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and gut changes in diabetic neuropathy

Forschungsgruppe Psychophysiologie

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1. Introduction.

Main function of the visceral afferent nerves reaching the central nervous system (CNS) at the level of the spinal cord, the medulla and the midbrain is maintenance of constancy of the internal environment. Information arising from visceral afferents is involved in the generation of a number of reflexes controlling gastrointestinal functions as smooth muscle contraction, secretion, absorption, and blood flow. Perception of hunger, thirst, urgency to urinate or defecate activates also appropriate behavioral patterns to satisfy our biological necessities. We do not need more than our common experience to believe that in such cases consciously perceived afferent information from the viscera reaches the central nervous system, where it is integrated at high levels to generate behavior, behavioral strategies, and behavioral learning. We can normally gain high control on visceral messages consciously perceived: we learn alimentary and bowel habits compatible with our social environment, and at any time we can modify our learned patterns under new environmental requirements. Painful or unpleasant messages from the gastrointestinal tract have as well functional utility as behavioral consequences, e.g. we learn to avoid unsuitable foods, to modify our habits, or to consult a physician.

Less evident, still experimentally widely proved, is the fact that also the unconscious afferent informations from the gastrointestinal tract reaches the brain, where they are integrated and generate physiological and behavioral output. On the footsteps of the pioniering experiments from Pavlov and Haidenhain, further evidence has been searched for a higher hierarchical level exists where also unconscious visceral afferences are integrated. It was demonstrated that painless distension of the duodenal wall causes EEG desynchronization proving that intestinal impulses may elicit electrical arousal reaching the ascending reticular activating system (1). In about 70% of the subjets the reticular activation was not accompanied by subjective sensations. Using verbal feedback it was possible to teach the experimental subjects to detect or to bring into consciousness, intestinal impulses which prior to conditioning had been unconscious (2). Later experiments using verbal feedback confirm that visceral local discrimination tasks can be learned and improved (3). This results indicate that conscious and unconscious intestinal signals can be detected by higher brain centers.

A good model of a feedback circuitry has recently been proposed, integrating at different hierarchical levels and regulating the gastrointestinal homeostasis (4). According to this model external sensory inputs (sight, sound, smell, somatosensory), internal sensory sources, and memory are integrated within neural circuits located in the CNS, the spinal cord, the prevertebral ganglia, and the enteric nervous system (ENS). Enteric motoneurons are the final common efferent pathway modulating intrinsic myogenic rhythmicity. Sensory input results both in conscious perception and in modulation of effector function via reflexes. Alteration in the sensitivity of afferent mechanisms at central or peripheral sites may result in a resetting of connections within the entire computational network, resulting in both inappropriate sensation (pain, distension) and dysmotility. Therefore, any alteration in afferent threshold would be expected to have significant effects on gastrointestinal motility and functions.

In vivo studies in humans are rare, which are able to verify the reciprocal influence of visceral afferent inputs and maintenance of functional homeostasis. A longstanding diabetic population complaining with autonomic and peripheral neuropathy and gastrointestinal symptoms seemed to us a good model for the investigation of 1) eventual impairment of perception and nociception in the lower gut; 2) influence of such impairment on the feedback mechanisms regulating intestinal motility and functions.

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2. Materials and Methods

The perception threshold and the pain threshold in colon was measured in 19 diabetic patients and 19 healthy controls. Assessment of local (motor) and central (sensory) response to phasic and tonic distension of the gut was possible by means of a computerized pumping system and a special colon probe (5).

2.1. Apparatus

The colon probe is a flexible silcolatex tube with a 14 mm external diameter. A ballon tube is fixed at both ends on the carrier tube and inflated trough a PVC tube inside the probe. It can be easily inserted in rectum and sigma-colon using a proper smoothing gel. In our study we inserted it 35 cm ab ano, thus positioning the ballon in the sigma-colon.

The pumping system consists of a syringe-type pump driven by a stepping motor with programmable control unit. It allows to pump volumes up to 500 ml with a resolution of 0.1 ml. Speed is adjustable from 0 to 50 ml/sec in steps of 0.1 ml/sec. We used 50 ml/sec for application of phasic stimuli and 20 ml/sec for tonic stimuli. The ballon predistension before application of the stimuli was done at a 0.2 ml/sec speed.

A specially developed software allows the application of well defined stimuli as well as the recording of intraluminal pressure, subject's answers given by means of a key-board after each trial, and other measured biosignals as heart rate, respiration, and electrodermal activity.

