REVIEW

Stress and animal models of inflammatory bowel disease—An update on the role of the hypothalamo–pituitary–adrenal axis

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Summary Chronic psychosocial stress has been repeatedly shown in humans to be a risk factor for the development of several affective and somatic disorders, including inflammatory bowel diseases (IBD). There is also a large body of evidence from rodent studies indicating a link between stress and gastrointestinal dysfunction, resembling IBD in humans. Despite this knowledge, the detailed underlying neuroendocrine mechanisms are not sufficiently understood. This is due, in part, to a lack of appropriate animal models, as most commonly used rodent stress paradigms do not adequately resemble the human situation and/or do not cause the development of spontaneous colitis. Therefore, our knowledge regarding the link between stress and IBD is largely based on rodent models with low face and predictive validity, investigating the effects of unnatural stressors on chemically induced colitis. These studies have consistently reported that hypothalamo–pituitary–adrenal (HPA) axis activation during stressor exposure has an ameliorating effect on the severity of a chemically induced colitis. However, to show the biological importance of this finding, it needs to be replicated in animal models employing more clinically relevant stressors, themselves triggering the development of spontaneous colitis.

Important in view of this, recent studies employing chronic/repeated psychosocial stressors were able to demonstrate that such stressors indeed cause the development of spontaneous...
colitis and, thus, represent promising tools to uncover the mechanisms underlying stress-induced development of IBD.

Interestingly, in these models the development of spontaneous colitis was paralleled by decreased anti-inflammatory glucocorticoid (GC) signaling, whereas adrenalectomy (ADX) prior to stressor exposure prevented its development. These findings suggest a more complex role of the HPA axis in the development of spontaneous colitis.

In the present review I summarize the available human and rodent data in order to provide a comprehensive understanding of the biphasic role of the HPA axis and/or the GC signaling during stressor exposure in terms of spontaneous colitis development.

1. General introduction

Inflammatory disorders, such as Crohn’s disease (CD) and ulcerative colitis (UC) represent a major health concern, particularly in Western societies, with a life-time prevalence of approximately 0.1% (for review see Singh et al., 2001). In addition to limiting quality of life due to abdominal cramps and pain, diarrhea, bloody stools, ulceration, fever, tiredness and other socially unacceptable symptoms, inflammatory bowel disorders (IBD) are further linked to an increased risk for developing inflammation-related colorectal cancer (CRC) (for review see Yang et al., 2009). Furthermore, early-onset IBD (i.e., in childhood) has been shown to cause growth problems and even to delay puberty, as an appropriate uptake of nutrients is not guaranteed during periods of severe gut inflammation (for review see Mamula et al., 2008).

The pathogenesis of IBD is still not completely understood. It is generally accepted that IBD has a complex and multifactorial aetiology, involving genetic and environmental factors (for review see Andus and Gross, 2000; MacDonald and Monteleone, 2005), which are in turn associated with a dysregulation of the mucosal immune system. One environmental factor that is often discussed in this context during the last decades is the perceived level of life stress (Salem and Shubair, 1967; Duffy et al., 1991; Bernstein et al., 2010). A similar link between stress and IBD has been suggested also by a large body of rodent data (Collins et al., 1996; Gue et al., 1997; Million et al., 1999; Qiu et al., 1999; Milde and Murison, 2002; Cakir et al., 2004; Gulpinar et al., 2004; Saunders et al., 2006; Melgar et al., 2008). However, the face and predictive validity of most animal models have to be valued with caution, as they often employ unnatural- and short-term-stressors and investigate their effects on artificially (chemically)-induced colitis. In contrast relevant human stressors in modern societies that are discussed as risk factors for the development of several affective and somatic disorders are mostly chronic and psychosocial in nature (Salem and Shubair, 1967; Duffy et al., 1991; Kiecolt-Glaser and Glaser, 1995; Kiecolt-Glaser et al., 1995, 1996, 1998; Agid et al., 1999; Coker et al., 2000; Herrmann et al., 2000; Buske-Kirschbaum et al., 2001; Heim and Nemeroff, 2001; Bitton et al., 2003; Wright et al., 2004; Amat et al., 2005; Post et al., 2005; Heim et al., 2009). It is, therefore, not surprising that, except from the generally reported colitis-ameliorating role of HPA axis activation during stressor exposure, there is a paucity of detailed neuroendocrine mechanisms underlying stress-induced development/ aggravation of IBD. Recent studies employing chronic psychosocial stressors to investigate the aetiology of stress-induced...
spontaneous colitis (Reber et al., 2007, 2008, 2011; Savignac et al., 2011), which are believed to be more clinically relevant (for review see Cryan and Slattery, 2007, 2010) have begun to address this dearth of knowledge. Interestingly, they suggest that while activation of the HPA axis in the initial phase of stressor exposure promotes the development of spontaneous colitis, its activation in the later phases of stressor exposure can actually be beneficial.

Therefore, the main aim of this review is to summarize available human and animal data linking stress and spontaneous or chemically induced colitis, and what we can deduce from these studies about the possible underlying neuroendocrine mechanisms. The focus will be put on the biphasic role of HPA axis activity and glucocorticoid (GC) signaling during stressor exposure on chemically induced and/or spontaneous colitis.

2. IBD in humans

In humans IBD classically includes two distinct disease patterns, UC and CD (for review see Blumberg et al., 1999; Lichtenstein, 2000; Blumberg and Strober, 2001; Brandtzaeg, 2001; Podolsky, 2002; MacDonald and Monteleone, 2005; Mawdsley and Rampton, 2006). Briefly, IBD can be classified as a chronic relapsing inflammatory condition of the intestinal tract and is characterized by mucosal ulceration. Patients suffer from chronic diarrhea, weight loss, abdominal pain, fever, and fatigue. Extra-intestinal manifestations can also occur, including skin ulcers, arthritis, and bile-duct inflammation, the last especially in UC. On top, patients suffering from IBD have also an increased risk for developing inflammation-related CRC (for review see Yang et al., 2009).

Epidemiologic studies showed that approximately 0.1% of the western population suffer from IBD (Singh et al., 2001) and that world wide, each year 5–18 out of 100,000 individuals additionally develop IBD (Yang et al., 2009). The peak incidence of IBD occurs in the third decade of life (Yang et al., 2009).

The salient features of UC and CD have been recognized for many years and are in detail described in a number of excellent review articles (for review see Podolsky, 1991a,b; Mawdsley and Rampton, 2006). Briefly, inflammation in UC is confined to the mucosa and superficial submucosa of the large bowel, with deeper layers of the bowel being not affected. This is reflected by a dense infiltration of the lamina propria by neutrophils and lymphocytes which produce extensive amounts of inflammatory mediators, finally driving development of superficial mucosal ulceration.

In contrast, active disease in CD is characterized by an infiltration of predominantly macrophages and lymphocytes into deeper layers of the bowel wall, often resulting in deep linear or even transmural ulcers. Inflammatory processes can even extend beyond the gastrointestinal tract, resulting in the development of fistulas.

Furthermore, mucosal ulceration in UC patients is continuous and restricted to the colon but patchy and possibly spread through the whole intestinal tract in CD (for review see MacDonald and Monteleone, 2005). Both UC and CD patients suffer from diarrhea and weight loss, abdominal pain, fever, and fatigue (Mawdsley and Rampton, 2006). Importantly, in UC patients loose stools are slimy and bloody, whereas in excrements from CS patients generally no blood is detected (Podolsky, 1991a; Fiocchi, 1998).

