorded for 3 h in the interdigestive state. All experiments were performed in the morning.

Experimental design

The choice of parameters reflecting the shape of the PD signal was based on the disturbances found in the celiac disease patients, a patient group in which we also know that the upregulated PD is associated with an increased net water secretion.²⁰ The chosen parameters were then used to analyse the relationship between phase III motor activity and PD in patients with IBS. In addition to the parameters implied by comparison with the celiac disease patients, we also included two parameters inferred from our previous work to reflect sensory network hyperactivity, i.e. the decay time of the transmural PD from peak PD to the point where PD again stabilized, and the propagation velocity of motor phase III. The control and IBS groups were age and gender matched.

Data analysis

Intestinal motor activity and PD were recorded for 3 h in the interdigestive state. All raw data were stored as ASCII files on a personal computer. The subsequent

data processing was done in Matlab (The Mathworks, Lowell, MA, USA; Release 14SP3).

1. A MMC phase III complex was identified in both the duodenum and the jejunum (defined as 10–12 contractions per min and characterized by an increase in pressure with a duration of at least 2 min and with distal propagation). Some individuals (both volunteers and patients) had more than one phase III period during the experiment. Fig. 1 shows that in the duodenum, similar maximal PD levels were reached when comparing the first and second phase III periods, whereas in the jejunum, the amplitude of the second phase III was slightly lower (slope of regression line = 0.75). As there were no major variations between phase III periods within a given subject and to avoid systematic bias, we restricted further analysis to the first phase III period from each subject in the subsequent analysis.
2. The pressure and PD data from a time window starting 17 min before the beginning of phase III and ending 32 min after the end of phase III were extracted and further analysed. In order to remove the effect of individual contractions, signals were low-pass filtered and resampled to 0.2 Hz (from 4 Hz) using the `idresamp` function in Matlab.
3. The following parameters were analysed (Fig. 2):
 - a. The mean transmural PD during late phase I–early phase II (= mean PD during the 5-min period starting 10 min after the end of motor phase III).
 - b. The mean transmural PD during late phase II (= mean PD during the 5 min preceding the beginning of phase III).
 - c. The maximum transmural PD during phase III (Max PD).
 - d. The rate of rise (mV s^{-1}) of the initial phase III evoked transmural PD increase.
 - e. The decay time of the transmural PD after the phase III peak (time from peak PD to the point where PD again stabilized).

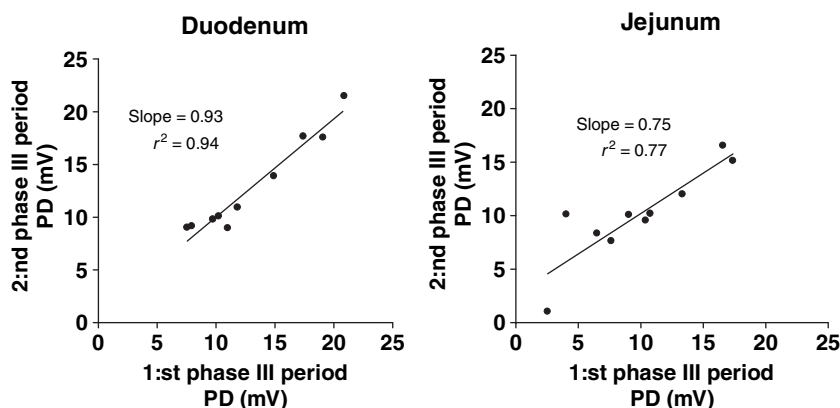


Figure 1 The correlation between the increase in potential difference (PD) after the first and second phase III period in controls and patients with celiac disease (duodenum, $n = 6$ and 4 , respectively; jejunum, $n = 7$ and 3 , respectively).

