

Chapter 7

Stress and the Gastrointestinal System

Bruno Bonaz

Abstract Stress was characterized by Hans Selye, in 1936, as the “stereotyped biological response to any demand.” Corticotrophin-releasing factor (CRF) and its related peptides urocortins (Ucns 1,2,3) are the mediators of stress. They are present both in the central nervous system and in the gastrointestinal (GI) tract where they exert their biological actions on target cells through activation of two receptors, CRF1 and CRF2. Stress is able to modulate numerous functions of the GI tract such as motility, secretion, permeability, sensitivity, and microbiota. Classically, stress delays gastric emptying while stimulating colonic transit and secretion, increases intestinal permeability and visceral sensitivity, and modifies intestinal microbiota. Through these various effects at the level of the GI tract, stress is involved in the pathophysiology of irritable bowel syndrome (IBS), a functional digestive disorder, as well as inflammatory bowel disease (IBD), Crohn’s disease, and ulcerative colitis. Targeting these CRF1/CRF2 signaling pathways by selective antagonists/agonists should be of clinical interest in the domain of IBS and IBD.

Keywords Autonomic nervous system • Corticotrophin-releasing factor • Gastrointestinal • Hypothalamus • Inflammatory bowel disease • Irritable bowel syndrome • Stress • Sympathetic nervous system • Urocortins • Vagus nerve

Stress-Definition

In 1936, Hans Selye defined the concept of stress as the “stereotyped biological response to any demand” [168] and elaborated the concept of the general adaptation syndrome. Later, the hypothalamic factor named corticotrophin-releasing factor (CRF) which stimulates ACTH release by the rat pituitary was discovered [77], positioning the hypothalamic–pituitary adrenal (HPA) axis as a key element in this concept. In 1981, Vale and his group [196] identified the 41-aa peptide CRF characterized

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from ovine hypothalami and later the cloning of CRF receptors and the development of specific CRF receptor antagonists [157]. In 2003, McEwen and Wingfield [121] defined the concept of allostasis as a process through which organisms actively adjust to both predictable and unpredictable events with allostatic overload being the cause of serious pathophysiology.

History of Stress Influence on Gut Functions and Diseases

In 1833, William Beaumont [15], a US Army surgeon, was the first to describe the influence of fear and anger on gastric acid secretion of his patient Alexis St. Martin, a Canadian trapper with a permanent gastric fistula caused by a musket shot. In 1902, Walter Cannon [42] observed gastrointestinal (GI) motility disturbances in the cat faced with an aggressive dog. Numerous other stress models have confirmed the effect of stress on GI motility in rodents [185]. Nowadays, the effect of psychological, physical, and immunological stressors on GI secretion, motility, epithelial permeability, visceral sensitivity, microbiota, and inflammation is clearly demonstrated, and stress has a major influence in irritable bowel syndrome (IBS) and most likely plays a role in inflammatory bowel disease (IBD). The circuitries and biochemical coding, particularly CRF and its receptors, involved in the stress response have been characterized through (i) the use of the immediate early gene *c-fos*, a marker of neuronal activation, and its relation to the autonomic regulation of gut functions [33] and (ii) its combination with the identification of neuromediators activated by stress models and (iii) the use of CRF-overexpressing as well as knock-out animals.

The present review will describe first the state of knowledge on the distribution of the CRFergic system including CRF and its related peptides urocortins (Ucns), and their CRF receptors, in the brain and in the GI tract and the effects and mechanisms of stress on gastrointestinal motility, secretion, permeability, visceral sensitivity, and microbiota. Lastly, the implication of stress in the pathophysiology of IBS and IBD [Crohn's disease (CD) and ulcerative colitis (UC)] will be highlighted.

Mechanisms of Stress

Stress Peptides and Their Receptors

CRF and CRF-Related Peptides (Fig. 7.1)

CRF is a member of a family of mammalian CRF-related peptides including Ucn 1, Ucn 2 (known as stresscopin-related peptide), and Ucn 3 (known as stresscopin) [12] with 40, 38, and 38 aa in length, respectively. During stress, CRF is released from