Substantial progress has been further made in characterizing immune-cell populations and inflammatory mediators in patients with IBD (for review see Podolsky, 2002; Mawdsley and Rampton, 2006). Briefly, the mucosa of CD patients is dominated by CD4+ lymphocytes and a typical and strong T helper type 1 (Th1) cytokine profile (increased secretion of tumor necrosis factor (TNF), interleukin (IL)-2, interferon (IFN)-γ, IL-12, IL-18). In contrast, the mucosa in patients with ulcerative colitis is dominated by CD4+ lymphocytes with an atypical Th2 phenotype, characterized by the production of transforming growth factor β (TGF-β) and IL-5. The presence of auto-antibodies, such as the anti-neutrophil cytoplasmatic antibody, also is suggestive of a Th2 pathogenesis in UC (for review see Singh et al., 2001).

Current treatments for IBD consist of TNF antibodies, IL-10 (Dejaco et al., 2000) and IL-11, and substances blocking specific gut-homing molecules for activated CD4+ T cells (antibody against the α4β1 subunit of the α4β1 and α4β7 integrin or antisense against intercellular adhesion molecule-1) (for review see Singh et al., 2001). However, 5-aminosalicylates and steroids constitute still a cornerstone of medical therapy in patients with IBD (Dejaco et al., 2006).

3. Risk factors for IBD

IBD has a complex and multi-factorial aetiology, comprising genetic and environmental factors (for review see Andus and Gross, 2000; MacDonald and Monteleone, 2005; Mawdsley and Rampton, 2006). IBD is predominantly associated with industrialized societies and temperate climates, and is rare in tropical countries with poor sanitation and a low level of overcrowding (for review see Elliott et al., 2000). The fact that migration to developed countries increases the risk for development of IBD (Probert et al., 1992, 1995) further supports the hypothesis that genetic factors are not solely responsible for the disease. Various environmental factors have been proposed to contribute to the enhanced risk of IBD in industrialized countries, including nutrition, infections, smoking status (Tan et al., 1992; Shapira and Tamir, 1994; Kleit et al., 1998; Russel et al., 1998; Neurath and Schurmann, 2000; Khan et al., 2002), and the level of perceived life stress (Salem and Shubair, 1967; Mitchell and Drossman, 1987; Robertson et al., 1989; Duffy et al., 1991; Levenstein et al., 1998, 2000; Bitton et al., 2003, 2008). As the latter finding is of central importance for the present review it will be outlined in more detail after introducing the reader to the "stress concept" and the adaptive physiological response of an organism to an acute stressor.

4. The stress concept

In the 19th century the French physiologist Claude Bernard (1813–1878) noticed that the relative constancy of the internal environment is critical for the functional integrity of an organism. Later, Walter Cannon (1871–1945) coined the term homeostasis for this internal equilibrium and described the disruption of it by fear- or rage-induced "fight or flight" reactions in his "emergency concept" (Cannon, 1939). In 1936 it was Hans Selye (1907–1982), who first defined stress

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and the stress response as “the non-specific response of the body to any physical demand” (Selye, 1936) and distinguished between “stress” and the “stressor” (Selye, 1975). According to him, stressors are defined as the specific challenges that cause the physiological stress response (Selye, 1975). Since then, an overwhelming number of studies have focused on the physiological, in particular neuroendocrine, and also behavioural consequences of an acute stress response, which are, in general, well understood (Sapolsky, 1992; Rabin, 1999; Sapolsky et al., 2000).

It generally accepted that the physiological and behavioural responses to acute stressors are adaptive and important to re-establish the homeostasis of the body (Sapolsky et al., 1986b; Dhabhar and McEwen, 1996; McEwen and Seeman, 1999; Dhabhar, 2002; McEwen, 2004). In contrast, repeated or chronic exposure to stressors over several weeks or months are thought to result in mal-adaptive alterations of numerous body and brain systems, which are less well understood partly due to the lack of appropriate and clinically relevant animal models.

In response to an acute stressor of any given quality, two major stress systems become activated, namely the autonomic nervous system (ANS), especially its sympathetic (SNS) tree, and the hypothalamic–pituitary–adrenocortical (HPA) axis. Stimulation of these emergency systems reflects the stress response of the organism with the major aim to re-establish the homeostasis of the body, a process referred to as allostatics (for review see Goldstein and Kopin, 2007). Rapid, within seconds, activation of the SNS via exclusively neuronal pathways originating in the thoracolumbar regions of the spinal cord (splanchnic nerve) results in the release of adrenalin from the chromaffin cells of the adrenal medulla into the blood. Elevated levels of adrenaline in the circulation act in synergy with an increased sympathetic noradrenergic innervation of essentially all organs in the body (for more details see Lewis, 1975; for brief summary see review Bartolomucci, 2007). As a result, cardiovascular and catabolic functions are promoted, and anabolic processes and digestion, for example, are inhibited.

In contrast to the very fast-acting SNS, induction of end-organ effects elicited by HPA axis activation, which is exclusively driven by hormones, takes longer to develop. The stimulation of the HPA axis is triggered by the secretion of corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus (PVN) of the hypothalamus into the portal blood stream of the pituitary stalk. CRH promotes the synthesis and the secretion of adrenocorticotrophic hormone (ACTH) into the peripheral blood, which, in turn, stimulates the adrenal cortical cells to produce and secrete glucocorticoids (GC; cortisol in humans, corticosterone in rodents) into the blood. Within minutes, termination of the acute stress response of the HPA axis is achieved by efficient negative feedback inhibition via GC acting at glucocorticoid (GR) and mineralocorticoid receptors (MR) at several brain levels (Reul and de Kloet, 1985). The degree and the time course of HPA activation are, and this is in contrast to what was proposed by Selye (1975), strongly dependent on the quality, intensity and duration of the acute stressor (for review see Koohlaas et al., 2011). For instance, exposure of adult male Wistar rats to different types of stressors for 15 min elicits totally different plasma GC responses, most pronounced following social stressors and least pronounced following noise stress or handling. In line, the group around Dhabhar showed that 2 h of combined restraint and shaking (severe) stress resulted in higher plasma corticosterone levels than 2 h of restraint (moderate) stress alone (Dhabhar and McEwen, 1997). In addition, they showed that in contrast to the immune stimulatory effects of plasma corticosterone released during 2 h of restraint stress, the repeated release of plasma corticosterone seen during daily 5 h exposures (3–5 weeks) to restraint stress (Dhabhar and McEwen, 1997) or chronic corticosterone administration (via drinking water) (Dhabhar and McEwen, 1999) have immunosuppressive effects. Stress-induced HPA axis activation is further dependent on prior experiences, resulting for instance in adaptation of the HPA axis response to repeated homotypic stressor exposure (Dhabhar and McEwen, 1997) and sensitisation to a novel heterotypic stressor (Berton et al., 1999; Chen et al., 2008; Reber et al., 2008). In addition, the acute neuroendocrine stress response was shown to be age-dependent (Sapolsky et al., 1986a; Meijer et al., 2005), altered under various physiological conditions (e.g. in the peripartum period (Neumann et al., 2000; Torner and Neumann, 2002; Torner et al., 2002)), and strongly influenced by the genetic background (Stenberg et al., 1989; Landgraf et al., 1999; Ohta et al., 1999; Touma et al., 2008). Moreover, the HPA axis response to acute challenges in genetically susceptible individuals (Alexander et al., 2009) can become dys-regulated by several factors including somatic and affective diseases as well as chronic stressor exposure (Wigger and Neumann, 1999; Veenema et al., 2008; Elzinga et al., 2010).