2.2. Patients and subjects

19 longstanding insulin-requiring diabetic in-patients, and 19 healthy controls, 8 males and 11 females in each group took part in this study. All patients complained with peripheral and autonomic neuropathy and gastrointestinal symptoms. 7 patients complained with diarrhea, 8 with constipation, and 4 patients with alternating diarrhea/constipation. Autonomic neuropathy was assessed by Airaksinen's (6) variability score for resting pulse frequency, and Ewing's index (7) of respiratory sinus arythmia. Peripheral neuropathy was assessed measuring warm and cold thresholds at foot. Gastric emptying (half-time) of a semi-solid meal was assessed with scintiscanning method. Results are summarized in Table I.

Table I: Description of patients and controls

	Mean ± SDEV	Range
Gastric emptying [*] [min]	39,3 ± 20,9	8,3 - 78,0
HbA$_{1c}$ [**] [%]	9,0 ± 1,8	5,5 - 12,2
Diabetes duration [yrs]	17,2 ± 6,7	9 - 36
Age [yrs]	48,6 ± 11,6	26 - 72
Age of controls [yrs]	41,6 ± 11,4	26 - 72

[*] Normal values: 12-19 min (8)   [**] Normal values: 4-6% (9)

3.   Procedure

Tests started at 7.30 after a 12-h fast. Enema was given to all subjects 1 hour before. Patients received 1/3 of their usual insulin dosis, and blood glucose was measured after 2 h. None of the sessions had to be suspended because of hypoglycemic risk. All subject lied comfortably on a bed. The application of the probe usually causes no pain, but an initial discomfort, as a cold, foreign body is inserted into the lower intestinal tract. Adaptation usually follows in a few minutes and none of our subject reported further complaints during the test.

Test consisted of a Staircase Procedure (SP) in two runs, which measured the perception threshold in colon at two different predistension levels (10 ml and 60 ml), and a Stepwise Distension Test (SWDT), to measure the pain threshold.

In the SP 30 stimuli are presented to the subject and their detection is tested. Stimuli are phasic volume incraeses of balloon, lasting 5 secs each. In principal, stimulus volume is increased if the stimulus is not perceived and reduced if perceived, so that after a while volume and pressure of stimuli represent the threshold intensity which then is tracked continuously. It is known that perceptual judgements are influenced by some nonperceptual variables which are collectively called response bias. Thus, in order to avoid psychological effects of personality traits on the judgments, we used a special forced-choice-design, which was developed by Wetherill et al. (1966, 10) and first applied in by Jamal (11) to determine thermal sensitivity. In this design each of the 30 trials consists of three subtrials A, B and C. The stimulus is presented either in A or in B. In interval C questions appear on a monitor facing the subject, asking him wether he has perceived the stimulus in A or in B. The subject is told to guess if he is not sure. Subjects give their answers using the key-board. Stimulus volume is decreased after three successive correct answers, and incresed after one wrong answer. Perception threshold is defined as the median volume of the last 15 stimuli. Stimuli range from 1 to 50 ml and are logaritmic scaled in 30 steps. We started with a first stimulus at 19.8 ml for all subjects and patients.

The SWDT consists of a progressive air inflation into the balloon in 20 ml steps given at 120 sec intervals on a balloon predistension level of 10 ml. After each stimulus the subject is asked through the monitor about his sensations. If his rating on the key-board corresponds either to a "definitely unpleasent" or to a "slightly painful" sensation, the SWDT is interrupted and air is pumped out. A maximal volume of 210 ml (10 stimuli) is never exceeded in order to protect subjects from colon overstretching.

4.   Evaluation

4.1.   Data recording

Continuous digital recording of balloon volume, pressure, heart rate, respiration and skin potential was obtained by means of a lab computer and, simultaneously, by means of a writing-recorder (1 mm/s, 1 mbar/mm). As reliable standard computerized methods for global evaluation of such data do not yet exist, results presented in this paper were obtained by means of manual evaluation based on paper-recordings. All pressure values were corrected by subtraction of correspondent values of balloon in air measured immediately before inserting the probe.

