RECTAL VISCERAL SENSITIVITY AND AUTONOMIC FUNCTION IN FEMALE PATIENTS WITH IRRITABLE BOWEL SYNDROME (IBS)

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2. LIST OF PAPERS

The thesis is based on the following papers referred to by their roman numbers.


Paper II: Spetalen S, Sandvik L, Blomhoff S, Jacobsen MB: Rectal visceral sensitivity in women with irritable bowel syndrome without psychiatric comorbidity compared with healthy volunteers. Submitted


3. ABBREVIATIONS

BMI       Body mass index
BMS       Between-mean square variation
CV_p      The pooled coefficient of variation
ECG       Electrocardiogram
EEG       Electroencephalogram
ERP       Event-related potential
EPQ       Eysenck Personality Questionnaire
HAD       Hospital Anxiety and Depression scale
IBS       Irritable bowel syndrome
ICC       The intraclass correlation coefficient
MDP       Minimal distending pressure
N1        First negative waveform of the ERP
P3        Third positive waveform of the ERP
RC        The repeatability coefficient
RSA       Respiratory sinus arrhythmia
VAS       Visual analogue scale
WMS       Within-mean square variation
4. INTRODUCTION

The management of patients with irritable bowel syndrome (IBS) is challenging, as the exact pathophysiology of the disorder is still unknown. Closer differentiation of different subgroups of IBS seems necessary to explore the pathogenesis. In this thesis we concentrated on a subpopulation of non-constipated IBS patients recruited outside secondary/tertiary care and without psychiatric comorbidity except phobic anxiety.

4.1. Irritable bowel syndrome (IBS) – definition and epidemiology

The irritable bowel syndrome (IBS) is characterized by chronic recurring abdominal pain associated with alterations in bowel habits. Owing to no detectable organic disease, it is referred to as a functional disorder. IBS is diagnosed according to symptom-based criteria, known as the Rome criteria (1 - 3). According to Rome III, IBS is defined as follows: Recurrent abdominal pain or discomfort at least 3 days per month in the last 3 months associated with 2 or more of the following three features: improvement with defecation, onset associated with a change in frequency of stool and onset associated with a change in form (appearance) of stool (3). The criteria should be fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis (3). Further, IBS may be subtyped by predominant stool pattern into IBS with constipation, IBS with diarrhoea, mixed IBS, and unsubtyped IBS (3).

About 5 – 15 % of adults and adolescents worldwide have symptoms consistent with IBS, and the female sex is most heavily affected (4 - 7). The prevalence seems to vary minimally with age (4). It appears that about one third to one half of the sufferers do consult a physician (7, 8), and that 16 – 29 % of those presenting to primary care require referral to a specialist (5, 7,
9). However, in gastroenterologic practice IBS patients constitute up to 30% of the patients (10, 11). One study in general practice indicates that denying a role for stress in symptom generation, frequent defecation, a long history of symptoms and the number of diagnostic tests were predictors of referral (9). In severe cases the illness can result in significant disability and impairment in patient quality of life (12).

4.2. Pathophysiology of IBS
The exact pathophysiology for IBS is still unknown, but today IBS seems to be best looked upon as a disturbed interaction of important biological and psychosocial factors (13). There is increasing evidence that no single factor is necessary to cause IBS and that multiple etiological variables may contribute to the disorder in a single patient (14). In the following, possible etiologic factors will be discussed with the main focus on visceral sensitivity.

4.2.1. Altered visceral sensitivity
4.2.1.1. Definition and background
Hypersensitivity refers to increased sensation of stimuli. Clinicians have for a long time registered that IBS patients are more likely than normal to report tenderness on abdominal palpation (15). IBS patients also have enhanced perceptual responses to physiological visceral events, such as contractions of the viscera (16). In 1973 it was for the first time reported that IBS patients were more sensitive than normal subjects to balloon distension of the colon (17). Currently, visceral hypersensitivity is the leading hypothesis to explain IBS. Indeed, it has been proposed that "altered rectal perception is a biological marker of patients with IBS" (18). Visceral hypersensitivity is found in about two thirds of adult patients with IBS (18 - 25). It has also been recognized in children suffering from this disorder (26).
The gut hypersensitivity in IBS is not stimulus-specific as hypersensitivity is demonstrated for rectal electrical (27, 28) and thermal stimuli (29) as well as for rectal distension. For simplicity, visceral sensitivity is most often examined in rectum in IBS studies, but visceral hypersensitivity has been documented along the entire gastrointestinal tract in IBS patients (30-32). However, one study suggests that visceral hypersensitivity away from colorectum is related to comorbid disorders, for example functional dyspepsia (33). Further, rectal hypersensitivity appears to be a stable phenomenon (34). Several authors have tried to explore if IBS patients in general tend to be more hypersensitive to pain. Most authors claim that the hypersensitivity is restricted to the gastrointestinal tract (19, 31), but more recently widespread somatic hyperalgesia has been associated with IBS (35, 36).

4.2.1.2. Methods in visceral sensitivity testing
Visceral sensitivity is often defined as threshold values for especially discomfort or pain, but threshold values for innocuous sensations such as the first sensation and the sensations of gas and stool have also been examined. Alternatively, visceral sensitivity is measured as scores of symptoms in response to standard stimuli. The literature seems to indicate that the intensity and unpleasantness of the sensations produced by noxious stimuli are relatively independent dimensions of subjective experience, and in some studies they are rated on separate scales (37). In recent years cerebral evoked potentials (38, 39) and brain imaging techniques (40, 41) have been used to measure the cerebral responses to different kinds of gut stimuli.

When testing visceral sensitivity in IBS research, the most common stimulus applied is distension of the gut. Inflating a balloon or flaccid bag can perform gut distensions using a handheld syringe, an automated pump (42) or a barostat (18, 21). Today, the computerized barostat developed about 30 years ago (37, 43) is the matter of choice.
The barostat maintains a constant pressure within an air-filled bag positioned in the gut lumen. When the gut contracts, the barostat aspirates air to maintain a constant intrabag pressure. When it relaxes, air is injected. The barostat may be programmed to distend the gut in a pressure- or volume controlled way. It is recommended to define sensory thresholds by pressure rather than volume because pressure is less vulnerable to measurement error (37). The rate of inflation also has to be defined. Sensory thresholds seem to be higher for rapid inflation than for slow inflation (44), and the differences between IBS patients and healthy controls appear to be greater for rapid distension than for slow distension (45). Further, the balloon may be distended at a continuous constant rate of infusion (ramp inflation), by stepwise increments or by stepwise increments separated by periods of bag deflation (phasic distension) (37).

When combining the distension type (volume or pressure), the inflation rate and the inflation mode (ramp, stepwise or phasic distension), several techniques are developed to test visceral sensitivity. Most simply, an ascending method of limits may be chosen. The bag volume or pressure is then progressively increased using ramp distension, stepwise distension or phasic distension until the subject reports the sensation of interest (37). However, this method is believed to be vulnerable to psychological biases, especially the fear of pain (37). To overcome the problem of psychological bias, methods are designed to deliver phasic distensions in a pseudorandom order. The two most frequent used pseudorandom methods are the tracking technique and the double random staircase method (37). Both these methods define the desired threshold as the average of multiple determinations of the threshold. One study did not find any difference in rectal sensitivity in healthy volunteers using an intermittent semirandom (phasic) and a continuous (slow ramp) distension (46).
All but one (42) of the validation studies designed to date indicates clinically acceptable reproducibility for both barostat (18, 45, 47 - 54) and nonbarostat visceral sensitivity testing in the lower gastrointestinal tract (44, 55 - 58). However, prior to our study (paper 1) data were not available for the double random staircase technique in the rectum.

Another aspect of the intraluminal distension studies is the viscerosomatic reference pattern, whereby the participants are asked to indicate in which area of a schematic drawing of a body they feel the discomfort or pain (59). Aberrant viscerosomatic referral means that IBS patients often notice pain over wider areas and at an increased distance from the site of distension compared to healthy volunteers (18, 60). Alteration of visceral perception may also be defined as sensitization to repeated stimuli (34).

The compliance of the gut reflects the capacity and distensibility of the gut (59), and is defined as the pressure-volume relationship. The barostat do not allow standardization of the distending stimuli when the compliance of the gut varies. Therefore a computerized tensostat has recently been developed that applies fixed tensions levels on the gut wall based on intraluminal pressure and intraluminal volume (61). Visceral sensitivity may also be studied applying transmucosal electrical stimuli to the gut (27,28) or thermal stimulation (29).

4.2.1.3. Clinical associations

It has to be questioned if rectal sensitivity testing may recognize IBS patients from other causes of abdominal pain. One study conducted in tertiary care answered yes, and reported that at a pressure of 40 mmHg the sensitivity of the rectal barostat to identify IBS patients from normal subjects and non-IBS patients was 95.5 % and its specificity was 71.8 % (20). However, rectal hypersensitivity has been documented in other conditions as in women with
dysmenorrhoea (62) and patients with active ulcerative colitis (63). On the other hand, rectal sensitivity was found in normal range in quiescent ulcerative colitis (63, 64) and was decreased in Crohn’s disease (65). IBS may also coexist with other disorders and add to the symptomatology in these patients.