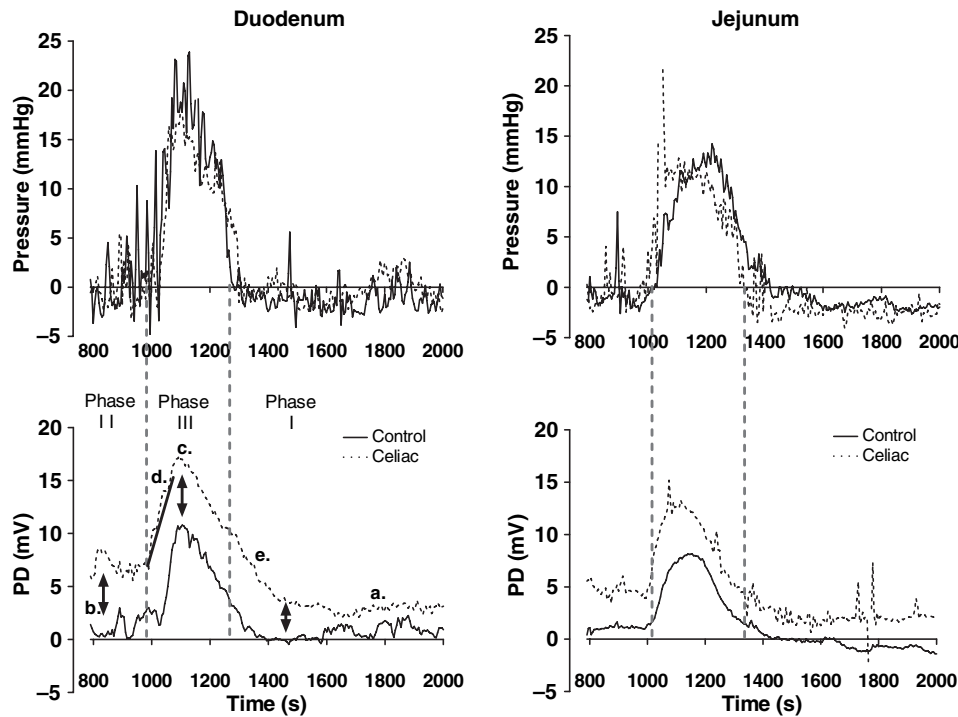


Figure 2 A representative migrating motor complex (MMC) phase III pressure signal and the corresponding changes in the transmucosal potential difference (PD). The average value from three healthy volunteers and three patients with celiac disease, all with similar phase III duration, are shown. The letters in the figure corresponds to respective parameters analysed.

- f. The amplitude of the mean pressure increase during phase III (= mean pressure during phase III minus mean pressure at phase I).
- g. The propagation speed of motor phase III (calculated by dividing the 30-cm distance between the duodenal and jejunal recording points by the time interval between the end of the motor phase III at these two points).

Please note that in all figures, the polarity of the PD signal is reversed, i.e. higher (positive) plotted PD values denote more lumen-negative PD.

Statistical analysis

The non-parametric Mann–Whitney U-test was used to identify the occurrence of significant differences between groups. A significant difference between the incidences of high PD values (defined as above 95th percentile) in different groups was tested by Fischer’s exact test. A *P* value of 0.05 or less was regarded as statistically significant. Two subjects displayed an abnormal PD decay to baseline conditions (one control in the duodenum and one celiac disease patient in the jejunum). The decay was very slow and the PD did not stabilize within the interval analysed. These subjects were therefore regarded as outliers and were removed

from the statistical analysis of the decay parameter (Fig. 4).

RESULTS

Dynamic behaviour of the PD signal in association with MMC phase III

No significant differences in any of the measured variables were seen between male and female controls (data not shown). Both control and patient data were therefore pooled without regard to gender.

The magnitude of the transmural PD increased at the onset of phase III (lower panels, Fig. 2). The PD signal tended to adapt after the initial increase in both the duodenum and jejunum, and returned towards baseline levels despite continued motor activity. On the basis of the changes in the shape of the PD curve in the celiac disease patients, the parameters described in the methodological section were chosen for the quantitative comparison.