4.2. Definition of pressure parameters

4.2.1 Components of Staircase Procedure tracing

Fig. 1 shows the components of a stimulus in the Staircase Procedure:

a) baseline immediately before stimulus
b) artefact, due to pressure rise in the tube between pump and balloon during inflation
c) minimum or short plateau immediately after inflation artefact
d) maximum before stimulus end
e) artefact during volume decrease in balloon
f) first maximum after the stimulus
g) end of stimulus evoked activity

For evaluation we used the pressure before stimulus p_a, which was considered as an index of tonic reaction to predistension and pressure during the stimulation $(p_c + p_d)/2$, which contains information about response to phasic stimuli. As the second artefact (e) interrupts the primary contraction (phasic response, d-f), its maximum could not be interpreted.
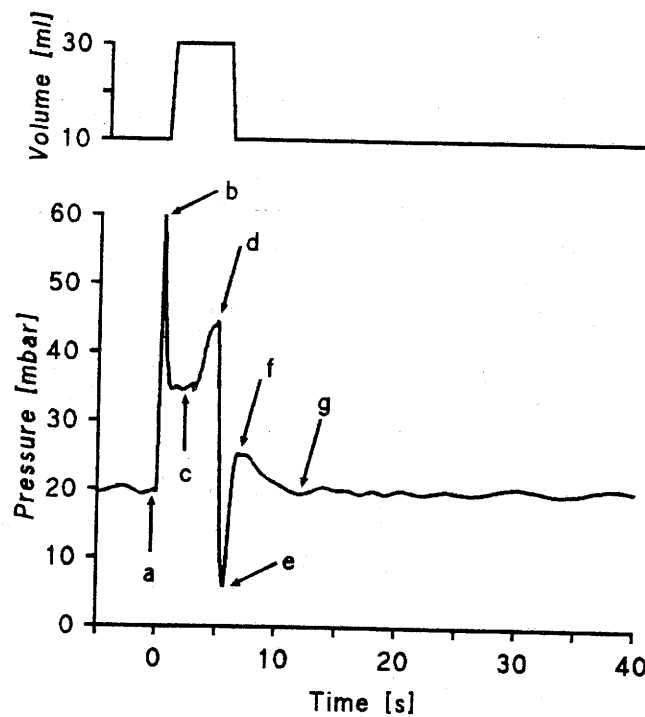

Fig. 1: Pressure tracings in Staircase Procedure

4.2.2 Components of SWDT tracing

Fig. 2 shows the components of a stimulus in the SWDT:

a) baseline immediately before stimulus
b) pumping artefact
c) minimum or short plateau immediately after inflation artefact
d) maximum of primary contraction

e) first minumum after the primary contraction

f) and g) Secondary contractions

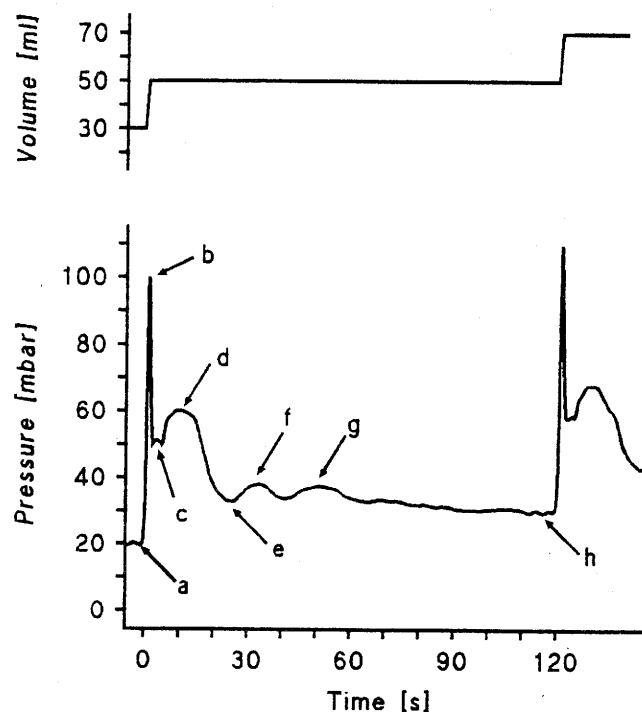h) baseline reached after 120 sec, just before next stimulus

Fig. 2: Pressure tracings in Stepwise Distension Test

In this case we could take in account all parameters, with exception of the pump artifact (b), of course. We considered the difference of pressure p(h) - p(a) as a static reaction of the bowel to the stimulus; the difference of pressure p(d) - p(e) as the amplitude of the primary contraction; the difference of pressure p(d) - p(c) as the active dynamic reaction; the difference of pressure p(c) -p(a) as the passive elastic reaction. We took also in account the latencies of (d) and (e).

4.3. Statistics

Statistical analysis was done by using SPSS-PC+ program. Group differences were tested by means of the Mann-Withney U-Test. ANOVA was also performed in order to test the influence of sex and age. Pearson's r was calculated for correlations between diagnostic results.

5. Results

5.1. Staircase Procedure

At both predistension levels perception thresholds and median of stimulus pressure for threshold stimuli did not significantly differ between patients and controls. They had a trend to be

higher in diabetics (Table II), but the main variance could be explained with "age" as a co-factor (ANOVA). Increase of threshold volume with age was highly significant (p<0.001 on both predistension levels). Distribution of threshold parameters is illustrated in fig. 3.