It remains unexplained why not all patients with IBS are affected by visceral hypersensitivity. Patient heterogeneity concerning predominant bowel pattern, symptom severity, psychological factors and subject recruitment procedure is supposed to explain some of this discrepancy. Studies suggest a possible association between sensory thresholds and predominant bowel pattern in IBS patients, but the results are conflicting (56, 66 - 72). Most studies, however, find rectal hypersensitivity in both diarrhoea predominant (56, 66 - 68, 71) and constipation predominant patients (66, 68 - 72). Some authors report increased visceral sensitivity in diarrhoea predominant patients compared to constipation predominant ones (56, 66, 67), others fail to find differences between subtypes (71). The knowledge about visceral sensitivity in IBS patients with alternating bowel habits is modest, but one study indicates that they are hypersensitive to rectal stimuli, lying in between diarrhoea predominant and constipation predominant patients (66). On the other hand, it has been suggested that increased sensitivity could be secondary to diarrhoea (73).

The relevance of visceral sensitivity to IBS symptoms is still uncertain. One study reported that severe abdominal pain was significantly more frequent in hypersensitive than normosensitive patients, but none of the individual IBS symptoms could accurately predict the presence of hypersensitivity (74). In other studies rectal hypersensitivity has also been found to be significantly associated with more severe IBS symptoms (21, 71, 75), but not in all (76).
Visceral perception is supposed to be influenced by psychological factors. A recent paper suggested that increased colonic pain sensitivity in IBS is the result of an increased tendency to report pain rather than increased neurosensory sensitivity (77). Further, IBS patients have altered visceral sensitivity during experimental stress compared with healthy controls (78, 79), and hypnotic relaxation has been found to decrease visceral sensitivity in IBS patients (80). Some authors report an association between psychological test scores and visceral sensitivity (18, 81), but the majority do not (19, 22, 82).

In addition, the chosen distension technique seems to be of importance for the conflicting results in visceral sensitivity studies. With slow ramp distension, IBS patients did not differ in their colorectal sensory thresholds from healthy controls (18, 45). In contrast, studies using rapid phasic distension report decreased sensory thresholds (18, 45). There is some evidence that IBS patients are normosensitive when using an unpredictable distension technique as the tracking method (23). However, the double random staircase method, another unpredictable distension technique, was used in one study reporting increased visceral sensitivity in IBS patients (67). The heightened sensitivity to stimuli of predictable magnitude in IBS patients may indicate hypervigilance, which means that the IBS patients have a greater propensity to label visceral sensations negatively and show lower tolerance for rectal balloon distensions (23).

Further, several other factors are known to influence visceral sensitivity. Rectal sensory thresholds have been found to increase with age (18, 83), but not in all studies (46). These thresholds seem to be reduced with increasing body mass index (84). There are conflicting data regarding gender related differences in visceral perception. In one study female appeared to be more sensitive than male (85), but this was not reported in another study (86). In
irritable bowel syndrome, but not in healthy volunteers, rectal sensitivity increases at the time of menses (87) Decreased colorectal sensitivity postprandially has been reported (25, 88 - 90), and some studies claim that this phenomenon is specific for the IBS patients compared to controls (25, 88, 89). Hyperglycemia is also reported to induce changes in visceral sensitivity (58, 91) and circadian variation of rectal sensitivity is documented as well (92).

When visceral barostat testing has been used to assess the impact of medications on visceral sensitivity and IBS symptoms, there is often little predictability between the two types of outcomes (93). However, μ- and κ- opiate analgesics have been found to have a robust analgesic effect on visceral sensitivity (94).

Although many aspects of visceral sensitivity are explored, little is known of visceral sensitivity in IBS patients without psychiatric disorders and outside secondary/tertiary health care.

4.2.1.4. Mechanisms of altered visceral sensitivity

In humans, visceral pain and discomfort are subjective, conscious experiences, which result from a central interpretation of the visceral afferent input that is influenced by context, memories, and emotional, motivational and cognitive factors (34). The bidirectional communication system between the gut and the brain is referred to as the brain-gut axis, and is mediated through the autonomic nervous system, the neuroendocrine hypothalamo-pituitary-adrenal axis and the neuroimmune pathways. These systems may interact (97).

Although the gut wall contains all of the machinery (intrinsic pacemakers and neural networks) necessary to programme the activity of the smooth muscle, it receives extrinsic
innervation as well (96). The distal colon and the rectum receive parasympathetic innervation via the sacral roots (S2-S4) through the pelvic nerves in opposite to the proximal gut that receives innervation through the vagus nerve (96). The sympathetic innervation is mediated through the spinal nerves (95), projecting to the thoracolumbar cord. Within the spinal horn, spinal afferents integrate with Lamina I neurons, which also receive descending (inhibitory and facilitatory) fibres from the brainstem and hypothalamus (97). The parasympathetic afferent nerves mediate rectal filling sensation (98, 99) whiles the physiologic role of the sympathetic afferents is poorly understood. However, there is evidence that these nerves mediate feelings of abdominal pain in IBS patients (18, 45). Visceral sensations are transmitted from the gut via afferent nerves to the spinal cord and to the brain where their painful characteristics are perceived.

The causes of the visceral hypersensitivity in IBS however, are still unclear. Principally, both peripheral and central mechanisms may be involved. Up to date, there are no valid instruments to measure the neural response to visceral distension at the level of the gut, the spinal cord or the brain stem in humans, but the cerebral responses may be measured using neuroimaging techniques and cerebral evoked potentials.

Several studies are supporting peripheral mechanisms involved in the development of visceral hypersensitivity in IBS. As will be discussed in section 4.2.5, IBS may develop after gastrointestinal infections (100, 101) and increased number of inflammatory cells has been reported in the gut wall in IBS patients (101, 102). The inflammation may lead to sensitization of primary afferent nerves due to the release of inflammatory mediators (95). Further, the development of such peripheral sensitization of visceral afferent fibres seems to cause long-lasting sensorimotor disturbances of the gut when the inflammation subsides.
Increased rectal sensitivity after rectal installation of glycerol (104) and decreased sensitivity after rectal installation of lidocaine (105) also support peripheral mechanisms in the development of visceral hypersensitivity. Changed expression of several receptors on visceral afferents seems to be involved in gut hypersensitivity, including the transient receptor potential vanilloid type I receptor (TRPV1 receptor) (95).

Enhanced nociceptor input to the spinal dorsal horn neurones leads to amplified responses to both noxious and innocuous inputs, referred to as central sensitization (95). This process alters the receptive field properties and pain sensitivity, causing tissue hypersensitivity far beyond the original site of injury (95).

There is increasing evidence supporting abnormal cerebral processing of visceral sensation in IBS patients. Several regions of the central nervous system are known to process visceral sensory information: brainstem nuclei, insula, anterior cingulate cortex, somatosensory cortex and orbitofrontal cortex (95). Different dimensions of visceral sensation and pain are processed at these different regions. For example, the insula processes information of the internal state of the organism and is the main integration center of the autonomic nervous system and visceral afferent signalling, the anterior cingulate cortex mainly processes pain affect and the somatosensory cortex is important for the sensory-discriminative pain dimension (95). In IBS, abnormal brain processing of rectal sensory signals has mostly been reported in subregions of the anterior cingulate cortex (95). However, conflicting results are reported in the brain imaging studies. Some studies report increased activity in the anterior cingulate cortex in IBS patients compared to healthy controls (40,106), others report decreased or absent activity (107,108). Both altered visceral afferent input to the brain and changed cognitive responses, for example hyperresponsivity to stress and hypervigilance,
may explain these altered cerebral processing. Animal models show evidence for visceral hypersensitivity induced by stress (109,110).

Further, visceral nociceptive transmission is also subject to central descending facilitatory pathways and inhibitory pathways (95). It is supposed that a disturbed balance between the facilitatory and inhibitory pathways may lead to changed visceral sensitivity.

4.2.2. Disturbed gut motility

Earlier studies on the pathophysiology of IBS focused on motor abnormalities of the small and large bowel, often examined by manometry. The most consistent motility disturbance recorded is probably increased contractile activity of the colon postprandially (111, 112). Other examples of reported disturbed gut motility in IBS include enhanced colonic motility in response to emotional stress (113), recto-sigmoid distension (114) and corticotropin-releasing hormone (115), and enhanced small bowel motility in response to meal ingestion (116). However, these results are often conflicting and no clear association between motility patterns and symptoms have been obtained (117, 118), except for stool patterns. Diarrhoea predominant IBS patients seem to have increased colonic motility (111, 114) while constipation predominant IBS patients appear to have reduced motility (119).

Compliance and tone are other important features of the rectal motor function, often assessed by a barostat. Previous studies have shown that IBS patients have lower rectal compliance compared with controls in response to balloon distensions (68, 72), but not all studies agree (25, 31). Changes in gut balloon volume when the pressure is kept constant, can be interpreted as change in visceral tone (68). Basal rectocolonic tone in IBS patients appears to be normal
(25, 88, 120) or increased (83, 121). Further, IBS patients are reported to have normal postprandial tone (88).

4.2.3. Dysregulation of the brain-gut axis

Dysregulation of the brain-gut axis has been implicated in the pathophysiology of IBS (122, 123). Altered function in one or the other of the two branches of the autonomic nervous system appears to exist in a subgroup (26 - 52 %) of patients with IBS (124 - 126). However, conflicting results exist as to how this imbalance is expressed. Some studies indicate altered sympathetic activity (127 - 130) and/or parasympathetic activity (125, 126, 131 - 137), or specific autonomic functional abnormalities only in particular symptom subgroups (138-143). However, it is uncertain to what extent disturbed autonomic function in IBS is a consequence rather than a cause of IBS.