5. Evidence for chronic stress to be a risk factor for IBD in human and non-human primates

In contrast to the so far reported adaptive and, thus, positive effects of the acute stress response, chronic stress and especially chronic psychosocial stress is a burden of modern societies and as such an acknowledged risk factor for numerous bodily and affective disorders, including stomach ulcers (Coker et al., 2000), diarrhea and digestive problems (Coker et al., 2000; Campbell et al., 2002), chronic pelvic and abdominal pain (Coker et al., 2000; Campbell et al., 2002), infections (Cohen et al., 1991; Kiecolt-Glaser et al., 1996; Coker et al., 2000; Campbell et al., 2002), impaired wound healing (Kiecolt-Glaser et al., 1995, 1998; Marucha et al., 1998), cancer (Kiecolt-Glaser and Glaser, 1995), irritable bowel syndrome (IBS), and IBD (for review see Mawdsley and Rampton, 2005, 2006).

Evidence for a role of stress in the modulation of disease onset and severity in IBD comes from numerous studies done in human and non-human primates. In 1958, Porter et al. (1958) reported the development of gastrointestinal lesions in 11/19 rhesus monkeys who were either restrained in chairs or placed in a conditioned anxiety situation (Drossman, 1985). Most of these were gastroduodenal erosions, but two of the monkeys, which were conditioned for anxiety, developed a wasting syndrome and chronic colitis (Drossman, 1985). The first hint for a link between stress and human IBD was provided by Salem and Shubair in 1967 who showed that Arab Bedouins frequently developed UC after they were forced to leave their familiar environment in the desert.
and live in houses provided by the government (Salem and Shubair, 1967). Two years later, Stout and Snyder (1969) described extensive colonic ulceration in Siamang gibbons that died within a few weeks after the death of their mating pair (Stout and Snyder, 1969).

The development of spontaneous colonic inflammation and carcinomas in different tamarin species (New World primates; *Saguinus oedipus* = cotton-top tamarin; *Saguinus mystax* = moustached tamarin) during captivity (Drossman, 1985; Gozalo and Montoya, 1992; Wood et al., 2000) further supports a possible link between stress and the development of colonic inflammation. Interestingly, the absence of colonic inflammations in the wild population (Wood et al., 1998) as well as in tamarins caged in the conditions of their natural habitat in Columbia (Wood et al., 1995) suggests that spontaneous colitis is caused by temperature-induced metabolic stress during captivity when caged in temperate climates throughout the world (Wood et al., 2000).

In 1991, Duffy and coworkers clearly demonstrated in a large prospective study, performed with 124 patients (UC and CD) over 6 month, an increased risk of IBD symptom exacerbation following severe, sustained life stress (Duffy et al., 1991).

Furthermore, in 1998 Leventstein and coworkers showed that among a group of patients with previously diagnosed UC who were currently in complete clinical remission, those with endoscopically visible rectal mucosal abnormalities reported higher levels of perceived life stress (Leventstein et al., 1998). A comparable study done by Bitton et al. (2003) also described that among 60 patients with UC in remission phase, those who relapsed showed significantly more severe life events during the time span prior to relapsing compared with patients that did not relapse. In a prospective follow up study they further showed that among patients with quiescent CD, those were least likely to relapse whose life was least stressful and who were least engaged in social diversion or distraction (Bitton et al., 2008). Further support for an impact of psychological factors and, thus, brain activity on intestinal disease state comes from the relatively high success rate of treating human colitis with placebo drugs (Thomas, 1994; Ilinskyi et al., 1997). In line Bernstein and coworkers just recently showed that only high-perceived stress was associated with an increased risk of flare in IBD patients (Bernstein et al., 2010). Evidence described so far showing that adverse life events, chronic stress, and depression are risk factors for IBD, is also supported by human studies employing experimental stressors. For further detailed information on the link between stress and IBD in humans the interested reader is directed to an excellent review articles done by Mawdsley and Rampton (for review see Mawdsley and Rampton, 2005).

With respect to the underlying neuroendocrine mechanisms only little is known so far from human studies. Available data suggest IBD, similar to rheumatoid arthritis, to be rather associated with hypocortisolism than hypercortisolism (for review see Mayer, 2000a; Mawdsley and Rampton, 2006). The prolonged lack of adequate levels of anti-inflammatory GC thereby might contribute to the generation of a pro-inflammatory milieu in the intestinal tract, finally favoring the development of IBD. Support for this hypothesis comes from the group around R. Straub. They showed that the positive correlation of the sympathetic tone and activation of the HPA axis found in healthy controls is shifted towards a more pronounced SNS and decreased HPA axis activation in UC patients (Straub et al., 2002). In addition, it has been reported that the release of GC in response to inflammatory cytokines becomes blunted in IBD patients (Straub et al., 1998).

Importantly, it has to be at least briefly mentioned in this context that besides IBD also IBS is discussed to be a consequence of physical and psychosocial stressor exposure (for review see Mayer, 2000a; Cumberland et al., 2003). In line, Bennett et al. (1998) provided evidence in a longitudinal human study that the presence of a highly threatening chronic stressor inhibits improvement from IBS, while its reduction or absence may be a prerequisite for a significant improvement of the disease. Furthermore, an increased rate of sexual and physical abuse during childhood together with high perceived life stress has been reported for IBS patients (Gaynes and Drossman, 1999). Interestingly, over the past years it became more and more clear that IBS does not solely represent a functional gastrointestinal disorder but is also linked with low-grade mucosal inflammation and innate and adaptive immune system activation (Ohman and Simren, 2010). The increased risk for developing IBS after infectious gastroenteritis and in patients with IBD in remission supports this hypothesis. In line with what has been so far reported for IBD, a disturbed HPA-axis activity has been found also in patients suffering from IBS (Patacchioli et al., 2001).

However, the role of psychosocial factors in the development and modulation of common gastrointestinal disorders, such as IBS and IBD, remains controversial (for review see Mayer, 2000b). For example Campbell et al. (1986) investigated in a prospective study whether there is a link between stressful life events and symptoms of pain or diarrhea and failed to find any significant correlations. In line, North et al. (1990) reported in their review article that only 8 out of 15 studies, done in patients with UC, support a link between stressful life events and the etiology of the disease, whereas the other 7 studies failed. In their own study they also failed to provide evidence for a link between stressful life events and disease activity in 24 patients with CD and 8 patients with UC (North et al., 1991). Furthermore, Riley et al. (1990) and Garrett et al. (1991) were not able to determine a clear correlation between stress and the pathogenesis of IBD. The existence of these contrary findings is particularly surprising in view of the unique well established bidirectional interactions between brain and gut, the prominent role of the gut associated lymphoid tissue in this brain—gut interaction, and in view of the common clinical impression that certain stressful life events frequently precede exacerbation of symptoms in all of these disorders (for review see Mayer, 2000b). As many of these studies investigating the effects of a defined stressor on IBD pathology do not explicitly distinguish between UC and CD patients (Duffy et al., 1991; North et al., 1991; Bernstein et al., 2010) or do only investigate stress effects on either UC (Salem and Shubair, 1967; Campbell et al., 1986; Leventstein et al., 1998; Bitton et al., 2003) or CD (Bitton et al., 2008) it is not possible to draw reliable conclusions on whether UC or CD is more heavily influenced by stress.

6. Rodent data supporting stress to be a risk factor for IBD

In addition to the already mentioned studies done in human and non-human primates, a growing number of rodent studies...
### Table 1
Summary of studies investigating the effects of different stressors on either reactivation of prior chemically induced colitis, aggravation/amelioration of chemically induced colitis, or development of spontaneous colitis.