Perception thresholds of the patients did not correlate with type of gastrointestinal symptoms. Pressure at threshold level did not differ between patients and controls (Table II), even though in the patient group there was a trend to higher pressure at lower predistension level.

Table II: Volume thresholds and threshold pressure in Staircase Procedure

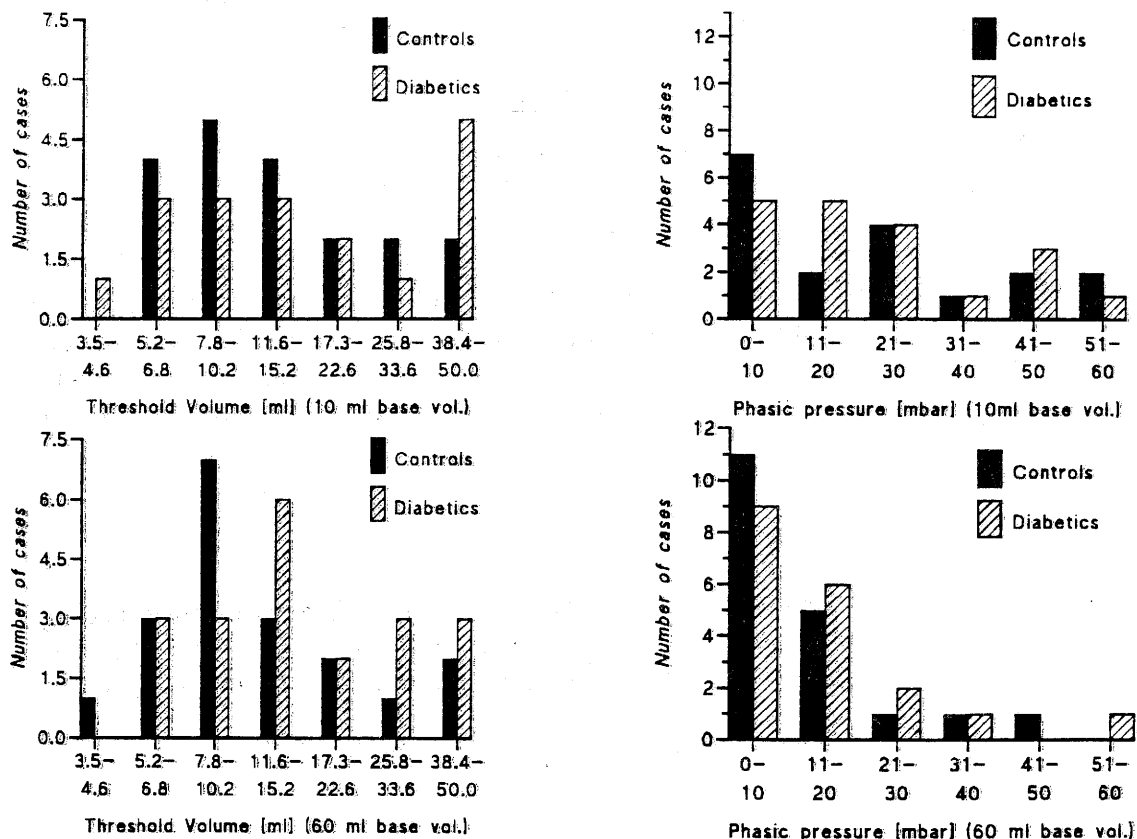|	Basal volume [ml]	Volume threshold [ml]		Threshold pressure [mbar]	
		Mean	σ	Mean	σ
Controls	10	16,0	13,6	21,1	18,8
Diabetics		23,0	16,3	22,4	17,6
Controls	60	15,2	12,3	13,3	10,6
Diabetics		14,3	13,6	14,3	13,6

Fig. 3: Volume thresholds and threshold pressure of controls and diabetics

According to these results, the perception threshold is no diagnostic criterion of colonic neuropathy, nevertheless recordings of intraluminal pressure can deliver useful information