The neuroendocrine hypothalamo-pituitary-adrenal axis is another important system in the communication between the brain and the gut. However, investigations of this axis are also inconsistent, and normal (78), increased (79) or decreased (144) reactivity is reported in IBS.

The gut contains the largest component of the immune system in the human body, and complex interrelationships exist between gut associated immune tissue, the central nervous system and the enteric nervous system (122). For example, the enteric mast cells are supposed to be an important mediator of such neuroimmune interactions (145), and increased number of mucosal mast cells has been documented in biopsies from patients with IBS (146,147).
Evidence for disturbed cerebral function is mainly derived from studies investigating cerebral responses to visceral stimuli, as discussed in section 4.2.1.4.

4.2.4. Psychosocial factors

Comorbid psychiatric disorders are common in patients with IBS. According to systematic reviews of the literature, 40% to 94% of the IBS patients meet criteria for any Axis I psychiatric diagnosis (148, 149). The most frequent psychiatric disorders in IBS patients are the same as the ones most prevalent in the general population (14). Depression is most common, followed by anxiety and somatization disorders (14). IBS patients have higher psychiatric comorbidity than general medical patients and patients with organic gastrointestinal disorders (14). However, it is still unclear if individuals with symptoms of IBS not consulting health care have increased psychiatric comorbidity (150, 151).

In addition, increased prevalence of both comorbid functional diagnosis, for example fibromyalgia and chronic fatigue, and biomarker – based somatic diagnosis, for example infections, are reported in IBS (152). It is suggested that this excess comorbidity in IBS is caused from a general amplification of symptom reporting and physician consultation (152).

Maladaptive personal characteristics are also associated with IBS. Neuroticism, a stable personality trait characterized by distress-proneness and negative bias in thinking (14), seems to be elevated in IBS (14, 153). Additionally, the cognitive trait of catastrophizing (14) and the subjective experience of emotional distress seem to be elevated (154, 155). It is claimed that neuroticism and catastrophizing may mediate the heightened distress and psychiatric symptoms seen among IBS patients (14). Recently, it has been suggested that anxiety about specific gastrointestinal sensations is important for the maintenance and perhaps the
development of IBS symptoms (153, 156). Stressful life events, especially a history of sexual abuse (157, 158), but also other threatening events as break-up of an intimate relationship (159), are reported to precede the onset of IBS more often than organic GI-disorders. Further, IBS patients tend to have a reduced threshold for experiencing illnesses as distressing and acting in response to them by health care seeking (160, 161). Altogether, IBS patients seem to have a generally negative emotional tendency in their cognitive processing strategies (59), and most of the adverse psychosocial factors have been shown to be related to symptom severity and the vulnerability to develop IBS (14).

Treatment responses support the influence of psychological factors in IBS. For example, a recent study showed that cognitive behavioural therapy in IBS patients was associated with reduced limbic activity, GI symptoms, and anxiety (168). There are also evidence for positive effect of hypnotherapy (169) and psychoactive medication (170) in the treatment of IBS.

4.2.5. Other suggested pathophysiologic mechanisms

Genetic factors alone do not seem to be able to explain IBS onset, but may be able to interact with environmental factors for the full clinical expression of the disease (165). Approximately 33 % of outpatients with IBS report a positive family history of IBS, compared with 2 % of outpatient controls (166). 26-34 % of the IBS patients have a parent with IBS compared with 13 % of control patients (167). In twin studies the genetic contribution to IBS appears to range from 0 – 20 % (168). Thus far, it has not been possible to identify an “IBS gene” although several candidate gene association studies have been performed (168).

Subtle microscopic abnormalities have recently been documented in IBS (169), and infectious gastroenteritis has been identified as the most significant environmental risk factor for the
development of this disorder (170). IBS seems to develop in 7% to 31% of infected patients (169) with the strongest risk factor being the duration of diarrhoea during enteritis (171). Other risk factors are female sex (171), the presence of depression, anxiety (172), and hypochondriasis (173), as well as reported more life events in the previous 12 months (173). Increased number of mucosal lymphocytes and enteroendocrine cells as well as increased mucosal permeability appear to persist for at least 1 year after infection in those developing postinfectious IBS (102). Further, the proinflammatory cytokine, interleukin-1β, appears to be increased in those developing postinfectious IBS (174). IBS patients without a history of gastrointestinal infection also seem to have immune activation, and increased number of mucosal lymphocytes has been reported (175). Interestingly, the abdominal pain in IBS correlated with the number of mast cells located in close proximity to the enteric nerves (147), and hypersensitive, diarrhoea predominant IBS-patients manifested increased number of enteroendocrine cells in their rectal mucosa compared with normosensitive IBS patients (176).

Recently, it has been shown that gastrointestinal microbiota are significantly altered in IBS and that their composition varies with the main symptoms of the patients (177, 178). The role of small intestinal bacterial overgrowth has also been studied in IBS pathogenesis, but the results are conflicting (179, 180). Additionally, abnormalities in the serotonin metabolism (181, 182), gas handling (123) and gastrointestinal secretion (123) have been suggested in IBS.

4.2.6. Models of the pathophysiology in IBS

To explain the association between IBS, sensorimotor function and psychosocial factors, several hypotheses have been suggested (95). As recently reviewed, these hypotheses may be
summarized as four main models (95): Firstly, there may be a direct biological interaction between psychosocial factors and gut sensorimotor function mediated by the brain-gut axis. Here, the psychosocial factors are supposed to be the initiating ones. Secondly, psychosocial factors may affect gut sensitivity through “psychological” processes that may influence symptom perception and/or reporting. Examples of such psychological processes are arousal, anticipation, interpretation of bodily feelings and visceral-specific anxiety. Thirdly, it is supposed that psychosocial factors do not have any direct influence on gut sensorimotor function, but perhaps only influence health care seeking or quality of life in IBS patients. Lastly, it may be claimed that psychosocial factors, gut sensorimotor function and IBS are manifestations of a common predisposition. Whatever model one believes in, most researchers agree that IBS is caused from an interaction of both biological and psychosocial factors. It is suggested that both visceral signals and the brain regions processing such signals, are crucial in the generation and regulation of emotions and feelings. Further, although the symptom picture at diagnosis seems to be functional, it may have had an antecedent peripheral initiating event as gut infection. It is also likely that different pathogenetic factors may be involved in different subgroups of IBS.

5. AIMS OF THE STUDY

The overall aims of the study were to:

1: evaluate the reproducibility of the rectal sensitivity using a barostat double random staircase method in healthy volunteers and patients with IBS (paper I).

2: evaluate rectal sensitivity in IBS patients without comorbid psychiatric disorders compared to healthy volunteers (paper II)
3: investigate if measures of autonomic function differ in IBS patients from healthy volunteers (paper III)

4: investigate how comorbid phobic anxiety influence brain information processing of auditory stimuli and visceral sensitivity thresholds in IBS patients (paper IV)

6. METHODOLOGY

6.1. Subjects

Twenty-two women recruited from the files of collaborating general practitioners and 210 female respondents to a newspaper advertisement in the Norwegian cities Oslo and Fredrikstad were screened for participation in the study. The screening process included mailed screening questionnaires with respect to IBS criteria, a mailed Hospital Anxiety and Depression (HAD) scale (183) to screen for psychiatric disorders and a telephone interview. Eighty-nine subjects who seemed eligible then underwent most of the following procedures: clinical assessment, screening blood tests, rectoscopy, a double-contrast barium enema or colonoscopy if not performed during the previous 2 years and a psychiatric examination, including the diagnostic MINI International Neuropsychiatric Interview (184). Symptoms according to the Rome criteria I (1) (appendix 1) of at least 1 year’s duration, were required for inclusion in the study. Only females were included.

To ensure that the disease was in an active phase of at least moderate severity, both the patient and the physician had to rate the disorder to at least 5 on a 0-10 visual analogue scale (VAS) measuring current global IBS severity, a score of 0 representing no IBS symptoms. Subjects were excluded if they had a constipation predominant IBS subtype, any organic disease of importance, a HAD score > 18, a previous history of psychotic disorder and any current
psychiatric disorder except for phobic anxiety in accordance with DSM-IV axis 1 criteria. The neuroticism subscale of the Eysenck Personality Questionnaire (EPQ) (191) were also filled out and reported in paper IV. The IBS patients recorded the daily number of evacuations and rated daily levels of pain and gas discomfort on a visual analogue scale ranging from 0 (no symptoms) to 10 (maximal severity) for 1 week immediately before the investigations (reported in paper IV).

Fifty-two IBS subjects constituted a pool of patients used in the studies. Eleven of the IBS patients had comorbid phobic anxiety disorders, and the rest was without psychiatric disorders. In the phobic anxiety group, seven subjects had a specific phobia (height, snakes, or spiders), two subjects had agoraphobia without panic, and two subjects had a nongeneralized social phobia. The IBS patients were classified as having diarrhoea predominant IBS or IBS with alternating stool habits. Eighteen IBS subjects examined twice were included in study I concerning reproducibility of the barostat measurements. In study II 38 IBS patients without psychiatric disorders and 11 IBS patients with comorbid phobic anxiety were included. In study III the 33 IBS patients with appropriate autonomic testing were included; also encompassing 5 women with comorbid phobic anxiety. The study population in study I and II included IBS patients both with and without comorbid phobic anxiety. The 11 IBS patients with comorbid phobic anxiety and 22 age-matched noncomorbid IBS patients were included in study IV.