<table>
<thead>
<tr>
<th>Drug to induce colitis</th>
<th>Type of stressor (duration)</th>
<th>Start of colitis induction relative to stressor exposure</th>
<th>Species (sex)</th>
<th>Stress effects on colitis</th>
<th>Stressor effects on histol. damage</th>
<th>Time of killing/blood drawing for determining plasma GC</th>
<th>Plasma GC levels</th>
<th>Further findings describing HPA axis activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effects of stressor exposure on prior chemically induced and already healed colitis</strong></td>
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</tr>
<tr>
<td>TNBS</td>
<td>Restraint (3 h/d; 3 d)</td>
<td>6 wk before Rats (male)</td>
<td>Reactivation</td>
<td>No</td>
<td>After last stressor</td>
<td>Increased (vs. non-stressed)</td>
<td>Plasma GC not different from VEH-treated rats exposed to the stressor</td>
<td>Collins et al. (1996)</td>
<td></td>
</tr>
<tr>
<td>DNBS</td>
<td>Restraint + sound (2 × 2 h/d; 5 d)</td>
<td>7—8 wk before Mice (male)</td>
<td>Reactivation when combined with sub-threshold dose of DNBS</td>
<td>Yes</td>
<td>After last stressor</td>
<td>Increased (vs. non-stressed)</td>
<td>Plasma GC not different from VEH-treated rats exposed to the stressor</td>
<td>Qiu et al. (1999)</td>
<td></td>
</tr>
<tr>
<td>DSS</td>
<td>WAS (1 h/d; 7 d)</td>
<td>4 wk before Mice (female)</td>
<td>Reactivation</td>
<td>Yes</td>
<td>After last stressor</td>
<td>Increased (vs. non-stressed)</td>
<td>Adrenal weight was increased (vs. non-stressed)</td>
<td>Melgar et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>DSS</td>
<td>WAS (1 h/d; 7 d)</td>
<td>4 wk before Mice (female)</td>
<td>Reactivation when combined with sub-threshold dose of DSS</td>
<td>Yes</td>
<td>After last stressor</td>
<td>Unaffected (vs. non-stressed)</td>
<td>Adrenal weight was increased (vs. non-stressed)</td>
<td>Melgar et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>DNBS</td>
<td>Restraint (3 h/d; 3 d)</td>
<td>6 wk before Rats</td>
<td>Reactivation when combined with sub-threshold dose of DNB</td>
<td>Yes</td>
<td>—</td>
<td>n.a.</td>
<td>Saunders et al. (2006)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Effects of stressor exposure on acute chemically induced colitis</strong></td>
<td></td>
<td></td>
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<tr>
<td>TNBS</td>
<td>PRS (2 h/d; 4 d)</td>
<td>1 d after Rats (male)</td>
<td>Aggravation</td>
<td>Yes</td>
<td>—</td>
<td>n.a.</td>
<td>ICV injection of CRH or AVP antagonists before each stressor worsened outcome</td>
<td>Gue et al. (1997)</td>
<td></td>
</tr>
<tr>
<td>TNBS</td>
<td>PRS (2 h/d; 4 d)</td>
<td>1 d before Rats (male)</td>
<td>Aggravation</td>
<td>Yes</td>
<td>—</td>
<td>n.a.</td>
<td>Gue et al. (1997)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNBS</td>
<td>WAS or PRS (alternatively; 3 h/d; 6 d)</td>
<td>24 h before Rats (female)</td>
<td>Aggravation (more pronounced in Lewis than Fischer rats)</td>
<td>Yes</td>
<td>20 min after 1st, 3rd, and 6th stressor</td>
<td>Increased in Fischer after 1st and 2nd stressor (vs. non-stressed)</td>
<td>-Daily (6 d) ICV injection of CRH reduced TNBS colitis in Lewis and Fischer rats -ICV CRH antagonist 10 min before each stressor worsened outcome in both strains</td>
<td>Million et al. (1999)</td>
<td></td>
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</tbody>
</table>
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<table>
<thead>
<tr>
<th>Model</th>
<th>Treatment</th>
<th>Time</th>
<th>Species</th>
<th>Outcome</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSS</td>
<td>Restraint (2 h/d; 4 d)</td>
<td>1 d after</td>
<td>Rats (male)</td>
<td>Faster symptom development</td>
<td>Yes</td>
</tr>
<tr>
<td>DSS</td>
<td>Restraint (2 h/d; 4 d)</td>
<td>Before (until fecal blood was detected)</td>
<td>Rats (male)</td>
<td>No effect</td>
<td>No</td>
</tr>
<tr>
<td>DSS</td>
<td>SD (2 h/d) or OC (24 h/d) over 19 d</td>
<td>1 d after</td>
<td>Mice (male)</td>
<td>Aggravation and impairment of regeneration</td>
<td>Yes</td>
</tr>
<tr>
<td>DSS</td>
<td>CSC (19 d)</td>
<td>1 d after</td>
<td>Mice (male)</td>
<td>Aggravation</td>
<td>Yes</td>
</tr>
<tr>
<td>DSS</td>
<td>MS (3 h/d; PND1—14)</td>
<td>9 wk after</td>
<td>Mice (male)</td>
<td>Aggravation</td>
<td>Yes</td>
</tr>
<tr>
<td>DSS</td>
<td>MS (3 h/d; PND1—14) and CSC (19 d; wk8—11)</td>
<td>1 d after termination of CSC</td>
<td>Mice (male)</td>
<td>Aggravation (vs. both resp. non-MS and non-CSC mice)</td>
<td>Yes</td>
</tr>
<tr>
<td>AA</td>
<td>WAS (30 min)</td>
<td>6 h before</td>
<td>Rats (male and female)</td>
<td>Amelioration</td>
<td>Yes</td>
</tr>
<tr>
<td>TNBS</td>
<td>PRS (2 h)</td>
<td>4 h after</td>
<td>Rats (male)</td>
<td>Amelioration</td>
<td>No</td>
</tr>
</tbody>
</table>
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Drug to induce colitis (duration)</th>
<th>Type of stressor induction relative to stressor exposure</th>
<th>Start of colitis induction relative to stressor exposure</th>
<th>Species (sex)</th>
<th>Stress effects on colitis</th>
<th>Stressor effects on histol. damage</th>
<th>Time of killing/blood drawing for determining plasma GC</th>
<th>Plasma GC levels</th>
<th>Further findings describing HPA axis activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNBS</td>
<td>Controllable ES (30 min)</td>
<td>4 h after</td>
<td>Rats (male)</td>
<td>Amelioration</td>
<td>Yes</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Injection of RU-486 (ip; 12 + 1 h before and 24 h after stressor) or CRH receptor antagonists (ICV; 10 min before stressor) reversed stressor effects</td>
<td>Gulpinar et al. (2004)</td>
</tr>
<tr>
<td>TNBS</td>
<td>Controllable ES (30 min; d0) + shock box (30 min/d; d1–3)</td>
<td>4 h after last stressor</td>
<td>Rats (male)</td>
<td>No effect</td>
<td>No</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Gulpinar et al. (2004)</td>
<td></td>
</tr>
<tr>
<td>TNBS</td>
<td>Controllable ES (30 min; d0) + shock box (30 min/d; d1–3) + PRS (2 h; d4)</td>
<td>4 h after last stressor</td>
<td>Rats (male)</td>
<td>Amelioration</td>
<td>Yes</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Gulpinar et al. (2004)</td>
<td></td>
</tr>
</tbody>
</table>

Effects of stressor exposure on the development of spontaneous colitis

- **CSC (19 d)**
  - Rats (male)
  - Induction of spontaneous colitis
  - Yes: d2, 3, 7, 14, 20 of stressor exposure
  - Increased only on d2 (morning)
  - Decreased on d20 (evening)
  - (all vs. non-stressed)
  - Adrenal weight was increased (d2, 3, 7, 14, 20)
  - Adrenal ACTH responsiveness was decreased (d20)
  - CRH mRNA in the PVN was only increased on d2 (vs. non-stressed)
  - ADX prior to CSC ameliorated spontaneous colitis
  - CRH-induced (ip; 30 min before killing) corticosterone production following 15 d of crowding was unaffected (vs. non-stressed)
  - Vicario et al. (2010)

- **Crowding (15 d)**
  - Rats (male)
  - Induction of colonic inflammation
  - No: d1, 3, 7, 12, 15 of stressor exposure
  - Increased at every time point assessed (vs. non-stressed)
  - Reber et al. (2007)

- **CSC (19 d)**
  - Mice (male)
  - Induction of spontaneous colitis
  - n.a.
  - n.a.