on the state of the gut. Fig. 4 shows example recordings of a control subject (left) and of a diabetic patient (right).
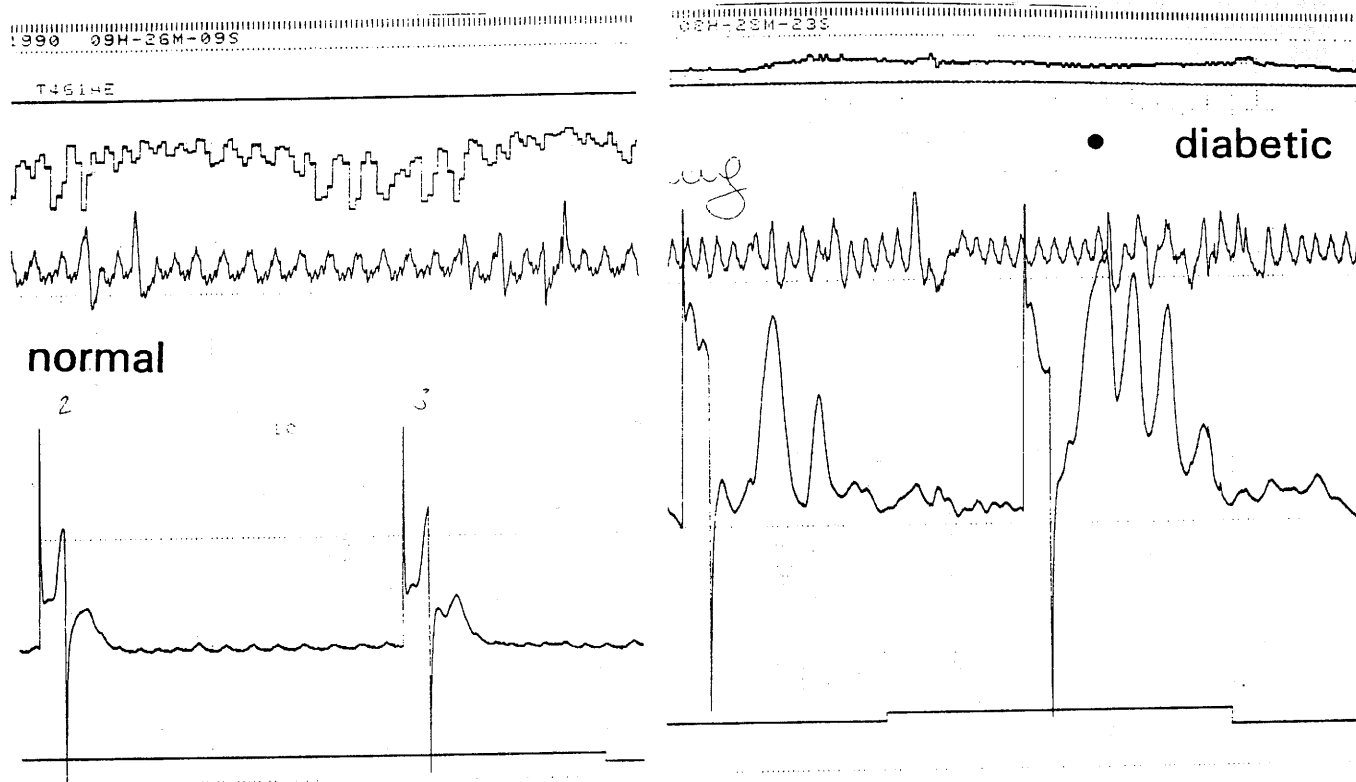

Fig. 4: Examples of records of physiological data in Staircase Procedure (at left: healthy control, at right: diabetic patient. Meaning of tracings, from bottom to top: Stimulus volume, balloon pressure, respiration, heart rate.

The pressure tracing of the subject corresponds to the pattern of fig. 1 (positive artefact, minimum, primary contraction, interrupted by negative artefact, finally return to baseline within few seconds). The patient's tracing however shows repeated contractions after the end of stimulus (at least two at the first stimulus, four at the second). As this pattern never was observed in healthy controls of this study and earlier ones of our group, total number of examinations being > 100, it was regarded as irregular. Such an irregular course of the pressure curve and an abnormal development of the dynamic reactions evoked by stimuli have been found in 10 out of 19 patients (53%), at the low predistension level of 10 ml.

5.2. Stepwise Distension Test

Like the perception thresholds, pain threshold measured by means of the SWDT did not differ in the two groups (Table III). Mean of colonic compliance, defined as tonic adaptation of the bowel to distension (compliance index measured as change of balloon pressure in relation to volume increase) is slightly lower in the patients' group, but the difference was not significant on the 5%-level (U-test). Histograms of these data are presented in fig. 5.