A total of 25 healthy women were enrolled as controls. All except one were students or health care workers. They had no history or symptoms of somatic or psychiatric disease.
The study was approved by our regional ethics committee (Ethics Committee in Health Region 2 of Norway) and performed according to the Declaration of Helsinki.

6.2 Methods

A computerized polygraph (Synectics Visceral Stimulator; Synectics, Stockholm, Sweden) was used to record data continuously and simultaneously from the barostat, ECG, pneumograph belt and skin conductance at a sampling rate of 32/s. The event-related potentials (ERP) used to evaluate cerebral function, were examined on separate occasions.

6.2.1. Volume-displacement device and anal manometry

A computer driven barostat (Synectics Visceral Stimulator; Synectics, Stockholm, Sweden) was used to inflate a rectal balloon. The balloon was an 8 cm long cylindrical plastic bag, infinite compliant when intrabag volumes were below 500 ml, and tightly fixed at both ends to a multilumen catheter. One lumen was used for inflation of the bag with air (38 ml per second) and another was used to measure pressure within the bag. Three lumens were perfused with saline in order to monitor the anal pressure and were connected to external pressure transducers and a Synectics polygraph. The manometric ports were located 4, 4.5 and 5 cm distal to the caudal end of the barostat bag.

Rectal barostat pressure and volume and anal manometry were continuously registered, and the sampling rate for the barostat-manometry assembly was 32 per second. The lubricated balloon was inserted into the rectum via an anoscope so that the saline-perfused manometry system monitored the pressure in the anal high-pressure zone. The tube was secured in its proper position with tape. To rule out any leak the barostat bag was inflated before use and
tested in water. Respiratory excursions were recorded on one channel from a pneumograph belt positioned around the lower chest.

6.2.2. Skin conductance

Many methods exist to examine sympathetic function. In this work we used skin conductance. It is considered to be a direct and undiluted representation of sympathetic (cholinergic) activity (186). The skin surface was cleaned carefully before placing one pair of Ag/AgCl cup electrodes on the volar surface of the distal phalanx of the dominant hand's fore and middle finger. The electrodes were placed in tight contact with the skin using NASA electrode pasta (hydroxyethyl cellulose 6%, NaCl 0.58 %, methyl parahydroxybenzene 0.1 %, propyl parahydroxybenzene 0.1 %, ethanol 2 %, water 91.2 %). Three measures of skin conductance are given: skin conductance level (baseline), skin conductance latency and skin conductance amplitude. The skin conductance latency was defined as the temporal interval between stimulus onset and initiation of the skin conductance response (186). The skin conductance amplitude was expressed at the phasic increase in skin conductance shortly following stimulus onset (186).

6.2.3. Respiratory sinus arrhythmia (RSA)

Respiratory sinus arrhythmia (RSA) is known as fluctuations of heart rate associated with breathing, with heart rate acceleration during inspiration and heart rate deceleration during expiration (187, 188). It is considered to be a selective index of parasympathetic cardiac vagal activity (187, 188), while the heart rate is modulated by both autonomic systems. Several methods for quantifying RSA exist, for example spectral analysis (188). We used another well-known method (the “peak-to-trough” method) because it was easier to implement in our software program thus allowing us to simultaneously record data from the gut and the
autonomic nervous system. This method quantifies RSA as differences in heart rate corresponding to phase of respiration and was manually derived from the screen on a breath-by breath basis (187). The highest heart rate for each breath was required to accompany inspiration, and the lowest heart rate had to occur during expiration. When heart rate fluctuations were not clearly associated with these specific phases of respiration for a particular breath, that breath was assigned a RSA score of zero and averaged into the analysis. We calculated the average RSA for ten respiratory cycles, and the values were transformed form heart rate to interbeat interval (ms). Standard cutaneous electrodes for ECG recordings were applied to the lower left side of the thorax and below the clavicula bilaterally, all placed on the mid-clavicular line. One computer recorded the ECG and the heart rate on two separate channels. Respiratory excursions were recorded on one channel from a pneumograph belt positioned around the lower chest.

6.2.4. Event-related potentials (ERP)

Event-related potentials (ERP) represent the brain electrical potentials in preparation for or in response to internal or external discrete events (189). Visually or auditory presented stimuli have usually been used in psychophysiological research. ERPs are registered through ordinary electroencephalogram (EEG) procedures, and often an oddball paradigm is used, in which a person is instructed to attend to a target stimulus in a mixed stimulus series (190, 191). The paradigm used in paper IV of this thesis was an ERP oddball distractor paradigm consisting of three conditions, each containing 200 stimulus presentations. A standard tone (800 Hz, 60 % probability), a target tone (1200 Hz, 20 % probability), and a distractor (everyday words, 20 % probability) were in each condition presented by headphones. The distractors were Norwegian words with emotional content, selected from validated translations of scales for anxiety, depression, and irritability (192 - 194). In the analysis, the ERPs from all three
distractor conditions were pooled together in the analysis. Continuous EEG activity was recorded from the frontal, central and parietal midline electrodes (195).

The first negative wave (N1) in the ERP in an auditory paradigm is a correlate of sensory processing and attention (196). Later occurring components, in particular the third positive wave (P3), are involved in stimulus evaluation and interpretation. N1 latency and amplitude to standard tones, target tones, and distractor words were analyzed at all electrodes. To capture the continuous nature of auditory language processing, the mean amplitudes to target and distractor stimuli in consecutive 50-msec time bins from 150 to 600 ms were additionally analyzed (197).

6.2.5. Study design

All experiments involving barostat examinations were carried out following a minimum 6-hour fast and following the application of one Klyx enema (120 ml, Ferring A/S, Kbh). The subjects were placed in the left lateral position in a bed. The examiner was always present, and the information given was standardized in a written protocol.

Every experiment started with unfolding the balloon to a volume of 200 ml or until the participants reported discomfort. The barostat balloon was then inflated to a minimum distending pressure (MDP), defined as the pressure at which respiratory excursions were regularly recorded, and the barostat pressure was kept constant for 15 min. All participants were then exposed to emotional stress. We used tones and everyday words with emotional meaning (emotionally loaded words) as described in section 6.2.4. Due to the technical facilities available, the stimuli were given in a fixed procedure, first with words representing
anger, then words representing sadness and at last words representing anxiety. The exposure
to each type of emotionally loaded words lasted for 9.5 min.

Visceral sensitivity was assessed using a double random staircase. This technique consisted of
a computer-controlled random application of two identical series of distension stimuli. In
protocol 1 the amount of each pressure increment was 1 mmHg lasting 20 s, and the pressure
within the rectal balloon was then lowered to a baseline pressure (MDP) for 5 s. In protocol 2
the amount of each pressure increment was 4 mmHg lasting 20 s, and the pressures within the
rectal balloon was then lowered to a baseline pressure (0 mmHg) for 30 s. The subjects were
asked to report the first feeling of gas and stool, and to press a button (after a signal 5 seconds
before the end of the step) when discomfort was experienced. At the discomfort level the
subjects rated the intensity of this feeling on a 100 mm visual analogue scale (VAS) ranging
from no discomfort (0 mm) to maximal imaginable pain (100 mm). The procedure was
stopped when the subjects had reported discomfort three times. After an at least 5-min rest,
the rectal balloon was inflated to the rectal discomfort pressure, and the pressure kept constant
for 1 min (the rectal stepwise discomfort test).

When we started our study, we designed a barostat protocol (protocol 1) that turned out to be
too detailed and time-consuming. Therefore we designed a shorter barostat procedure
(protocol 2). Twenty-one healthy women went through protocol 1. All IBS patients and 9
healthy volunteers were examined using protocol 2. Five of the volunteers were tested with
both methods. As reported in study I fifteen healthy volunteers and three of the IBS patients
were retested after two days. In addition 15 IBS patients, all receiving placebo in a double-
blinded clinical study, were retested at the end of the placebo-period (3 months). This study
has not been published.
In study II we evaluated the position’s influence on the pressure-volume relationship in 5 of the healthy volunteers. A device working as a back support controlled the position of the participants. The subjects were investigated in the supine position (0°) and in the left lateral position at 45°, 70°, 80° and 90°. The barostat bag was distended with a stepwise, ascending method of limits in every position. Each pressure increment was 5 mmHg and lasted 60 seconds. The procedure was stopped when 40 mmHg was reached or if the participant asked for it because of discomfort. Between every position there was a pause of 5 minutes.

The ERP registrations were done on separate occasions as briefly described in section 6.2.4.

6.2.6. Data analysis

Sensory thresholds were expressed as target pressures. The corresponding volume and the calculated tension at the end of the stimulus were also reported (paper I and II). The discomfort threshold was defined as the average of the first three steps on which the subject gave a positive response. The intensity of the discomfort sensation is reported as the mean VAS value. Wall tension in the rectal wall was calculated using Laplace’s law assuming that the distended bag had a cylindrical shape (47).

Additionally, in paper IV the differences in pressure between sensations of stool and gas were taken as the gas-stool tolerance; the differences between gas and discomfort as the gas-discomfort tolerance. In paper IV the rectal wall compliance defined as the volume/pressure relationship was calculated at all thresholds.