PD changes in celiac disease

In celiac disease, there was no significant difference from controls in motor phase III amplitude (Tables 1

Table 1 Pressure and corresponding PD changes in healthy controls and patients with celiac disease or IBS during a MMC phase III period in the duodenum

Variable	Control (<i>n</i> = 14)	All IBS (<i>n</i> = 18)	d-IBS (<i>n</i> = 11)	c-IBS (<i>n</i> = 7)	Celiac (<i>n</i> = 4)
Mean pressure phase III (mmHg)	17.9 ± 2.1	22.6 ± 4.4	22.6 ± 4.4	19.3 ± 2.0	20.5 ± 2.4
Phase III duration (s)	299 ± 36	356 ± 56	396 ± 63	293 ± 63	228 ± 50
Mean PD phase II (mV)	3.3 ± 0.6	4.1 ± 0.4	4.1 ± 0.6	4.0 ± 0.4	7.7 ± 2.4
Mean PD late phase I-early phase II (mV)	1.0 ± 0.3	1.8 ± 0.3	1.9 ± 0.4	1.7 ± 0.6	3.2 ± 1.0*
Rate of rise PD phase III (mV/s)	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.10 ± 0.02

PD, potential difference; IBS, irritable bowel syndrome; d-IBS, diarrhoea-predominant IBS; c-IBS, constipation-predominant IBS; MMC, migrating motor complex. Results are expressed as mean ± SEM. Asterisks indicate significant differences compared with controls (**P* < 0.05).

Table 2 Pressure and corresponding PD changes in healthy controls and patients with celiac disease or IBS during a MMC phase III period in the jejunum

Variable	Control (<i>n</i> = 12)	All IBS (<i>n</i> = 22)	d-IBS (<i>n</i> = 13)	c-IBS (<i>n</i> = 9)	Celiac (<i>n</i> = 6)
Mean pressure phase III (mmHg)	14.7 ± 2.7	13.8 ± 0.8	14.3 ± 1.1	13.0 ± 1.1	13.0 ± 1.7
Phase III duration (s)	403 ± 37	407 ± 30	426 ± 40	378 ± 46	341 ± 48
Mean PD phase II (mV)	0.8 ± 0.6	1.9 ± 0.3	1.8 ± 0.4	2.1 ± 0.6	4.5 ± 0.9**
Mean PD late phase I-early phase II (mV)	-0.5 ± 0.7	1.1 ± 0.4*	0.7 ± 0.5	1.5 ± 0.6*	2.4 ± 0.4**
Rate of rise PD phase III (mV/s)	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.09 ± 0.01**

PD, potential difference; IBS, irritable bowel syndrome; d-IBS, diarrhoea-predominant IBS; c-IBS, constipation-predominant IBS; MMC, migrating motor complex. Results are expressed as mean ± SEM. Asterisks indicate significant differences compared with controls (**P* < 0.05, ***P* < 0.01).

and 2) or phase III propagation velocity ($7.1 \pm 1.2 \text{ cm min}^{-1}$ (celiac disease) compared with $6.5 \pm 1.2 \text{ cm min}^{-1}$ (controls). As described previously in the duodenum,¹⁷ maximal PD during phase III and during late phase I-early phase II were significantly higher in both the duodenum and jejunum in patients with celiac disease compared with controls [Fig. 3 (*P* < 0.01) and Tables 1 and 2 (*P* < 0.05)]. Late phase II PD and the rate of rise of the PD signal at the onset of phase III was elevated in the jejunum of celiac disease patients, but did not differ significantly from controls in the duodenum (Tables 1 and 2). The time needed to reach a stable late phase I-early phase II PD was significantly prolonged in the duodenum of celiac disease patients, but not in the jejunum (Fig. 4, *P* < 0.05).