- **SDR (2 h/d; 6 d)**
  - Mice (male)
  - Induction of spontaneous colitis
  - Yes: 2 h after last stressor
  - Not affected

- **SDR (2 h)**
  - Mice (male)
  - No effect
  - No: 2 h after last stressor
  - Increased (vs. non-stressed)

- **Savignac et al. (2011)**
provide evidence for a link between exposure to different types of stress procedures and the pathogenesis of an experimentally induced and/or spontaneous colitis. In the current review the term colitis is used when animals developed cellular infiltration and/or histopathology, whereas more subtle indications of colonic inflammation as for instance increased cytokine production or myeloperoxidase (MPO) activity are referred to as colonic inflammatory processes. However, before enlightening the effects of stressor exposure on gut inflammation, relevant rodent models for the induction of IBD-like pathology will be introduced briefly. For further details on these models and on additional models the reader is directed to a recent review article by Singh and coworkers from 2001 (for review see Singh et al., 2001).

6.1. Rodent models for IBD

Besides adoptive transfer models in which colonic inflammation is induced in immunodeficient animals by the transfer of CD4+ Tcell subpopulations, and immune models in which colitis is induced by the generation of a single deletion of a target gene (knockout animals) or increased expression of a particular gene (transgenic animals) (for review see Singh et al., 2001), there is also a variety of chemically induced colitis models. Colitis thereby is induced by oral or rectal administration of different chemicals (inducible models). Although rectal administration of dinitrobenzene sulfonic acid (DNBS) (Qiu et al., 1999), trinitrobenzene sulfonic acid (TNBS) (Morris et al., 1989; Elson et al., 1996), acetic acid (AA), or oxazolone (Boirivant et al., 1998) reliably induces colitis, the application procedure itself poses an additional and uncontrollable stressor for the rodent and, thus, seems not to be the gold standard for investigating the effects of a defined stress procedure on the pathogenesis or severity of colonic inflammation. Despite of that disadvantage chemically induced colitis models so far are the only ones that have been used in this context in rodents. However, this is different for colitis induction by the oral administration of the sodium salt of a sulphated polysaccharide, dextran sulphate sodium (DSS), via the drinking water, which has been described for mice (Okayasu et al., 1990), rats (Kishimoto et al., 1992; Kimura et al., 1995), and hamsters (Ohkusa, 1985; Yamada et al., 1992). DSS colitis is restricted mainly to the large intestine and, therefore, was considered to be a useful animal model for human UC (Okayasu et al., 1990; Kitajima et al., 1999).

Interestingly, the finding of a Th1 shifted immune response in animals treated with DSS also suggests a similarity to human CD (Strober et al., 2002). In general, animals treated with DSS show a marked loss of body weight, rectal bleeding, reduction of colonic length, and destruction of the epithelial layer and glandular architecture of the large intestine (Okayasu et al., 1990; Cooper et al., 1993; Kitajima et al., 1999).

As stated by Singh and coworkers (for review see Singh et al., 2001) spontaneous animal models of IBD are uncommon, with the most studied being the already mentioned non-human primate tamarin model (Drossman, 1985; Madara et al., 1985; Podolsky et al., 1985; Gozalo and Montoya, 1992; Elson et al., 1995). However, recently we and others could show that also repeated/chronic psychosocial stressors are able to cause the development of spontaneous colitis (Reber et al., 2007, 2008, 2011; Savignac et al., 2011). Besides offering a new way of inducing spontaneous colitis in male mice, these findings provide important evidence for a link between repeated/chronic psychosocial stress and the development of IBD and, thus, will be discussed in more detail below.

6.2. Rodent data supporting (or at least not contradicting) the concept that stress-induced HPA axis activation has ameliorating effects on experimentally induced or spontaneous colitis (for summary see Table 1)

6.2.1. Stress-induced reactivation of chemically induced colitis

Collins et al. (1996) showed in rats that a previous colitis, which was induced by TNBS administration 6 weeks prior to stressor exposure, was reactivated by repeated restraint stress (3 h/d for 3 consecutive days). This was indicated by a significant increase in MPO activity. However, stress-induced inflammatory processes were not accompanied by tissue damage, suggesting that changes in colonic histology may take longer to establish (Collins et al., 1996). Restraint stress thereby had no effect in rats prior treated only with vehicle (VEH; EtOH). Interestingly, similar plasma corticosterone levels in TNBS and VEH-treated rats immediately following the third round of restraint stress indicate that factors different from HPA axis activation probably have mediated stress-induced reactivation of prior TNBS colitis (Collins et al., 1996).

In line, Qiu et al. (1999) demonstrated that DNBS colitis in mice resolves by 6 weeks, but can subsequently be reactivated by stress (2 x 2 h/d of combined restraint and sonic stress for 5 consecutive days) plus a sub-threshold dose of DNBS, but not by a sub-threshold dose of DNBS alone. Here, the reactivation of colitis was also paralleled by mucosal inflammation and ulceration and essentially dependent on the presence of CD4+ lymphocytes (Qiu et al., 1999). In addition, they have shown that stress impairs the colonic barrier function by decreasing mucus production and increasing colonic permeability (Qiu et al., 1999). Again, similar plasma corticosterone levels in DNBS and VEH-treated rats immediately following stressor termination indicate that the reactivation of colitis is unlikely to reflect an altered hypothalamic–pituitary response to stress in animals that had previous colitis.

It has been further shown that DSS colitis can be reactivated by water avoidance stress in mice (VAS; 1 h/d for 7 consecutive days) (Melgar et al., 2008) and DNBS colitis by restraint stress (3 h/d for 3 d) (Saunders et al., 2006), both applied in addition to a sub-threshold dose of the respective hapten. Interestingly, in the DSS study, plasma corticosterone levels seem blunted in response to repetitive VAS exposure in previously DSS-treated compared with VEH-treated mice, although the adrenal weight was increased in DSS mice. This suggests that reactivation of DSS colitis might be due to a functional loss of the adrenal glands and the consequent reduction in anti-inflammatory GC signaling in these mice (Melgar et al., 2008). However, it has to be mentioned here that reactivation of prior DSS colitis, although to a less pronounced extent, was also possible without adding the sub-threshold dose of DSS (Melgar et al., 2008). Interestingly,
both adrenal weight and plasma corticosterone levels were increased in WAS compared with non-stressed control mice. Although a role of the HPA axis cannot be excluded (not assessed) the DNBS study suggests noradrenergic and cholinergic neural stress pathways to be critical for the stress-induced relapse of colitis (Saunders et al., 2006).