Rectal tone was expressed as the mean 1-min value of the barostat volume after 13-min baseline registration. During the double random staircase procedure the mean volumes at two
distensions reached by most of the participants (8 mmHg and 24 mmHg) were also registered (reported in paper II).

In paper III measures of autonomic function are reported. Heart rate, RSA and skin conductance at rest were estimated as the average of the mean 1-min values at 3-, 8- and 13-min baseline registrations. The baseline volume was only estimated at 13 min because this time was required for baseline stabilization. As a response to emotionally loaded words the same parameters were estimated as the average of the mean 1-min values at 5 and 7 min after the beginning of each word series. The autonomic responses to the emotionally loaded words were defined as the differences in autonomic measures between baseline and the different emotional states. The heart rate and RSA response to rectal discomfort was defined as the difference between the values before and during rectal discomfort. The skin conductance response to rectal discomfort was measured as skin conductance latency and amplitude.

6.2.7. Statistics
For comparison of means paired or unpaired Student’s t-test was used when appropriate, with a 5 % significance level. Associations were investigated using the Pearson’s correlation coefficient.

In paper I the following three indexes were used as measures of reproducibility: the intraclass correlation coefficient (ICC), the pooled coefficient of variation (CVp), and a repeatability coefficient (RC). The ICC (198) was calculated using the equation

$$ICC = \frac{(BMS-WMS)}{BMS}$$
in which BMS is between-mean square variation and WMS is within-mean square variation. ICC is considered to demonstrate acceptable reproducibility (for group comparisons) if it is larger than 0.80, and ideal reproducibility if it is larger than 0.90 (199).

Pooled CVs in percent for a group of observations (200) were calculated using the equation

\[ CV_p = 100 \% \cdot \frac{\sqrt{(\Sigma d^2)/2n}}{x} \]

in which \( d \) is the difference between two results obtained from one participant, \( n \) is the number of the subjects, and \( x \) is the mean of the results obtained from all the participants.

The RC (201, 202) was defined as

\[ RC = 2 \times \sqrt{2s_w^2} = 2.83 \times s_w \]

in which \( s_w \) is the within-subject standard deviation, the common standard deviation of repeated measurements. It was calculated using one-way analysis of variance. The difference between two measurements for the same subject will then be less than the RC for approximately 95% of the subjects.

In paper I plots of the difference between test and retest against the mean of test and retest ("Bland-Altman plots") were also constructed according to published recommendations (203). Approximately 95% of the differences between test and retest will lie between the "limits of agreement" (mean ±2 SD of the differences) on the Bland-Altman plot.

In paper IV group differences were reanalyzed using the GLM-general factorial model with duration of present episode and self-reported symptoms of anxiety, depression, and neuroticism as covariates. General linear model (GLM)-repeated measurement statistics were used in the time bin analysis. Greenhouse-Geisser corrections were made when appropriate.
In addition, all patients were included in a forward linear-regression analysis where ERP data from all electrodes combined with significant visceral sensitivity data were used as independent variables: number of evacuations, VAS-levels of pain, gas discomfort, and global severity as dependent variables.

The software package SPSS Statistics (SPSS International BV, Chicago, IL) was used for the statistical analyses.

7. SUMMARY OF PAPERS

7.1. Paper I
Eighteen IBS patients and 15 healthy volunteers were recruited for this study evaluating the reproducibility of barostat assessed rectal visceral sensitivity. All healthy volunteers and three of the IBS patients were retested after 2 days. The remaining 15 IBS patients, all receiving placebo in a double-blinded clinical study, were retested at the end of the placebo period (3 months). The barostat double random staircase method was used (protocol 1 in the healthy volunteers and protocol 2 in the IBS patients). Pressure, volume and tension were measured at first sensation of gas, stool and discomfort. Sixteen IBS patients and 12 controls had complete examinations and were included in the analysis. The patients and the controls were analysed separately. No significant differences were found between the two test sessions, except for the VAS score in the healthy volunteers, which was significantly lower on day 1. Three different indexes were used as measures of reproducibility: ICC, CV_p and RC. The ICCs ranged from 0.76 to 0.93 in the healthy volunteers and from 0.53 to 0.88 in the patients. The CV_p ranged from 10 to 24 % in the healthy volunteers and from 11 to 49 % in the IBS patients. For the gas and stool thresholds the ICCs were higher and the CV_p and RC values
were lower for the healthy volunteers than for the patients. The reproducibility of the discomfort threshold seemed to be the same for both groups, and in the patient group the reproducibility was better for the discomfort threshold than for the gas and stool thresholds. Plots of the difference between test and retest versus the mean of test and retest were also constructed. We did not find any obvious relation between the difference and the mean for any of the thresholds. The results indicate that barostat visceral sensitivity measurements in the rectum may be applicable when comparing group of subjects.

7.2. Paper II

Thirty-eight IBS patients without comorbid psychiatric disorders, 11 IBS patients with comorbid phobic anxiety disorder and 9 healthy volunteers were included in this study. The IBS patients without comorbid psychiatric disorders were classified as having diarrhoea predominant IBS or IBS with alternating stool habits. Visceral sensitivity using a barostat double random staircase method (protocol 2), rectal baseline volume (tone), HAD scores and VAS scores measuring current global IBS severity were registered. The noncomorbid IBS patients had normal visceral pressure thresholds. However, the volume at discomfort threshold was reduced. The baseline tone was also reduced and it was significantly correlated with the volume at discomfort threshold. There were no significant differences in visceral sensitivity between diarrhoea predominant IBS patients and IBS patients with alternating stool habits, but the volume at discomfort threshold tended to be lower in the diarrhoea predominant patients (p = 0.062). Compared with healthy volunteers, baseline tone, volume at the stool threshold and volume and tension at the discomfort threshold were significantly reduced in the diarrhoea predominant patients. Further, there were no significant differences between the IBS patients with alternating stool habits and the healthy volunteers. IBS patients with comorbid phobic anxiety did not differ in visceral sensitivity from healthy volunteers,
but they had increased pressure and volume at the gas threshold compared to the noncomorbid IBS patients. There were no significant correlations between rectal discomfort thresholds and HAD anxiety and depression scores and VAS scores measuring global IBS severity. The IBS patients in both groups were significantly older than the controls, but the age and discomfort threshold were not significantly correlated. The effect of the body position on the rectal pressure – volume relationship was also recorded in 5 of the healthy volunteers. Visual inspection of the curve demonstrated that the position was of greater importance when low pressures were applied to the rectum compared to high pressures.

7.3. Paper III

This study encompassed 33 IBS patients in whom comorbid psychiatric disorders except from phobic anxiety was excluded and 21 healthy volunteers. The IBS patients were classified as having diarrhoea predominant IBS or IBS with alternating stool habits. Autonomic function (heart rate, RSA and skin conductance) at rest and in response to emotional and rectal stimuli was reported as well as HAD scores and VAS scores measuring current global IBS severity. Baseline heart rate was higher and RSA tended to be lower (p = 0.063) in the IBS patients compared to the healthy volunteers, but there was no significant difference in skin conductance level. Both groups had decreased RSA and increased heart rate when exposed to emotionally loaded words compared to baseline, and for the first word series representing anger the skin conductance level was also increased. However, the two study groups did not differ significantly in their autonomic responses to emotionally loaded words. At discomfort threshold the patients had increased heart rate response and skin conductance amplitude when compared to the healthy volunteers while the RSA response was similar. The diarrhoea predominant IBS patients had significantly increased heart rate and decreased RSA at rest compared to healthy volunteers. The IBS patients with alternating stool habits had
significantly increased heart rate response and skin conductance amplitude to the rectal stepwise discomfort test compared to the healthy volunteers. There were no significant correlations between the measure of autonomic function (both at baseline and as a response to the rectal stepwise discomfort test) and the rectal discomfort threshold, the VAS scores representing global IBS severity or HAD scores in the total IBS population. However, correlations between autonomic responses and HAD depression differed markedly between diarrhoea predominant IBS patients and the IBS patients with alternating stool habits.

7.4. Paper IV

Eleven IBS patients with comorbid phobic anxiety disorder and 22 age-matched IBS patients without any current psychiatric disorders were compared with respect to ERP, visceral sensitivity, and symptom levels the last week before assessment. IBS patients with comorbid phobic anxiety experienced significantly less symptoms of gas discomfort than the noncomorbid IBS patients, but there were no differences concerning the level of pain or the daily number of evacuations. Further, they had a significantly enhanced first negative ERP wave (N1) to all stimuli, indicating increased use of brain attentional resources. When adjusting for duration of present episode, anxiety, and depression, the threshold for the sensation of gas was significantly increased in the comorbid IBS patients compared with the noncomorbid patients. Additionally, the gas-stool and gas-discomfort tolerances were significantly reduced. Enhanced N1 amplitude at the frontal electrode and reduced gas-stool tolerance significantly predicted subjective gas complaints, explaining 47 % of the symptom variation.
8. DISCUSSION

8.1. Methodological aspects

In this section some selected methodological issues will be discussed.

8.1.1. Subjects

The IBS patients recruited were diagnosed according to international accepted criteria (the Rome I criteria (1). However, since we included our patients, the criteria have been revised twice. This means that it is not sure that our included patients would have fulfilled the accepted criteria of today (Rome III) (3). As pointed out in a recent review, it is a general problem in IBS research that the patients are selected on the basis of strictly subjective criteria (204). As far as no objective definition of IBS has been achieved, this problem cannot be solved, but this diagnostic uncertainty cannot stop us from exploring this common and bothersome disorder. Another challenge is that the IBS symptoms are fluctuating in nature, implying that the timing of the study may be crucial. To reduce this problem, we claimed the disease to be in an active phase of at least moderate severity, assessed by VAS-rating.