PD changes before, during and after an MMC phase III in patients with IBS

In the whole IBS group, irrespective of dominating bowel symptom, the MMC propagation velocity was significantly increased compared with the controls ($9.3 \pm 1.0 \text{ cm min}^{-1}$ vs $6.5 \pm 1.2 \text{ cm min}^{-1}$, *P* < 0.05). Despite similar increases in pressure, the maximal PD

during phase III was significantly higher in both the duodenum and jejunum in IBS patients compared with controls (Fig. 3, *P* < 0.05). In the jejunum, after phase III cessation, the PD subsequently returned to a stable late phase I-early phase II level that was significantly higher in IBS patients than in controls under the same conditions (Table 2, *P* < 0.05). In addition, the time to reach this level in the jejunum was significantly longer in IBS patients than in controls (Fig. 4B, *P* < 0.01). In the duodenum, neither the late phase I-early phase II PD (Table 1, *P* = 0.06) nor the time to reach a stable late phase I-early phase II PD (Fig. 4A, *P* = 0.24) differed significantly between the IBS patients and controls.

PD changes in IBS subgroups (d- and c-IBS)

To tentatively evaluate the relationship between PD values and bowel habits, we also compared diarrhoea- and constipation-dominant patients separately with controls. The d-IBS group (*n* = 14) had an elevated maximal PD in both the duodenum and jejunum compared with controls (Fig. 3, *P* < 0.05). In both duodenum and jejunum, after phase III cessation, the PD eventually stabilized to a level that was not

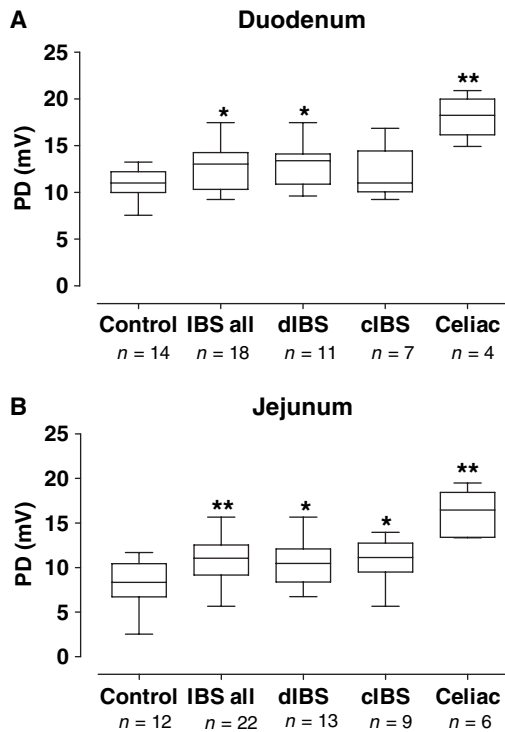


Figure 3 Maximum potential difference (PD) during migrating motor complex (MMC) phase III contraction in healthy controls and patients diagnosed with celiac disease or irritable bowel syndrome (IBS) in the duodenum (A) or jejunum (B). The IBS patients (IBS all) are also subgrouped into diarrhoea-predominant IBS (d-IBS) and constipation-predominant IBS (c-IBS). Data are shown as box plots with median, upper and lower quartiles and range. The different disease groups are statistically compared with the control group. * $P < 0.05$, ** $P < 0.01$.

significantly different from that in control segments under the same conditions (Tables 1 and 2, $P = 0.07$ and 0.10 , respectively). However, the time to reach this level was significantly longer in d-IBS than in controls, and this applied to both the duodenum and jejunum (Fig. 4, $P < 0.05$). The propagation speed of phase III in d-IBS patients was $9.8 \pm 1.3 \text{ cm min}^{-1}$, which was significantly faster ($P < 0.05$) than the propagation speed observed in controls ($6.5 \pm 1.3 \text{ cm min}^{-1}$). There were no significant differences, neither in the duodenum nor the jejunum, in the d-IBS group compared with controls with respect to the following variables: mean pressure increase during phase III, mean PD during late phase II or the rate of rise of the PD increase during phase III (Table 1 and 2).