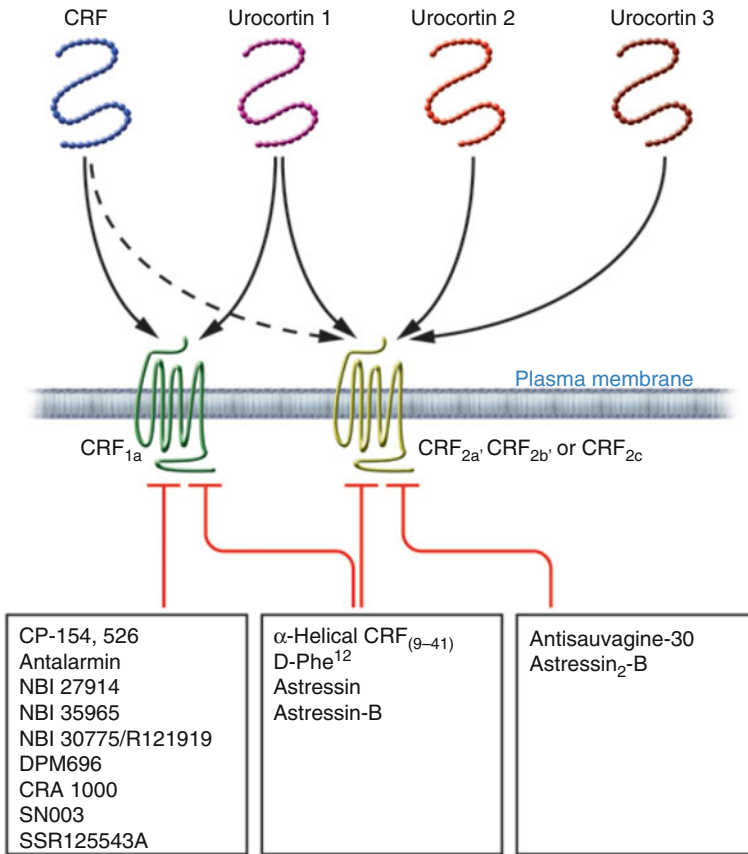


Fig. 7.1 The CRF peptide family and its receptors and receptor antagonists (With permission from Taché and Bonaz [185]). *CRF* corticotrophin-releasing factor

the hypothalamus and is vehiculated through a portal venous network to the anterior pituitary where it triggers the release of ACTH which stimulates the release of cortisol, the stress steroid, by the adrenal glands, i.e., the HPA axis (Fig. 7.2). CRF also acts directly in the central nervous system (CNS) as a neurotransmitter and neuro-modulator through the projections of CRF-containing neurons to areas of the central autonomic network that controls the central response to stress. The intracerebral injection of CRF mimics behavioral (anxiety/depression, alterations of feeding), autonomic (sympathetic and sacral parasympathetic activation, vagal inhibition), immune, metabolic, and visceral responses induced by various systemic or cognitive stressors [12, 188]. The hypothalamic peptide arginine–vasopressin (AVP) acts in synergy with CRF to stimulate ACTH secretion in acute stress conditions, while a habituation of the hypothalamic CRF is observed in chronic stress conditions where AVP takes over from CRF [31].