The complexity of confounding factors influencing pain perception and report convinced us that careful patient description is mandatory in studying disease mechanisms. This is the reason why we tried to include a quite homogenous IBS population. It can be argued that excluding IBS patients with comorbid psychiatric disorders may fail to reflect clinical reality, where overlap is the rule rather than the exception. We believe however, that better characterising of distinct subgroups of IBS is a necessary step on the way to explore the pathogenesis of IBS.
We additionally excluded IBS patients with predominant constipation, and in paper II and III the IBS patients were further subdivided into diarrhoea predominant patients and patients with alternating stool habits. However, the Rome III criteria of IBS use stool consistency, not defecation frequency, to define stool subtype (3) in opposite to the Rome I criteria used in this study. Patient reports of “diarrhoea” and “constipation” may be misleading, for example may the stool be solid although the defecation is frequent and vice versa (205). Most patients with IBS have a normal frequency of bowel movements (206), but stool consistency reflects intestinal transit time (207). Another important aspect of stool based subtyping of IBS patients is that the bowel pattern appears to be highly unstable. One study reported that 75 % of the patients changed subtype over 1 year (208). In spite of this uncertainty, results from several studies in IBS indicate that stool based subtyping may be meaningful (56, 67, 141).

Further, we carefully tried to exclude somatic disorders that could mimic IBS symptomatology by clinical assessment, screening blood tests (including serologic testing for celiac disease), a double-contrast barium enema or recto-/coloscopy. Some concern may be given to the lack of biopsy taking introducing the possibility of including patients with for instance microscopic colitis.

It may be argued that our population of healthy volunteers is not representative for the general population. For example, persons participating in this kind of research may have some gastrointestinal problems although denying them. Ideally, the controls should have been recruited from the general population by chance. Although not ideal, advertisement is the most common form of recruiting controls for this kind of research.
At last, the sample sizes are relatively small, implying that some caution is needed in the interpretation of the findings.

8.1.2. Methods

Barostat distension is the most widely used technique for determining visceral sensitivity, but it is supposed that psychological influences including anticipation might modify responses. Therefore, we used a double random staircase method to determine visceral thresholds. As far as we know, this technique is only used in a couple of other rectal studies (67, 68) making it more difficult to compare our results with others. A recent review article (59) strongly recommends use of randomized stimulus.

The mode of barostat distension may be important for the visceral thresholds reported (18, 45). Therefore, when we compared visceral sensitivity in IBS patients and healthy controls (paper II), we only included those controls that had gone through exactly the same distension protocol as the patients (protocol 2). However, in paper III we compared the 21 healthy volunteers examined with protocol 1 with the IBS patients examined with protocol 2 when exploring the autonomic response to rectal distension. In the last setting we think it is highly unlikely that the difference in protocol will affect study outcome, as we were interested in the autonomic responses to the subjective feeling of rectal discomfort. Further, five of the healthy women and five men were tested twice with both staircases, and no significant difference in pressure at the discomfort threshold was found (data not shown).

According to guidelines published in 1997 (37) the duration of each step and each interval in barostat phasic distension procedures was recommended to be at least one minute. We chose shorter steps and intervals because in our opinion, otherwise the procedure would have been
too time-consuming. The motivation of the participants is very important in this kind of study because the reported endpoints are subjective. Indeed, two healthy women did not complete the retest because of lack of motivation.

Factors known to be able to influence visceral sensitivity such as meals (88 - 90), sex (85) and body mass index (84) are well controlled for in our studies. The patients and the controls were of similar age, except from in paper II. In this paper the IBS patients were significantly older than the healthy volunteers. In some studies older people appear to be less sensitive than younger (18, 83). In our opinion, it is highly unlikely that a mean difference in age of only 8 years is of clinical importance, and we found no significant correlation between age and the discomfort threshold. It is claimed that rectal sensitivity may change with menstrual cycle (87). Our experiments were carried out independently of the menstrual cycle, but we did not observe any correlation between visceral sensitivity and the day of menstruation in paper II.

A potential limitation of using conventional autonomic tests in IBS is the assumption that systemic autonomic function mirrors gastrointestinal autonomic function. However, one group has shown that cardiovagal tone, as measured by heart rate variability, correlates with rectal mucosal blood flow and colonic transit, supporting the relationship between cardioautonomic measures and gastrointestinal function (209). Further, autonomic measurements, especially the cardiovascular ones, depend on confounding factors like age, gender, resting heart rate (210) and physical fitness (211). The two first factors were well controlled for in our study (paper III) while baseline heart rate differed significantly between groups and the degree of physical fitness was unknown. The baseline registrations may also have been influenced by anxiety of the test stimuli (78, 79), the level of chronic pain and the presence of the rectal balloon during the rest condition.
In the literature, it is an ongoing discussion whether respiratory factors should be controlled for when measuring RSA (212). This was not done in our study. A newer study showed that the amplitude of RSA is not affected by respiration frequency under baseline conditions (213).

Considerable within-group variance with respect to ERP measures was found (paper IV). This is probably due to a methodological problem in ERP research, caused by the difficulty in assessing CNS function with electrodes located outside the skull, in addition to individual variation (196).

8.2. Findings

The identification of biological markers in IBS has proven difficult. This study was conducted to see if altered function in the brain-gut axis characterizes IBS patients. The main focus in this thesis was on gut visceral sensitivity, but autonomic and cerebral functions were also explored.

8.2.1. Reproducibility of the rectal barostat

Paper I focused on reproducibility of the barostat double random staircase method in the rectum, as to our knowledge, this distension technique had not been validated in the rectum earlier. Repeated measurements of visceral sensitivity in the same subject will vary. Several statistical approaches can be used to evaluate the reproducibility (within-subject variation) of a test. The different methods aim at measuring different aspects of reproducibility.

There were no significant mean differences between the test and retest, except from the VAS-score in the healthy volunteers at discomfort threshold. Although the test sessions were
similar on average, comparison of means at two sessions gives no information about how well the measurements agree within individuals. For this the intraclass correlation coefficient (ICC) may be better suited. It provides an index of the information content of the measurement, since it is essentially a ratio of the variability between subjects to the total variability (214). ICC is considered to demonstrate acceptable reproducibility (for group comparisons) if it is larger than 0.80, and ideally if larger than 0.90 (199). Accordingly, in the healthy volunteers the reproducibility was acceptable for the volume and tension thresholds and the gas and stool pressure thresholds. In the IBS patients the ICC was only acceptable for the discomfort volume and tension thresholds. The ICC depends on the range of the measurements (214). It is obvious from the equation for the ICC that as BMS increases ICC approaches 1. Our two study groups were quite homogenous regarding age, gender and diagnosis, leading to expected lower variability between subjects than in a more heterogeneous study group. Thus, in our study the ICC’s were not artificially high due to heterogeneity in the study groups.

The precision of the measurements may be quoted as being within twice the coefficient of variation (202). However, the coefficient of variation is dependent on the numerical value of the mean as is obvious from the denominator in the equation for $CV_p$. This means that the volume thresholds in this study will be favoured. Also, when the measured interval is large compared to the size of the smallest observations, quoting the error as a percentage is not so meaningful. The $CV_p$ ranged from 10% to 24% in the healthy volunteers and from 11% to 49% in the IBS patients. These values are in agreement with Hammer et al (47), but higher than reported by Varma et al (55). They are also similar to the coefficients of variation reported in a gastric barostat study using the double random staircase technique (215).
The repeatability coefficients and the Bland-Altman plots (fig. 2, paper I) in this study demonstrate that the within patient variation of barostat measurements were relatively high. If the difference between two measurements lies between “the limits of agreement” or is below the RC, it is uncertain if this difference is real or is caused by measurement error. Therefore, when used for diagnostic purposes, special attention is needed for patients with barostat scores close to the diagnostic limits.

Most of the validation-studies designed up to date indicate clinically acceptable reproducibility for both the barostat (18, 45, 47 - 54) and the non-barostat visceral sensitivity testing (44, 55 - 58). Despite utilizing different test populations and methods, all but one study (42) conclude that the intraindividual variation between test and retests is acceptable. In a recent study, it was concluded that reproducibility varies with sensation type, pressure level and distension method (ascending method versus phasic distensions in random order), and the reproducibility was better for the higher distension pressures (54).

As the distension stimuli are applied to the gut in an unpredictable way using the double random staircase method, we may expect less reproducible results than using the ascending method of limits. In fact, in a study comparing reproducibility of gastric visceral sensitivity using both stepwise and double random staircase distensions, there was a tendency towards lowest variability of the stepwise mode (215). However, in healthy volunteers rectal sensory pressure thresholds were equivalent when elicited with the ascending method of limits, the tracking technique or the double random staircase method (216).