There were no significant differences between controls and c-IBS patients ($n = 9$) in any of the measured variables in the duodenum (Table 1, Figs 3 and 4). In contrast, in the jejunum, both late phase I–early phase

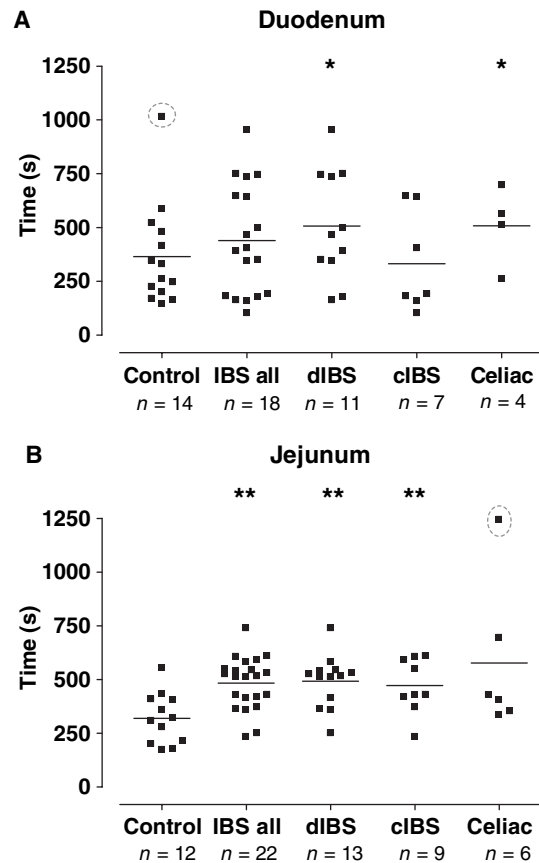


Figure 4 The time required to reach the late phase I–early phase II potential difference (PD) (i.e. the plateau where the PD starts to stabilize) after an migrating motor complex (MMC) phase III period in healthy controls and patients diagnosed with celiac disease or irritable bowel syndrome (IBS) in the duodenum (A) or jejunum (B). The IBS patients (IBS all) are also subgrouped into diarrhoea-predominant IBS (d-IBS) and constipation-predominant IBS (c-IBS). Data are shown as scatter plots with a mean value. Outliers are marked with a circle. The different disease groups are statistically compared with the control group. * $P < 0.05$, ** $P < 0.01$.

II PD and the maximal PD during phase III were significantly elevated (Table 2 and Fig. 3, $P < 0.05$). Furthermore, the time to reach a stable late phase I–early phase II PD was significantly prolonged in the jejunum of c-IBS patients (Fig. 4, $P < 0.05$). The MMC propagation velocity in c-IBS patients was $8.4 \pm 1.4 \text{ cm min}^{-1}$, which was not significantly different from that seen in controls ($P = 0.09$).

Discriminative power of PD signal

We thus observed an elevated maximal PD value in a substantial subgroup of IBS patients. To evaluate the

Table 3 Number of subjects in each group (normals and patient subgroups) outside 'normal range' (defined as above the 95th percentile) of maximal PD during MMC phase III in the duodenum

Variable	Controls (<i>n</i> = 14)	All IBS (<i>n</i> = 18)	d-IBS (<i>n</i> = 11)	c-IBS (<i>n</i> = 7)	Celiac (<i>n</i> = 4)
No	13	9	5	4	0
Yes	1	9	6	3	4
Percent abnormal	7.1%	50.0%*	54.5%*	42.9%	100%**
				(<i>P</i> = 0.09)	

PD, potential difference; MMC, migrating motor complex; IBS, irritable bowel syndrome; d-IBS, diarrhoea-predominant IBS; c-IBS, constipation-predominant IBS. Asterisks indicate significant differences compared with controls (**P* < 0.05, ***P* < 0.01).