6.2.2. Stress-induced aggravation of chemically induced colitis

Besides the stress-induced reactivation of resolved colitis, Gue et al. (1997) further showed that 4 d of partial restraint stress (PRS; 2 h/d) applied either prior to or after TNBS instillation, increased the severity of experimental colitis in rats. Interestingly, this effect was not mediated by increased brain levels of either CRH or AVP (Gue et al., 1997), key hormones in the activation of the HPA axis during stress. In contrast, brain CRH and AVP seemed even to restrain colitis aggravation during stress, as daily central injection of either CRH or AVP antagonist before PRS (applied prior to TNBS instillation) resulted in further enhancement of colitis severity (Gue et al., 1997). The authors, thus, hypothesized that mechanisms different from activation of the immunosuppressive HPA axis during the 4 d of PRS must be involved in aggravation of a subsequent TNBS colitis (Gue et al., 1997). In line, daily intracerebroventricular (ICV) injections of CRH during the 6 consecutive days following TNBS instillation resulted in a decreased colitis severity in inbred rat strains with hypo (Lewis/N) and hyper (Fischer344/N) CRH responsiveness to stress (Million et al., 1999). Further underlining the protective role of an increased HPA axis activity during stressor exposure on stress-induced aggravation of colitis, TNBS colitis was found to be more severely worsened by subsequent daily intermittent stress (3 h/d water avoidance stress or wrap restraint alternatively for 6 d) in CRH hyper-responsive Lewis/N compared with CRH hyper-responsive Fischer344/N rats (Million et al., 1999).

In 2002, Milde and Murison also showed that restraint stress (2 h/d for 4 consecutive days) further aggravates subsequent DSS colitis in rats. In contrast to earlier studies restraint stress did not aggravate or sustain the inflammatory condition, when applied following colitis induction (Milde and Murison, 2002). However, as in the Milde and Murison study visible bloody stools and not colonic MPO activity were taken as readouts for colitis severity, comparison with earlier studies is difficult.

Support for an aggravating effect of a more naturalistic and clinically relevant stressor, namely repeated/chronic psychosocial stress, on DSS colitis was recently also provided by our own group. Mice that were exposed for 19 d to either the social defeat/overcrowding (SD/OC) (Reber et al., 2006) or the chronic subordinate colony housing (CSC) (Reber et al., 2007; Veenema et al., 2008) stress paradigm and subsequently treated with DSS (1%, 7 d) showed an aggravated colitis compared with non-stressed single housed control mice. This was indicated by an increased body weight loss, inflammatory reduction of colon length, and histological damage score of SD/OC and CSC mice compared with respective controls on day 8 of DSS treatment (Reber et al., 2006, 2007; Veenema et al., 2008). Although a causal involvement still has to be proven, the decreased GC signaling — caused by adrenal insufficiency (Reber et al., 2006, 2007) and/or GC resistance (Reber et al., 2007) — in both SD/OC and CSC mice following stressor termination is very likely to be involved in the overshooting immune response in the colon of these mice during DSS treatment. This is indicated by the lack of an increase in plasma corticosterone levels on day 2 of DSS treatment although cytokine secretion from isolated mesenteric lymph node cells was already increased at that time point. In contrast, an increased cytokine secretion from isolated mesenteric lymph node cells in control mice was first detected after 7 d of DSS treatment and paralleled by high plasma corticosterone concentrations (Reber et al., 2008). Activation of the immunosuppressive HPA axis caused by inflammatory stimuli thereby can occur at various levels, including hypothalamic CRH/AVP neurons, pituitary corticotrophs, and the adrenal cortex (Sapolsky et al., 1987; Gue et al., 1997; for review see Tracey, 2002). As AVP, instead of CRH, has been suggested to be the main activator of the HPA axis during chronic inflammatory conditions like adjuvant-induced arthritis (Chowdrey et al., 1995; Harbuz et al., 1995), the decreased AVP mRNA expression in the PVN of CSC mice (Reber and Neumann, 2008) might additionally contribute to the generation of the overall pro-inflammatory milieu following CSC exposure. Further support for colitis aggravating/promoting effects of decreased GC signaling following chronic stress comes from our recent data showing that combination of early life stress (maternal separation, MS; 3 h/d, from postnatal days (PND) 1–14) and 19 d of CSC during adulthood have additive effects on DSS colitis. In contrast to CSC mice which just cannot adequately generate the diurnal rise in plasma GC, mice exposed to both MS and CSC suffer from hypocorticism even during the morning hours, although their adrenals are significantly enlarged (Veenema et al., 2008).

6.2.3. Stress-induced amelioration of chemically induced colitis

Besides studies providing evidence for stress exacerbating the severity and/or causing reactivation of an experimentally induced colitis, there are also studies showing contrasting effects. For example, Cakir et al. (2004) showed that 30 min of water avoidance stress, applied during the 6th hour after colitis induction by acetic acid (AA) infusion, ameliorated colitis severity. Interestingly, by the injection of the GR antagonist RU486 1 h before stressor exposure, they could demonstrate that this effect was mediated by GCS, the most potent endogenous inhibitors of inflammation (Cakir et al., 2004). In addition, Gulpinar et al. (2004) showed that acute controllable electric shock (ES) and to a lesser extent also PRS prior to colitis induction by TNBS reduced its severity, possibly via the stimulation of the HPA axis and/or the SNS. This was suggested by a more pronounced stress-induced aggravation of colitis severity in rats which were injected ICV with a CRH receptor antagonist or ip with the GC receptor antagonist RU-486 or the ganglion blocker hexamethonium 10 min prior to ES exposure (Gulpinar et al., 2004). They further hypothesized that the colitis aggravating effect of prior repeated restraint stress ( restraint; 2 h/d for 4 d) described by Gue et al. (1997) and the colitis ameliorating effect of their repeated stress paradigm (PRS after repeated ES exposure; day 1: exposure to ES; days 2, 3: exposure to the same boxes but receiving no shock; day 4: 2 h of PRS) might be due to a difference in stressor controllability (Gulpinar et al., 2004). However, based on the above reported colitis
ameliorating effects of HPA axis activation during stressor exposure, another explanation seems to be more likely. Daily homotypic stressor exposure — restraint stress (Gue et al., 1997) or ES/shock box exposure (Gulpinar et al., 2004) — might have resulted in an adaptation of the HPA axis and, thus, equal or just slightly increased GCs levels in comparison to unstressed control animals. In contrast to the Gue study, the additional exposure to the heterotypic PRS in the Gulpinar study before colitis induction (Gulpinar et al., 2004), probably has dramatically increased the levels of anti-inflammatory corticosterone at the time of colitis induction.

6.2.4. Stress-induced development of spontaneous colitis
As already mentioned, 19 d of CSC aggravate the severity of subsequent DSS colitis (Reber et al., 2008). Interestingly, in contrast to non-stressed controls, CSC mice, already on the second day of DSS treatment, showed an increased cytokine secretion from isolated mesenteric lymph node cells. This finding indicated that chronic psychosocial stressors themselves might trigger the development of spontaneous colitis. In agreement, CSC mice not subsequently treated with DSS also showed an increased cytokine secretion from isolated mesenteric lymph node cells 8 d following CSC termination (Reber et al., 2008). Furthermore, exposure to CSC caused an increase in the histological damage score, which is first detectable after 14 d of CSC (Reber et al., 2007), in the number of colonic macrophages, dendritic and Th cells (Reber et al., 2011), and in the cytokine secretion from isolated mesenteric lymph node (Reber et al., 2007) and lamina propria mononuclear (Reber et al., 2011) cells. Together, these findings clearly indicated, for the first time, that clinically relevant chronic psychosocial stressors in male mice cause the development of mild spontaneous colitis. Further evidence that psychosocial stress exposure can lead to spontaneous colitis in rodents comes from the group of John Cryan. These groups have demonstrated that a modified version of the social-disruption stress paradigm (SDR) (Avitsur et al., 2001; Bailey et al., 2006, 2007; Engler et al., 2008) caused increased plasma levels of pro-inflammatory cytokines and mild histological damage in the colon (Savignac et al., 2011) of male mice. Similar to CSC exposure (Reber et al., 2007, 2011; Schmidt et al., 2010), SDR stress has been repeatedly shown to result in decreased GC signaling (Avitsur et al., 2003; Engler et al., 2005, 2008), increased bacterial translocation into the host (Bailey et al., 2006), and changes in the structure of the intestinal microbiota (Bailey et al., 2011).