Table 3: Results of Stepwise Distension Test in Controls and Diabetics

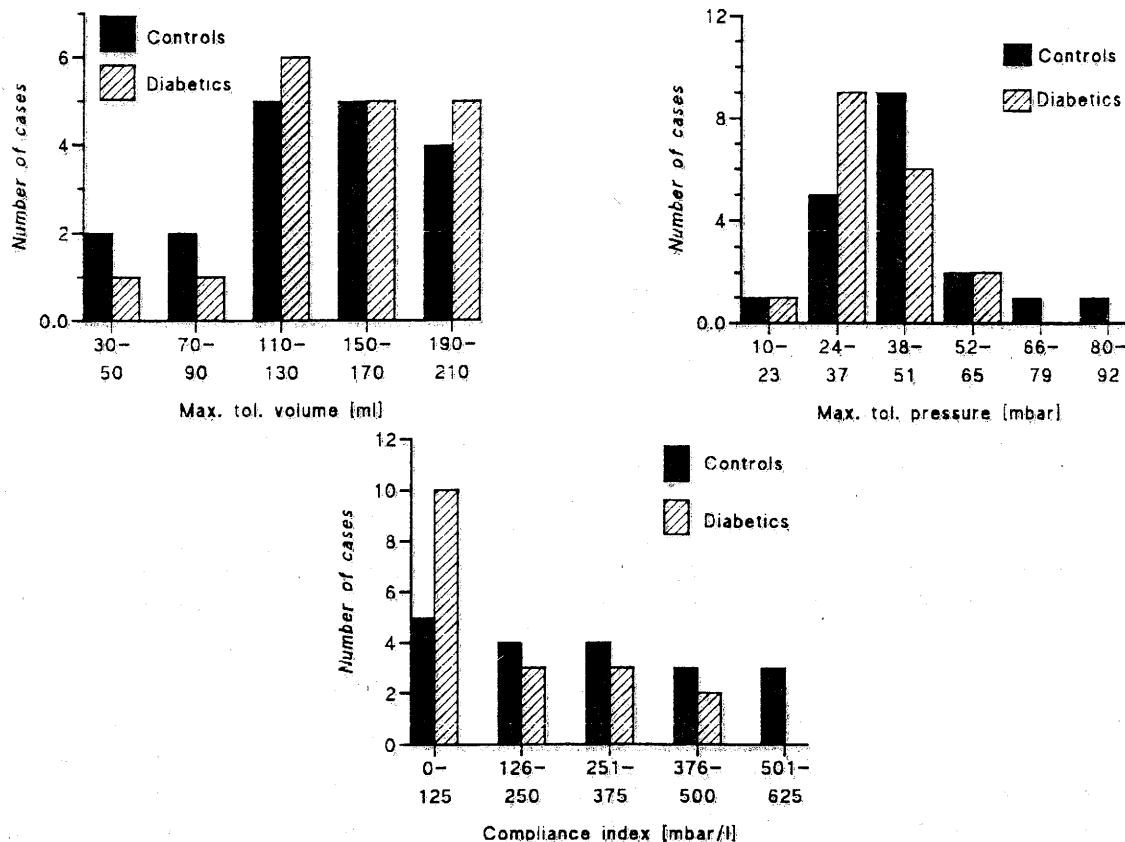|	Controls		Diabetics	
	Mean	σ	Mean	σ
Max. tolerated volume [ml]	139,5	49,6	147,8	48,0
Max. tolerat. pressure [mbar]	43,6	18,4	36,7	10,8
Compliance index [mbar/ml]	0,29	0,18	0,18	0,15

Fig. 5: Max. tolerated vol. (left), max. tol. pressure (right) and compliance index (bottom) in diabetics and controls (no differences)

In the patient curve we can notice an abnormal delay of the primary contraction. Latency of the primary contraction (defined as the time-interval between stimulus begin and peak of the primary contraction) ranges in normal subjects between 5-13 sec and is not influenced by aging, but it was sometimes over 40 sec in patients. It has to be regarded, that not all subjects show a primary contraction in the SWDT, e.g. in this study 3 patients and 3 controls (16%) did not show it. Out of the remaining patients, 44% had latencies higher than the maximum of the controls. Fig. 7 summarizes these results.

Delay of primary contraction was tested by U-Test and found to be highly significant (p<0,002 both for maximum and end of primary contraction).