There are several sources that can impair agreement between our two data sets. It is reported that repeated responses on a single day were most reproducible after a “conditioning” distension (47). We did not repeat the measurements twice, but we started every experiment
with unfolding the balloon. This unfolding may act like a "conditioning" stimulus. However, our study was not designed to evaluate this aspect. We have not made any distinctions between transient and constant sensations, but according to one publication this should not be necessary (47). In our study the interstimulus intervals are short, especially for the healthy volunteers. Longer intervals between stimuli have been reported to give more reliable thresholds than short intervals for stepwise distension (37). The subject’s psychological state can alter the threshold for perception compared with baseline (82), and the psychological state may vary from day to day. The participants were tested in a fasting state and did not use medication potentially affecting GI-motility and sensitivity. Body position has influence on rectal volume during graded distension (217 and figure 1, paper II). Positioning was standardized in our study to avoid this source of variation. Although our experiments were carried out independently of the day in menstruation cycle and time of day, both conditions reported to affect visceral sensitivity (87, 92), we found no test-retest differences.

Interestingly, we did not find any significant change in perceptions thresholds between test and retest in the placebo-treated IBS patients. Thus a period of three months with placebo-treatment did not influence the barostat measurements. There was a tendency towards lower reproducibility in the IBS group for the gas and stool thresholds compared to the healthy volunteers. Rectal sensitivity may not be a constant feature of patients with IBS over time, and the long interval between the two test sessions for most of the IBS patients may have disfavoured reproducibility. However, a recent review concludes that rectal hypersensitivity in IBS appears to be a stable phenomenon (34).

The results demonstrate that visceral sensitivity measurements may be applicable when comparing groups of subjects. However, the relatively large CVs and RCs and the relatively
low ICCs indicate that special attention is needed when using the barostat method for individual diagnostic purposes.

8.2.2. Visceral sensitivity in IBS patients without comorbid psychiatric disorders

The main focus in paper II was to examine rectal visceral sensitivity in the IBS patients without comorbid psychiatric disorders. Compared with healthy volunteers they seemed normosensitive in their rectum as far as pressure thresholds were concerned, but the volume was reduced at the discomfort threshold.

We had to explain this discrepancy between volume and pressure at the discomfort threshold in order to answer one of the main questions in this thesis: is rectal sensitivity different in IBS patients and controls? Discrepancy between pressure and volume thresholds has been described earlier (46, 67), and it has been demonstrated that after prolonged isobaric distension, the volume increased but the perception did not (218). It is still under debate what the relevant stimuli for the intestinal mechanoreceptors are. There is evidence that pressure (46) and tension (219) are better candidates than volume, and volume thresholds seem to be more vulnerable to measurement errors than pressure thresholds (37). Therefore, we used a pressure controlled distension protocol in our studies, and we defined the rectal thresholds as pressure values. The calculated tension at the discomfort threshold was not changed in these IBS patients. However, the baseline tone was reduced and the volume at a chosen fixed pressure (24 mmHg) reached by most of the participants, tended to be reduced in the patients. Further, the baseline tone and the volume at the discomfort threshold were significantly correlated. In our opinion, our findings indicate that the reduced volume at the discomfort threshold is not due to increased visceral sensitivity, but may be due to increased rectal tone.
Increased colorectal tone in IBS patients has been reported earlier (83, 121), but not in all studies (25, 88, 120).

Visceral hypersensitivity is supposed to be a biological marker of IBS (18), but it is still unknown why not all IBS patients are affected. We suppose that the lack of visceral hypersensitivity in our IBS patients without psychiatric comorbidity is due to patient characteristics, especially the lack of psychiatric comorbidity and the way they were recruited. It has been argued that visceral hypersensitivity in IBS may be explained by psychological bias (77, 80 - 82); others do not agree (220). If lower pain or discomfort threshold in IBS patients were due to psychiatric influences, one would expect IBS patients without comorbid psychiatric disorders to be normosensitive. Our findings were consistent with this hypothesis in opposite to most of the limited number of comparable studies involving non-psychiatric IBS patients only (74, 221, 222). In this study there was no association between HAD depression and anxiety scores and visceral thresholds. Some authors report an association between psychological test scores and visceral thresholds (18, 81), but the majority do not (19, 22, 82). As briefly discussed in section 4.2.4, several maladaptive personal characteristics are associated with IBS (14). We did not examine such psychological factors.

Since published studies excluding IBS patients with psychiatric disorders report visceral hypersensitivity in IBS, it can be suspected that our recruitment of IBS patients from outside secondary/tertiary care has been decisive for finding visceral sensitivity in the normal range. Although most of our IBS patients were recruited by advertisements, they seemed to be comparable with non-psychiatric IBS patients met in general practice. The low referral rate to specialists in our IBS patients is consistent with that. We have only been able to identify one study of rectal sensitivity in community IBS patients (31) although only 17-30 % of the IBS
patients in general practice are referred to specialists (5, 9). In that study diarrhoea predominant, non-psychologically disturbed, community patients with IBS had normal rectal sensitivity although they had increased gastric perception (31). Our study cannot explain how different patient recruitment can affect visceral sensitivity. However, in addition to a higher degree of psychological symptoms in IBS patients seen in referral centers versus primary care, a higher degree of fatigue and lower quality of life are reported (223). Inclusion of a control group of non-psychiatric IBS patients from secondary/tertiary care would have strengthened our study and is warranted in future studies.

Because our study included a relatively small number of participants, especially healthy volunteers, it is not possible to exclude a type II error. However, the observed pressure difference at the discomfort threshold was small and hardly of clinical importance. Few rectal studies using the double random staircase method are reported, but even lower discomfort threshold in healthy volunteers has been found in one study using a similar distension protocol to ours (67). Further, a recent study using the double random staircase method reported pain thresholds at 16.9 mmHg in IBS patients with diarrhoea or constipation and 19.0 mmHg in IBS patients with alternating subtype (224), that is lower values than reported for the discomfort threshold in our IBS population. It may also be argued that our choice of a more unbiased distension method can explain why the discomfort pressure threshold seemed to be normal in our IBS patients. We believe this explanation is unlikely because other studies using the double random staircase method report increased rectal sensitivity in IBS (67, 68).

We did not find any significant differences in visceral sensitivity between diarrhoea predominant IBS patients and IBS patients with alternating stool habits, but the volume at discomfort threshold tended to be lower in the diarrhoea predominant patients. Compared
with healthy volunteers, baseline tone, the volume at stool and discomfort thresholds and the tension at discomfort threshold were significantly reduced in the diarrhoea predominant IBS-patients. As discussed above this volume reduction may reflect increased rectal tone. Lower colorectal volume at discomfort threshold in diarrhoea predominant IBS patients is consistent with previous studies (66, 67), but lower pressure at discomfort or pain threshold is reported as well (66, 71) and some studies do not find any disturbed visceral sensitivity in these patients (69, 70). In opposite to our findings, it is claimed that IBS patients with alternating stool habits are hypersensitive (66, 71).

8.2.3. Visceral sensitivity in IBS patients with comorbid phobic anxiety disorder

Most researchers have not addressed the possible role of concurrent psychiatric disorders for their findings in visceral sensitivity studies. In this thesis, IBS patients with comorbid phobic anxiety were compared to healthy volunteers and non-psychiatric IBS patients. In paper IV, the pressure threshold for the sensation of gas was significantly increased in the IBS patients with comorbid phobic anxiety compared to IBS patients without comorbid psychiatric disorders when adjusted for duration of the present episode, neuroticism, anxiety and depression. Because a healthy control group was not included in paper IV, the comorbid group was also included in paper II. Without statistically adjusting for covariates in paper II, we reproduced the finding of increased gas threshold in the comorbid IBS patients compared with the larger and not age-matched population of included noncomorbid IBS patients. However, there were no significant differences in visceral sensitivity between the comorbid IBS patients and the healthy volunteers. Interestingly, the threshold values in the healthy volunteers were lying in between the threshold values in the two IBS patient groups, supporting that the two patient groups had different gas thresholds. However, our finding was
not consistent with the reported inversed correlation between severity of phobic anxiety and sensory thresholds (18).

The IBS patients with phobic anxiety had similar stool and discomfort thresholds to both the noncomorbid IBS patients and the healthy volunteers. The gas threshold is far less examined and thought to be less interesting than discomfort and pain thresholds. In paper I we demonstrated that this threshold seemed to be less reproducible than the discomfort threshold in the IBS patients. One reason may be the body position’s influence on the pressure – volume relationship as described in one earlier study not using barostat methodology (217). As demonstrated in figure 1 in paper II, the position is of greater importance when low pressures, as at the gas threshold, are applied to the rectum compared with high pressures.

Even if the finding of increased gas tolerance must be interpreted with some caution because of the fairly small number of subjects included and poorer reproducibility of the gas threshold, it raised an interesting hypothesis. Perhaps comorbid IBS patients have increased gas threshold compared to noncomorbid patients because of a competition between attentions towards external or internal stimuli caused by the two disorders. The stool and discomfort thresholds were similar in the two patient groups indicating that with increasing strength of the visceral stimuli, the internal stimuli seem to be overshadowed by the external ones.

8.2.4. Autonomic function in IBS patients

In paper III we focused on one of the primary gut-brain communication pathways; the autonomic nervous system. IBS patients without psychiatric comorbidity except from phobic anxiety were compared with healthy volunteers at rest and in response to emotional and rectal stimuli. At rest, the IBS patients had significantly increased heart rate and tended to have
decreased respiratory sinus arrhythmia (RSA) compared to healthy volunteers. Increased heart rate at rest in IBS patients has been reported earlier (225). This finding may be due to increased sympathetic tone, decreased parasympathetic tone or both. Our results indicate that the increased heart rate probably is due to decreased vagal tone, at least in the diarrhoea predominant IBS patients. However, other studies found decreased (135, 226) or no (128, 132 – 134, 137, 141, 227) difference in baseline heart rate. Reduced vagal tone in women with IBS has been reported by others (126), and parasympathetic provocation tests such as cardiac response to deep breathing have shown vagal withdrawal in most studies (132 – 134, 139), but not in all (128, 135).