Support comes also from a recent study done by Vicario et al. (2010). They showed that 15 d of crowding stress causes mild colonic inflammation, indicated by an increased MPO activity, and increased mast cell degranulation and mucosal content/luminal release of mast cell protease-II. However, whether or not the lack of histological damage following 15 d of crowding stress is due to a pronounced immunosuppression by the continuously increased plasma GC levels still remains elusive. It might also be possible that crowing stress is less severe than for instance CSC and, therefore, histological damage might not be detectable after 2 week of stressor exposure but might take longer to develop.

It is, therefore, very likely that during conditions of repeated/chronic psychosocial stress a reduction in the immunosuppressive signaling capacity of GC and increased number or changed community structure of intestinal/translocated bacteria contribute to an overall pro-inflammatory milieu, which in turn promotes the development of mild colitis.

6.2.5. Conclusions to be drawn from these studies on the underlying HPA axis-dependent mechanisms
While it appears at first glance that the effects of stress on the pathogenesis of experimentally induced colitis in rodents are contrasting, closer inspection reveals that stress-induced HPA axis activity and, thus, anti-inflammatory GC signaling is beneficial rather than detrimental. Although the face and predictive validity of many of these animal models have to be viewed with caution, these findings seem to be in line with the limited human data available (see above; for review see Mawdsley and Rampton, 2006). Support comes also from the fact that adrenocorticotropic hormone and corticosteroids are not just commonly used for treatment of IBD but have also been proven to be highly effective (for review see Singh et al., 2001; Sands, 2007). However, although the ameliorating effect of HPA axis activation on stress-induced colitis aggravation/development seems to be generally accepted, recent evidence suggests that an increase in GC signaling, especially during the initial phase of chronic stressor exposure, might also have opposite effects.

6.3. Rodent data supporting the concept that stress-induced HPA axis activation has colitis-promoting effects (for summary see Table 1)
A detrimental role of HPA axis activation on experimentally induced colitis, at least during the initial stress phase, is suggested by our recent finding that adrenalectomy (ADX) prior to CSC exposure results in amelioration of subsequent DSS colitis (Reber et al., 2008). Furthermore, ADX mice exposed to chronic psychosocial stress only showed a slight increase in cytokine secretion from isolated mesenteric lymph node cells and no histological abnormalities (Reber et al., 2007). Thus, given that ADX prior to CSC ameliorates the CSC-induced aggravation of DSS colitis (Reber et al., 2008) and prevents the development of a full-blown spontaneous colitis (Reber et al., 2007), activation of the HPA axis during the initial phase of chronic psychosocial stress, while the adrenals are still fully functional and the target cells are still responsive to GC, seems to have a colitis-promoting effect (Reber et al., 2007).

Assessment of several functional levels of the colon during the initial stress phase (10 h of CSC) revealed a pronounced, adrenal hormone-mediated, local immune suppression in the colonic tissue; probably allowing luminal- and translocated-bacteria to proliferate without constraint (Reber et al., 2011). Immune suppression was indicated by a reduced cytokine and immunoglobulin (Ig) A secretion from isolated and anti-CD3/IL-2-stimulated lamina propria mononuclear cells (LPMC), a decreased percentage of CD3+ cells within all isolated LPMC, a decreased pro-inflammatory colonic cytokine mRNA expression, and a lower number of F4/80+ macrophages, CD11c+ dendritic cells, CD3+ T cells, and CD4+ T helper (Th) cells in colonic tissue of CSC compared with non-stressed mice. Whether or not this effect is mediated by
cortical GC or medullary catecholamines still needs further investigation. However, given the well documented immunosuppressive effects of prolonged high doses of GC (Dhabhar and McEwen, 1999) it is very likely that this initial immunosuppression is mediated by cortical GC and, thus, the activation of the HPA axis, instead of medullary catecholamines.

6.4. HPA axis-independent mechanisms underlying colitis-promoting effects of stress

It is known from clinical studies that during IBD, excessive penetration of antigens through a leaky epithelial barrier results in an inappropriate immune stimulation, which leads in turn to chronic gastrointestinal inflammation (Hollander, 1992; Wyatt et al., 1993). For instance, increased epithelial permeability has been shown to play a role in the onset (Hollander et al., 1986; May et al., 1993; Yacyshyn and Meddings, 1995), and relapse, of CD (Wyatt et al., 1993). Support in this direction is also provided from animal studies. For example, injecting luminal bacterial wall extracts directly into the sub-mucosa of rats, thus bypassing the epithelial barrier, initiates a chronic relapsing inflammatory syndrome similar to CD (Yamada et al., 1993; Meddings and Swain, 2000). Furthermore, simply decreasing epithelial barrier function in mice, by altering the expression of intracellular adhesion molecules, prompts the spontaneous generation of intestinal inflammation (Hermiston and Gordon, 1995; Meddings and Swain, 2000). Altering luminal constituents by housing these animals in a germ free environment can delay, or even prevent, disease onset (Rath et al., 1996).

Interestingly, experiments employing prolonged antibiotic treatment revealed also a causal role of such bacterial translocation/proliferation during the initial phase of CSC in initiating colonic inflammation (Reber et al., 2011). In line, exposure to SDR failed to increase for instance circulating IL-6 levels when mice were treated with an antibiotic cocktail (Bailey et al., 2011). However, in contrast to SDR causing bacterial translocation into secondary lymphoid organs (Bailey et al., 2006) 10 h of CSC only increased the number of bacteria in the stool and colonic tissue (Reber et al., 2011). This suggests that stress-induced changes in the composition of colonic microbiota and/or adherence to/ translocation into the lamina propria of the colon might be enough for induction of inflammatory processes. However, many stressors have been shown to change the composition of the intestinal microbiota in both humans and laboratory animals (Holdeman et al., 1976; Everson and Toth, 2000; Bailey et al., 2004, 2010, 2011) but do not cause development of spontaneous colitis. This suggests additional factors to be vital in developing these stress-induced changes in the colonic microbiota into a full-blown colitis. In support, the above mentioned ADX-CSC data (Reber et al., 2007) clearly indicate an important role for the immune-modulatory effects of fluctuating levels of adrenal hormones, i.e. the pronounced immunosuppression during the initial and the over-reactive immune system during the late CSC phase, in spontaneous colitis development (Reber et al., 2011). This overshooting immune response to bacterial antigens during later stressor exposure might further be promoted by a chronic stress-induced up-regulation of Toll-like receptor 4 (TLR4) expression in the colonic mucosa (McKernan et al., 2009), which is one of the pattern recognition receptors of the innate immune system activated by bacterial cell wall products.

Importantly, all different compartments of the intestinal barrier, i.e. physical-diffusion barriers, regulated physiological and enzymatic barriers, and immunological barriers, are under neuro-hormonal control and, thus, possible targets for the inflammation-inducing influence of stress (for review see Söderholm and Perdue, 2001). Therefore, it is not surprising that both animal and human studies demonstrate that various stressors affect the functional integrity of the gastrointestinal tract, resulting in an altered mucin production (Castagliuolo et al., 1996, 1998; Pfeiffer et al., 2001), secretion of ions and water (Barclay and Turnberg, 1987, 1988; Santos et al., 1998), and intestinal permeability (Saunders et al., 1994, 1997; Kiliaan et al., 1998; Santos et al., 1999, 2000; Ferrier et al., 2003; Vicario et al., 2010). Furthermore, Meddings and Swain (2000) showed that two very different forms of stress exposure, namely 3 h of immobilization or 20 min of forced swimming, both increased intestinal permeability of all segments of the gastrointestinal tract (Meddings and Gibbons, 1998; Meddings and Swain, 2000).