No correlation was found between abnormal latencies, abnormal oscillatimg pattern and type of gastrointestinal symptoms, metabolic state or time of gastric emptying. No correlation was found also between latencies of the dynamic reaction and perception threshold.
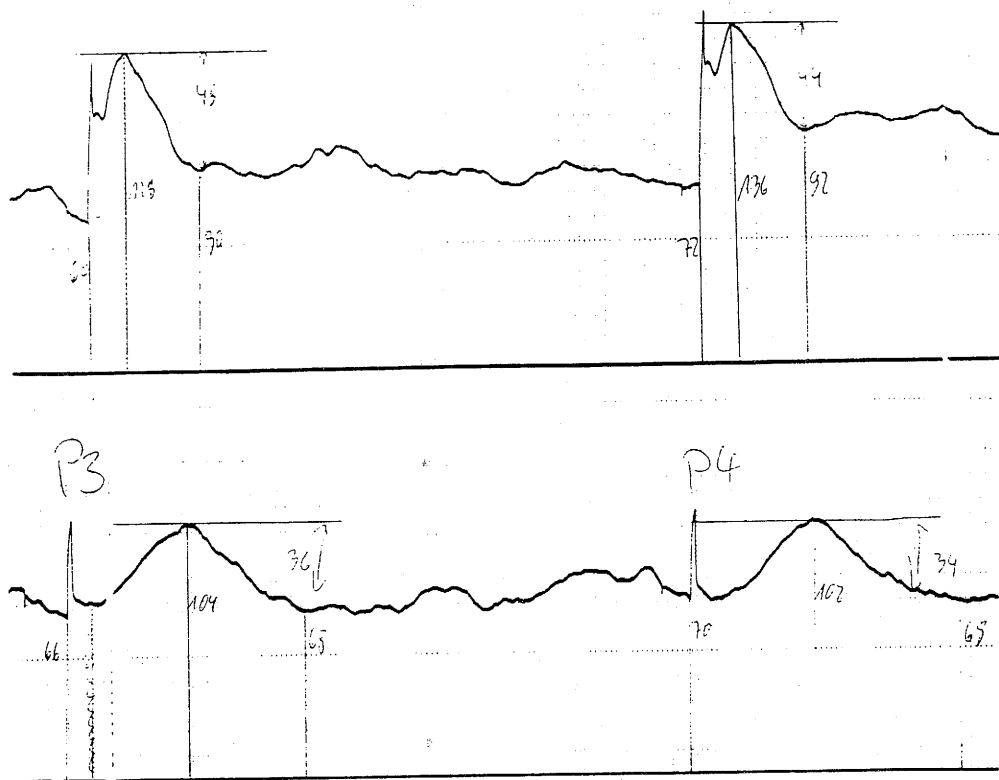
Fig. 6: Examples of pressure tracings of Stepwise Distension Test in a control (top) and a diabetic (bottom). To better compare, examples are from same subjects shown in fig. 4. Amplitudes of primary contraction are comparable, but latency are increased in the patient.
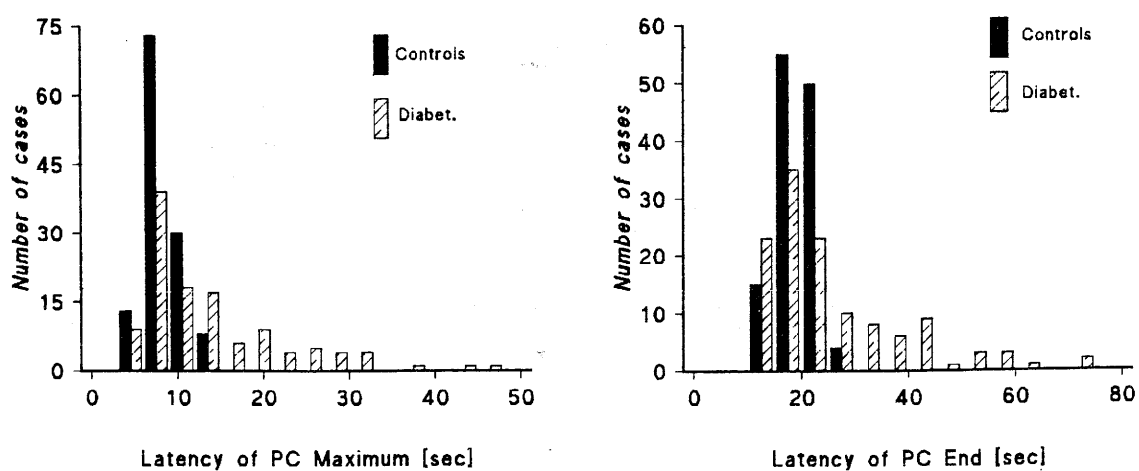

Latency of PC Maximum [sec]

Latency of PC End [sec]

Fig. 7: Latencies of primary contraction maximum (left) and end of primary contraction (right) in controls and diabetics

6. Discussion

The main result of this study is that colonic perception and nociception are preserved in diabetics with autonomic and peripheral neuropathy despite pronounced gastrointestinal symptoms usually attributed to neuro-gastro-enteropathy. A clear explanation of these results is not easy, also because

1) colonic perception and nociception in diabetic neuropathy has been until now very little investigated in human in vivo studies, and

2) the absence of correlation between gastrointestinal symptoms, metabolic state, perception and nociception thresholds and tracings abnormalities stretches the complexity of the clinical feature of the diabetic illness and the multicausal origin of the symptoms.