In the present study there were lower parasympathetic activity and higher sympathetic activity in response to emotional words, indicating a stress response, but the alterations were similar in patients with IBS and controls. Thus, presenting emotional loaded words were effective in causing autonomic changes and therefore appropriate for investigating responses to a psychological challenge. However, our results did not support the hypothesis that the IBS patients were hyperresponsive to emotional stimuli. There have also been negative findings in other studies (78, 226, 228), but our findings are inconsistent with one report supporting the connection of stress and autonomic dysfunction in IBS (227).

Until the last couple of years, there has been a paucity of data on autonomic responses to enteric stimulation in patients suffering from IBS. In our IBS population, rectal distension induced an increased sympathetic response (increased heart rate and skin conductance amplitude) although the rectal discomfort threshold was not changed compared with controls. Our finding is consistent with two recent studies (136, 137) that reported increased skin conductance (136) and systolic blood pressure (137) in response to visceral distension in IBS
patients. In contrary to our results a decreased parasympathetic response has been demonstrated as well (136). Postprandial increased sympathetic dominance in IBS patients has also been described (141, 228). It may be speculated that this change in autonomic response to distension and eating may explain the exacerbation of IBS symptoms postprandially.

Although the IBS patients and healthy controls reported similar visceral sensitivity (paper II), their autonomic response to the distension differed (paper III). In an earlier report we found evidence for ERP-assessed hyperreactivity to auditory stimuli in non-psychiatric IBS patients compared with healthy volunteers (229). Altogether, this may indicate that in our population of IBS patients, autonomic measures are more sensitive to detect disturbances in visceral sensitivity than the subjective verbal responses. Further, changes in autonomic and cerebral responses seem to characterize this population of IBS patients better than ordinary reported changes in visceral sensitivity.

There is no consensual explanation for the discrepant autonomic findings in the literature. The use of different batteries of autonomic tests, fluctuation in symptoms, inclusion of patients with different stool habits and psychological factors are some possibilities. Therefore, the diarrhoea predominant IBS patients and the IBS patients with alternating stool habits were analyzed separately. Among the diarrhoea predominant IBS patients the resting heart rate was higher and the resting RSA lower than in the healthy volunteers, and among the patients with alternating stool habits the autonomic responses (heart rate and skin conductance amplitude) to rectal discomfort were higher. In an earlier report IBS patients with diarrhoea-predominant and alternating stool habits had increased sympathetic and decreased parasympathetic responses postprandially although no differences were found at baseline (141). Diarrhoea
predominant IBS patients were recently found to have vagal withdrawal during sleep compared to IBS patients with alternating stool habits (142). Contrary to our results it has been reported that vagal activity is normal (139) or increased in IBS patients with diarrhoea (230) and decreased in those with constipation (138, 139).

The autonomic responses may differ between IBS patients with and without psychiatric comorbidity. For example, a history of anxiety and depressive disorders have been linked to reduced parasympathetic activity in both women with IBS and healthy women (231) while another study did not find any differences in autonomic activity between women with IBS with and without comorbid depression (232). In addition, chronic levels of anxiety and depression are associated with altered activity of the autonomic innervation to the gut measured as rectal mucosal blood flow in women with idiopathic constipation (233). Studies of autonomic function conducted in non-psychiatric IBS patients present conflicting results (127, 135, 136, 139, 141, 142, 227, 234). In our study, patients with psychiatric disorders were excluded except from a few patients with comorbid phobic anxiety. Thus our main results suggest that the autonomic abnormalities in IBS patients cannot be explained by psychiatric comorbidity.

Except from one study (138) no correlation has been found between autonomic function and psychological parameters (134, 139, 141). We observed increased HAD scores for anxiety and depression in our IBS population compared to the control group, but the scores were well within the reference values. In the total IBS population there was no significant correlation between autonomic function and HAD scores. However, when the two IBS subgroups were analysed separately, significant correlations were found between HAD scores and some of the measures of autonomic function. For example, in both groups HAD depression was

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significantly associated with baseline RSA, but this correlation was negative in the diarrhoea predominant subgroup and positive in the subgroup with alternating stool habits. These results indicate that psychological factors may influence autonomic function or vice versa and suggest that important differences exist between these subgroups.

We do not know whether the altered autonomic balance in IBS reflects a primary disorder in the autonomic pathways or whether it reflects alterations in communication along the brain-gut axis. There is increasing evidence for aberrant CNS processing in IBS patients (40, 106, 229), and the abnormal autonomic responses to rectal distension in our IBS patients may have cerebral causes. An association between cortical and subcortical brain activation and heart rate responses to rectal stimuli has been reported (235). Another interesting hypothesis, not further explored in this thesis, is that the altered autonomic function may induce changes in gut mastcells via the close intestinal mast cell-nerve interaction (147). The vagal nerve also exerts tonic anti-inflammatory action on visceral inflammation (236) indicating that reduced vagal tone may explain the mild inflammation in IBS patients as reflected by systemic proinflammatory cytokines (237).

8.2.5. Influence of comorbid phobic anxiety on the brain-gut axis in IBS

In paper IV, we showed that the IBS patients with comorbid phobic anxiety had enhanced ERP-assessed N1 wave to all auditory stimuli compared with the noncomorbid patients. The differences in N1 wave between the two IBS groups were seen to all stimuli, indicating that the enhanced N1 wave most probably is a characteristic of the IBS group studied. Because N1 represents an automated shift in attention towards a new stimulus, the enhanced wave in the IBS-patients with phobic anxiety probably represented the environmental scanning for the feared object or situation, which is a clinical characteristic of phobic anxiety patients (196). In
a previous study, we found that non-psychiatric IBS patients had a significantly enhanced frontal N1 wave compared with healthy volunteers (229). This may mean that IBS patients with comorbid phobic anxiety have two disorders sharing an enhanced frontal N1 wave as an essential disease characteristic.

Changed cerebral processing in IBS has been described to gastrointestinal stimuli (40, 106). Our study supports research indicating that altered cerebral response pattern in IBS is not specific for gastrointestinal stimuli, as it also is reported for somatic stimuli (238) and auditory stimuli (239, 240). In fact, our finding that phobic IBS patients were more reactive for auditory stimuli than non-phobic IBS patients indicates that differences in emotional state might be an important underlying factor concerning cerebral processing in IBS patients.

As discussed in section 8.2.3 phobic IBS patients had increased rectal gas threshold and reduced gas-stool and gas-discomfort tolerances compared with the noncomorbid group. Enhanced N1 amplitude frontally and reduced gas-stool tolerance significantly predicted subjective gas complaints, explaining 47 % of the symptom variation. Thus, phobic anxiety appeared to interfere with the processing of visceral information in the frontal cerebral region and thus affected visceral sensitivity and IBS symptoms. This finding may suggest a causal relationship between brain processing, visceral sensitivity and subjective IBS symptomatology.

Psychiatric disorders differ with respect to ERP characteristics. Enhanced N1 amplitudes have been described in panic (241) and somatization disorders (242). Other anxiety disorders may enhance the P3 wave, whereas a major depressive disorder seems to attenuate the same wave (243). It is therefore possible that other psychiatric disorders may influence the visceral
sensitivity differently. In paper IV we did not distinguish between patients with diarrhoea predominant IBS and IBS with alternating stool habits. There is some evidence that IBS patients with different bowel pattern have different cerebral response patterns to rectal stimulation (107).

9. CONCLUSIONS

Our results demonstrate that the reproducibility of visceral sensitivity measurements in the rectum using the barostat double random staircase method may be applicable when comparing groups of subjects. However, special attention is needed when using the barostat method for individual diagnostic purposes. Further, in this female population of non-constipated, mainly non-psychiatric IBS patients outside secondary/tertiary care rectal visceral sensitivity appeared to be normal, but the autonomic function was altered. Altogether, this thesis suggests that in this subgroup of IBS patients, disturbances of autonomic function better characterize the patients than alterations in visceral sensitivity. Additionally, comorbid phobic anxiety seems to interfere with the processing of visceral information in the frontal cerebral region in IBS patients and thus may affect visceral sensitivity.
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11. APPENDIX 1

Irritable bowel syndrome (IBS): Rome I criteria (1)

The following criteria apply if (1) symptoms are chronic or recurrent for at least 3 month, and (2) based on adequate medical evaluation and the symptoms are not attributable to other gastrointestinal disease.

A Continuous or recurrent symptoms of

1. Abdominal pain or discomfort that is
   a. relieved with defecation; and/or
   b. associated with a change in frequency of stools; and/or
   c. associated with a change in consistency of stool; and

2. Two or more of the following, at least one-fourth of occasions or days:
   d. altered stool frequency (defined as < 3 movements per week (constipation) or > 3 per day (diarrhoea);
   e. altered stool form (lumpy/hard of loose/watery stool);
   f. altered stool passage (straining, urgency, or feeling of incomplete evacuation);
   g. passage of mucus;
   h. bloating of feeling of abdominal distension