Although there is limited evidence in rats showing endogenous GC (Meddings and Swain, 2000) and dexamethasone (Spitz et al., 1996) to be involved in the stress-induced decrease in colonic-barrier function, ADX mice exposed to 10 h of CSC still display signs of leaky barrier function, which argues against this hypothesis. Therefore, it is more likely that the initial decrease in colonic sulphomucins and development of an obolescent mucosa during CSC exposure (Reber et al., 2011) are mediated by mechanisms different from HPA axis activation.

As activation of the noradrenergic and cholinergic pathways were already suggested to be involved in the stress-induced relapse of colitis (Saunders et al., 2006), it should be mentioned in this context that tyrosine hydroxylase mRNA expression is significantly up-regulated in the colonic tissue of CSC mice during the initial stress phase (Reber et al., 2007). Tyrosine hydroxylase is the rate-limiting enzyme in the production of catecholamines, which suggests that CSC-induced barrier defects might be mediated by a local activation of the sympathetic nervous system instead of the HPA axis. In support, elevated plasma norepinephrine (NE) levels were observed after 19 d of CSC exposure, which suggests that, in contrast to the HPA axis, the SNS is still able to respond when challenged. This uncoupling of the HPA axis and the SNS during chronic psychosocial stressor exposure may also promote pro-inflammatory processes, since the synergism between steroid hormones and SNS neurotransmitters will be dissipated (Straub et al., 2002).

Increased basal plasma NE levels following 19 d of CSC are in line with the general accepted idea that during conditions of stress predominantly the sympathetic/catecholaminergic tree of the ANS is activated and the parasympathetic/cholinergic tree is inhibited (for review see Goldstein and Kopin, 2007; Tracey, 2009). It has been further shown that, besides tonic inhibition of heart rate and cardiac contractility during resting conditions, the cholinergic ANS maintains homeostasis by limiting pro-inflammatory responses within a healthy, protective, and non-toxic range (for review see Tracey, 2002, 2009). Thus, inhibition of this cholinergic anti-inflammatory reflex following/during 19 d of CSC might further contribute to the development of an overall pro-inflammatory milieu.

Several studies also suggest an important role for mast cells, peripheral CRH, and cholinergic/parasympathetic mechanisms in the pathophysiology of stress-mediated barrier disturbances (Castagliuolo et al., 1996; Saunders et al., 1997; Kiliaan et al., 1998; Santoss et al., 1999, 2000). It is hypothesized that mast cells may be activated in parallel with various other factors for instance by CRH and/or acetylcholine released by peripheral gut neurons. As mechanisms different from HPA axis activation underlying stress-induced break down of colonic barrier function are not in the focus of the current review, the interested reader is directed to excellent studies done by the groups around Santos, Saunders, Söderholm, Tache, and Perdue (Perdue et al., 1991; Santoss et al., 1999, 2000, 2001; Söderholm and Perdue, 2001; Saunders et al., 2002a,b; Söderholm et al., 2002a,b; Tache et al., 2002, 2004). In line, just recently a role of mast cells in chronic stress-induced development of spontaneous colonic inflammation has been suggested by an increase in colonic MPO activity and mast cell-mediated barrier dysfunction in the rat bowel following 15 d of crowding stress (Vicario et al., 2010).

Stress-induced opening of colonic epithelial tight junctions might also be mediated by a stress-induced increase in IFN-γ secretion from CD4+/CD8+ T cells, which in turn activates myosin light chain kinase (Ferrier et al., 2003).

7. Summary (see Fig. 1)

Independent of how the above described barrier defects are induced by stress, there have been repeatedly shown to result in increased bacterial translocation from the gut lumen into colonic tissue (see Fig. 1, grey line), mesenteric lymph nodes, the liver, and the spleen (Ando et al., 2000; Eversion and Toth, 2000; Ding et al., 2004; Wang et al., 2004; Bailey et al., 2006; Reber et al., 2011). A pronounced adrenal-hormone mediated suppression of the local gut immune system (see Fig. 1, green line) allows unhindered proliferation of luminal and trans-located bacteria, which leads to a further increase in the antigen load found in colonic tissue (Reber et al., 2011) during the initial phase of stressor exposure. It is further very likely that stressor-induced alterations in the structure of intestinal microbiota (Bailey et al., 2010, 2011) are mediated by such pronounced local immunosuppression.

As most stress paradigms initially result in a pronounced activation of all stress-relevant neuroendocrine systems, the above described scenario, i.e. local immunosuppression, barrier defects, and bacterial translocation, is likely to be a common feature of stressor exposure, independent of the type of stressor.

However, whether or not this finally results in a full-blown spontaneous colitis strongly depends on the type and duration of the stressor and the activity/response patterns of the neuroendocrine systems. In detail, these responses include the regulation of plasma corticosterone by the HPA axis activity (see Fig. 1, blue line), regulation of plasma and colonic NE levels (for colonic TH mRNA levels see Fig. 1, turquoise line) by the SNS, and the GC sensitivity of target cells (see Fig. 1, purple line) during stressor exposure. Stressors that result in an overall decrease in GC signaling, induced by either adrenal insufficiency (Reber et al., 2007, 2008; Veenema et al., 2008) or GC resistance (Avitsur et al., 2001, 2002; Engler et al., 2005; Reber et al., 2007; Schmidt et al., 2010), have been shown to promote the development of a full blown spontaneous colitis in this context (Reber et al., 2007, 2008, 2011; Savignac et al., 2011), characterized by histological damage and leukocyte infiltration (see Fig. 1, red line). HPA axis activation during stressor exposure has been consistently reported to have ameliorating effects on chemically induced colitis. Thus, the colitis-promoting effect of decreased GC signaling during repeated/chronic psychosocial stressors is probably due to a lack of immunosuppression and a consequent overshooting of the immune response (see Fig. 1, green line) to the increased antigen load (see Fig. 1, grey line) in the colonic tissue. Importantly,
animal models of stressor exposure that result in this decrease in GC signaling mainly seem to be repeated/chronic and psychosocial in nature (Reber et al., 2007, 2011; Savignac et al., 2011), like human stressors that are discussed to be risk factors for the development of many affective and somatic disorders, including IBD. Repeated/chronic psychosocial stress paradigms, thus, seem to represent the most promising tools to learn more about the mechanism underlying stress-induced development of IBD. As colitis induced by these chronic social stressors in general is very mild, and as IBS over the last years has been repeatedly shown to go along with low-grade mucosal inflammation, I finally just want to raise the possibility that CSC and SDR might also pose adequate models for IBS instead of or in addition to IBD-like pathology. Investigation of IBS key symptoms, as for instance visceral pain sensitivity using the colorectal distension model, would help to clarify this aspect.

Taken together, these animal and human studies have greatly increased our understanding on the neuroendocrine mechanisms underlying stress and IBD. Further, they substantiate the recently phrased hypothesis by Karin and coworkers (for review see Karin et al., 2006) that an aberrant epithelial barrier instigated not only by genetic and microbial, but also stress factors and followed by microbial invasion and the failure to control leukocyte activation, might indeed be central to the pathogenesis of IBD.

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Conflict of interest

None.

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