The not impaired colonic perception and nociception sustains the hypothesis of an integer afferent system. However, since we have not measured concomitant evoked potentials (EP), we do not know if the travel speed of the afferent information is normal or delayed, we can only infer that the information correctly reaches the CNS.

The prolonged latencies of the primary evoked contraction in the SWDT which were found in 44% of the patients resembles delays of many EP components found in similar percentages of diabetics in different studies using acoustic, visual and somatosensory EPs. Such delays have been interpreted as signs of CNS lesions by some authors (12-17) and of peripheral lesions by others (18, 19). Considering the model that we have taken of a feedback circuitry regulating the internal homeostasis, we can only say that the information in input is correct, but not at which level the circuitry is deranged. Therefore, according to our model, we cannot exclude central disorders; nevertheless the hypothesis seems to us improbable. We can formulate at least two possible hypotheses which might explain our results:

1) Since the primary contraction evoked by the stimulus has the pattern and functional features of the spontaneous peristaltic reflex, which is regulated by ENS (20-23), a derangement in the colonic intrinsic innervation can be infered. This peripheral hypothesis is also supported by the oscillations noticed in 53% of the patients in the SP. We have found that the healthy gut displays an evoked contraction after stimulus and rapidly returnes to baseline pressure values, and the the neuropathic gut does not succeed to return to baseline so easily and oscillates in the attempt to regain balance. This too suggests a derangement of the efferent motoric regulation at local site. Morphological animal studies have already shown alterations of the intrinsic nervous system in the intestine as a consequence of diabetes, e. g. reduced number of Auerbach's plexus and degenerated intrinsic axons, as well as extrinsic nerves of the gastrointestinal tract (24, 25). Diabetic changes in the gut are not limited to the nervous system, but extend to other tissues, such as deformed villi, lynphocyte aggregation, blood vascular lesions, increased mucosal weight, decreased muscle thickness, etc. (26, 27). An interesting hypotesis of main local damage defined as pacemaker disturbance has been made (28, 29), which seems to us the best to explain our results.

2) Although the peristaltic reflex can be elicited at local site in the absence of signals from the CNS, the bowel does not normally function without such signals (30). Distal colon receives both sympathetic and parasympathetic innervation, the former supplied by nervus splanchnicus, the latter by pelvic nerve. Thus, centrally mediated reflexes,as well as locally mediated reflexes, are important in normal gastrointestinal function.

Basic motor patterns may be dependant on the activity of intrinsic neurons, but be subject to modification by signals from the CNS acting on command neurons controlling intrinsic enteric circuits (31). Indeed, vacuolation of cell cytoplasm and degeneration of cell bodies has been described in the sympathetic ganglia together with segmental demyelinization and axonal degeneration in the rami communicantes (32-36); morphological changes in the splanchnic nerve, with loss of fibers and paranodal and segmental demyelinization have also been found (37). Therefore, we cannot exclude a damage of the efferent nervous pathways controlling the intrinsic enteric circuits at an higher hierarchical level.

Unfortunately, in none of the above mentiond studies a correlation could be unequivocally assessed between structural and functional damages.

Since also in our study no correlation was found between the abnormalities shown by the tracings and a particular type of gastrointestinal symptom, it is difficult to say which role the neurological impairment plays in the pathogenesis and decourse of the diabetic gastrointestinal complications, also because similar symptoms are often common to other clinical patient subgroups, e. g. IBS, in which such impairments were not found (38). Other authors held also the opinion that autonomic neuropathy or neural changes cannot alone account for the variety and complexity of the gastrointestinal complication of diabetes and stretch the role of metabolic changes (39-43) and / or altered gut hormone and peptide release (44-45).

Therefore we may conclude that neural damages of the colon in longstanding diabetic patient consist mainly of local motor efferent impairment, but metabolic changes and altered hormone release probably play a main role in determining the gastrointestinal symptomatology.

It has to be noticed that the delayed latencies and oscillating patterns that we found in diabetics colon suggest a similarity with the features of other signs of autonomic neuropathy, i.e. orthostatic response, respiratory sinus arithmia, pupil reflex, as in all those tasks which require a rapid adaptation diabetics are impaired.

The neurological findings of the present work require further investigation. Studies on patients with spinal injury or neurological deseases could help to clarify the role of the ENS and of the extrinsic innervation respectively in regulating the colonic reaction to applied stimuli.

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