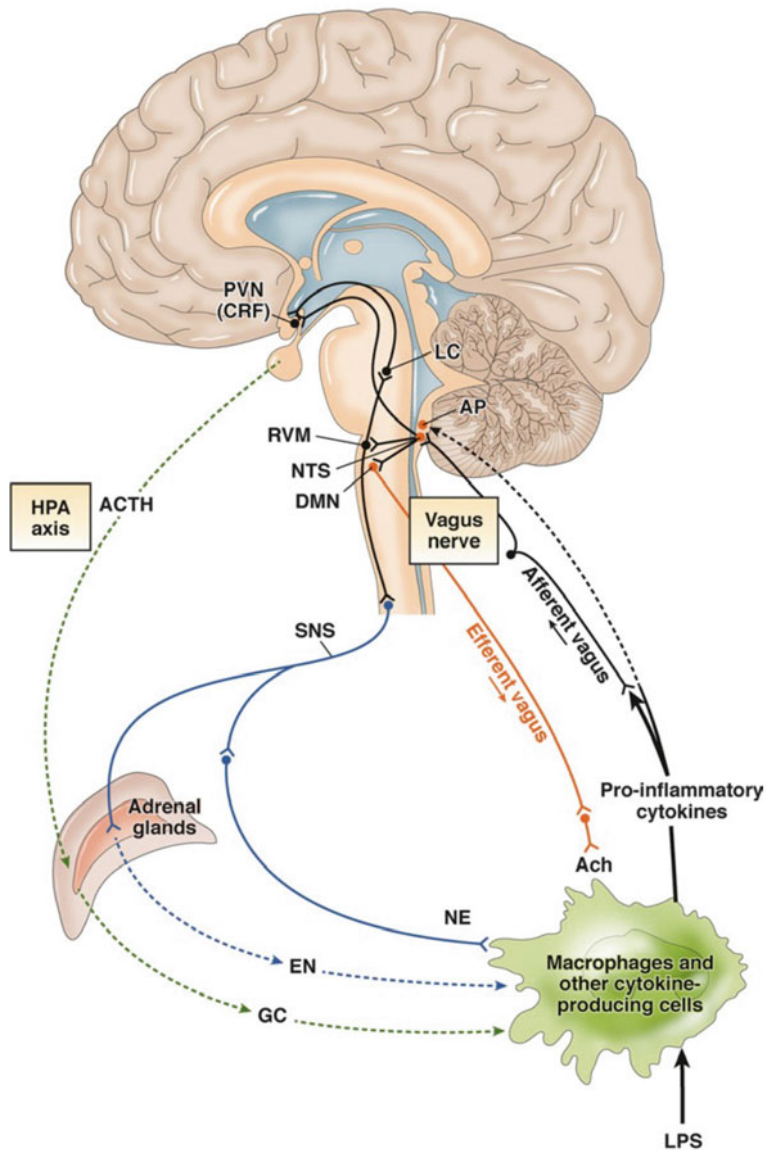


Fig. 7.2 The hypothalamic–pituitary–adrenal axis and the autonomic nervous system (With permission from Bonaz and Bernstein [27]). *ACTH* adrenocorticotrophic hormone, *AP* area postrema, *CRF* corticotrophin-releasing factor, *DMN* dorsal motor nucleus of the vagus nerve, *EN* epinephrine, *GC* glucocorticoids, *HPA* hypothalamic–pituitary adrenal axis, *LC* locus coeruleus, *LPS* lipopolysaccharides, *NE* norepinephrine, *PVN* paraventricular nucleus of the hypothalamus, *RVM* rostral ventrolateral medulla

CRF Receptors

CRF and Ucn exert their biological actions on target cells through activation of two receptors, CRF1 (415 aa) and CRF2 (411 aa), that belong to the B1 class subfamily of 7-transmembrane domain G protein-coupled receptors and are encoded by two distinct genes [83].

CRF1a, the main functional variant of CRF1, is greatly expressed in the brain and some peripheral tissues in mammals [83]. Alternative splicing of the primary transcript encoding CRF1 can lead to other variants ($n=8$), named CRF1b–i, all of which display impaired signaling [83]. The functional significance of these other transcripts is still poorly characterized. Selective peptide CRF1 agonists (cortagine and stressin1-A) have been developed [155]. Three functional *CRF2* variants (2a,b,c) have been identified in humans [83], whereas only 2a and 2b are expressed in other mammals.

CRF1 and CRF2 have distinct affinities for the CRF family of peptides [12, 83]. CRF has a higher (10- to 40-fold) affinity for CRF1 than for CRF2. Ucn 1 binds CRF2 with a greater (100-fold) affinity than CRF and CRF1 with greater (sixfold) affinity than CRF. Ucn 2 and 3 exhibit high selectivity only for CRF2, with a slightly higher affinity for CRF2b *versus* CRF2a. CRF2a, CRF2b, and CRF2c are almost identical with high affinity for Ucn 1, Ucn 2, and Ucn 3 and lower affinity for rat/human CRF. In non-primate mammals, CRF2a is expressed only by neurons and CRF2b mainly in the periphery and by nonneuronal cells of the brain, whereas CRF2c is found only in the amygdala of the human brain. The binding of CRF family peptides to their receptors has been characterized [83]. The long N-terminal extracellular domain of CRF receptors primarily interacts with the C-terminal residues of CRF, and the N-terminal residues of CRF interact with the transmembrane region of the receptor, resulting in conformational changes that enable G protein activation. Stimulation of CRF1a, CRF2a, and CRF2b activates adenylyl cyclase/cAMP–protein kinase A signaling pathways through coupling and activation of G α s proteins. CRF receptors modulate various kinases, including phosphokinases A, B, and C, and can phosphorylate and activate mitogen-activated protein kinase (MAPK), particularly the ERK1/2 and p38/MAPK pathways. CRF receptors also stimulate transient calcium mobilization in certain cell types by phospholipase C activation *in vitro* and PKC activation *in vivo*.