Table 4 Number of subjects in each group (normals and patient subgroups) outside 'normal range' (defined as above the 95th percentile) of maximal PD during MMC phase III in the jejunum

Variable	Controls (<i>n</i> = 12)	All IBS (<i>n</i> = 22)	d-IBS (<i>n</i> = 13)	c-IBS (<i>n</i> = 9)	Celiac (<i>n</i> = 6)
No	11	13	7	6	0
Yes	1	9	6	3	6
Percent abnormal	8.3%	40.9%	46.2%	33.3%	100%*
		(<i>P</i> = 0.06)	(<i>P</i> = 0.07)		

PD, potential difference; MMC, migrating motor complex; IBS, irritable bowel syndrome; d-IBS, diarrhoea-predominant IBS; c-IBS, constipation-predominant IBS. Asterisks indicate significant differences compared to controls (**P* < 0.01).

ability of this phenomenon to discriminate between controls and IBS patients, we calculated the number of controls and patients with a maximal PD value above the 95th percentile of control values. The resulting contingency tables are shown in Table 3 and 4. In the duodenum, one healthy subject was incorrectly identified as pathological. 9/18 (50%) of IBS patients (*P* < 0.05) and all (4/4) celiac disease patients (*P* < 0.05) were correctly identified. In the jejunum, the same discrimination algorithm identified 9/22 (41%) of the IBS patients (*P* = 0.06), six of whom were of the d-IBS type (*P* = 0.07) and three of the c-IBS type (n.s.). All celiac disease patients were correctly identified (*P* < 0.01).

DISCUSSION

The design of the present study was based on the use of motor and secretory components of the MMC as a

marker for network behaviour of enteric neurons. In the IBS patients included in the current study, we found an increased propagation speed of motor phase III, an elevated maximal PD in both duodenum and jejunum, and a prolonged time required for PD to return to a steady state level after the end of phase III.

An elevated lumen-negative transmural PD may be due to increased electrogenic absorption of cations (mainly sodium), to increased mucosal electrical resistance or to increased intestinal secretion of anions (mainly chloride). The most important electrogenic transport mechanisms in the upper small intestine are sodium-coupled solute transport (e.g. glucose and amino acids) and electrogenic chloride secretion. In the fasting state, there are no substrates for glucose or amino acid transport, implying that the contribution of sodium-coupled transport to the PD signal is minimal.^{21,22} The change in PD might theoretically be due to modifications in paracellular resistance, which can be measured indirectly with permeability markers²³ or directly on mucosal biopsies in Ussing chambers.²⁴ However, patients with celiac disease have a decreased epithelial resistance,^{24,25} which would reduce but not increase the transmural PD. Thus, it is unlikely that the change in PD seen in these patients is due to changes in epithelial resistance only. Moreover, patients with a defective cystic fibrosis transmembrane conductance regulator (CFTR) mechanism have a normal motility pattern, but do not exhibit changes in PD during phase III contractions.²¹ If the PD response during phase III was due to increased electrical resistance, one would expect an intact PD response in CFTR-deficient patients. Thus, it is likely that the PD response measured in the current study mainly reflects the activation of electrogenic chloride secretion via the CFTR.

Celiac disease increases motility-related PD,¹⁷ and this is accompanied by an increased net fluid secretion.²⁰ Celiac disease was therefore used as an 'internal standard' in the present study. As duodenal data on PD and fluid secretion in celiac disease have been reported previously, we restricted the sample size in this group to six patients, only four of whom actually had a duodenal phase III. This was not considered to be a major problem, as the duodenal data were confirmatory only. In celiac disease, there is a strong inflammatory reaction in the mucosa, which leads to crypt hyperplasia and villous atrophy.²⁶ Mucosal electrical resistance is actually reduced,²⁴ which would *per se* tend to reduce PD in these patients. Accordingly, in Ussing chambers, biopsies from patients with subtotal or total villus atrophy have a reduced, not an elevated PD.²⁴ It is therefore difficult to explain the large PD increase

in vivo solely on the basis of changes in epithelial phenotype or changes in mucosal architecture. In view of the similarities in the shape of the PD curve in celiac disease and that in IBS, it is tempting to postulate the involvement of similar mechanisms. By definition, IBS patients do not have any major degree of mucosal inflammation (this is usually checked in d-IBS only). However, a low-grade upregulation of inflammatory mediators cannot be excluded. Increased levels of celiac disease-associated antibodies, although less severe, have been reported in a subgroup of patients with d-IBS.²⁷

We found striking similarities between the MMC-associated PD response in celiac disease and IBS. For reasons already given, it is hard to account for this pattern solely on the basis of epithelial factors. An alternative mechanism is dysregulation of sensory neuron networks that might, in turn, be due to neuromodulation by inflammatory mediators. Indeed, others have reported lymphocyte infiltration in the myenteric plexus in patients with severe IBS.²⁸ It is well established that the autonomic nervous system can reflexively regulate inflammatory responses²⁹ and that neuroimmune signalling can affect intestinal ion transport.³⁰ It has also been shown that the excitability of a subpopulation of enteric neurons (AH neurons) is increased in a model of colonic inflammation,³¹ and that this increased excitability persists even after the resolution of inflammation.³² There is consequently some support in the literature for interactions between the immune system and enteric sensory network behaviour.

The complex shape of the PD response in association with motor phase III is well compatible with network behaviour of submucous neurons. Motor phase III itself may be due to maximal coordinated firing of AH neurons in the myenteric plexus,^{12,15} which will of course generate a very intense activation of mechanosensitive submucosal neurons. An increased synaptic efficacy in myenteric sensory neuron networks will be expected to increase phase III velocity.^{12,15} If the submucous sensory networks are hyperactive, they will be able to sustain network activity when a mechanical stimulus is terminated,^{12,13} a pattern that might account for the slow decay of the PD signal in some IBS patients. The present results encourage further studies of enteric network behaviour in inflammatory and non-inflammatory intestinal disorders.

The findings that c-IBS patients also had increased maximal PD values in the jejunum may seem contradictory. However, phase III mainly occurs at night (i.e. during prolonged fasting), and the amount of fluid generated during this period can be readily absorbed in

the colon, provided that it is intact and that the transit time is not dramatically reduced. The importance of the colon in relation to bowel habits is illustrated by the fact that constipation occasionally occurs also in patients with celiac disease.²⁴ A concomitant disturbance in colonic mucosal function may indeed occur in d-IBS, as Pienkowski *et al.*³³ have shown that a group of d-IBS patients had a decreased colonic PD compared with controls, a pattern similar to that in ulcerative colitis,^{34,35} although of lower magnitude. When interpreting the current data, one should regard the motility-related PD signal as a marker for submucous plexus activity at the measuring site only.

As clearly shown by the contingency analysis, the duodenal PD signal alone was only able to identify 50% of the IBS patients, and one control out of 14 was erroneously considered pathological. In the jejunum, its discriminative power only reached borderline significance ($P = 0.06$). This situation is by no means unique. In our analysis, we used one of the statistical tests (above the 95th percentile) on which prevalence values for visceral hypersensitivity is based.⁹ Incidentally, the prevalence of this particular disturbance, measured as an elevated sensory response to colorectal distension, was also found to be about 50% in IBS patients.^{3,8,9} Whether PD-positive and visceral hyperalgesia-positive IBS patients represent separate pathophysiological entities, with different clinical profiles, is an intriguing question that needs to be addressed by further research.

In conclusion, we found that the maximal PD during phase III is significantly elevated; the decay time to baseline conditions is prolonged; and the propagation speed of phase III contractions is elevated in a substantial subpopulation of IBS patients. This pattern is compatible with the dysfunction of enteric sensory neuron networks. A substantially larger material is needed to assess the prevalence of this phenomenon, its relation to visceral hyperalgesia, and its relation to symptom profile and clinical outcome.

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