CRF receptor antagonists have been developed to characterize the functions of CRF receptors in the behavioral (anxiety, decreased feeding, and drug seeking), cognitive (arousal and anxiety), neuroendocrine (ACTH release), autonomic (activation of the sympathetic nervous system), immunological, and visceral (hypertension and alterations in gut motor function) responses to stress [50]. α -helical CRF9–41 was the first CRF receptor antagonist [157] followed by subsequent CRF antagonists such as d-Phe¹²CRF^{12–41} and astressin. Astressin-B was developed later, as the most efficacious and still effective 24 h after a single peripheral injection [158]. Peripheral administration of these peptide antagonists has a poor penetration into the brain and could thus be used to selectively block physiological processes of the GI tract. Selective CRF2 peptide antagonists, binding equally to CRF2a, b, and c with little or no affinity for CRF1 receptors, have been developed

[156] such as antisauvagine-30 and the long-acting analog astressin2-B. Non-peptide selective CRF1 antagonists that cross the blood–brain barrier depending on their lipophilicity have been developed, e.g., CP-154,526, antalarmin, NBI 30775, and NBI 35965 [50]. The activation of the brain CRF/CRF1 signaling pathway plays a major role in the coordination of many physiological responses to adaptive stress through the activation of the HPA axis, autonomic nervous system (ANS), and changes in cardiovascular, GI, and immune functions in rodents and primates [80, 115]. The abnormal increase of central CRF1 signaling could contribute to the pathogenesis of anxiety and depression as well as to IBS [11, 80, 161]. CRF2 receptors in the brain dampen and/or facilitate the proper recovery of the CRF1-initiated behavioral, endocrine, and visceral responses to stress [154] and have a primary role in the anorexic response to exogenous administration of CRF and Ucn1 [177]. CRF-binding protein (332-aa) isolated in different species functions as an endogenous antagonist by sequestering CRF ligands, thus modulating the access of CRF and related peptides to CRF receptors [16].

Expression of the CRFergic System in the Central Nervous System and the Gastrointestinal Tract

In the brain, these peptides are expressed in distinct regions. CRF is essentially expressed by the neurons of the parvocellular part of the paraventricular nucleus of the hypothalamus (PVH) involved in the activation of the HPA axis, in the prefrontal and cingulate cortices which mediate behavioral and cognitive components of stress, as well as in the central nucleus of the amygdala (CeA) [182], a limbic structure involved in the processing of emotion [29]. CRF is also present in the Barrington's nucleus, which is located adjacent to the locus coeruleus (LC) and regulates the sacral parasympathetic nucleus and thus the autonomic control of the rectum, left colon, and bladder [199]. CRF is also found in the hippocampus, bed nucleus of the stria terminalis, nucleus accumbens, several thalamic areas, substantia nigra, raphe, periaqueductal gray, cerebellum, and spinal cord.

Ucn 1 neurons are mainly localized in the Edinger–Westphal nucleus (EWN) and lateral superior olive of the pons in rodents, humans, and nonhuman primates and at a lesser extent in the olfactory bulb, supraoptic nucleus (SON), ventromedial and magnocellular regions of the PVH, lateral hypothalamic area, several cranial nerve motor nuclei, and in neurons of the ventral horn of the spinal cord in experimental animals and humans [25, 97]. In the rat, the highest density of Ucn 1 immunoreactive fibers is found in the lateral septum, with more moderate levels in the SON, PVH, periaqueductal gray, EWN, dorsal raphe, nucleus of the solitary tract (NTS), parabrachial nucleus, substantia nigra, and interpeduncular nucleus, as well as throughout the length of the spinal cord. The only Ucn 1 projections are those from the EWN to the spinal cord and lateral septum. There is little neuroanatomical overlap between the brain distribution of CRF and Ucn 1.

Ucn 2 mRNA is expressed in the PVH, SON, and arcuate nucleus of the hypothalamus and in the LC as well as in several cranial nerve motor nuclei and the

ventral horn of the spinal cord [113, 152]. The neuroanatomical distribution of Ucn 2-containing fibers has not been characterized.

The distribution of Ucn 3 in the brain differs from that of CRF and Ucn 1 and Ucn 2. Ucn 3 is essentially located in the median preoptic nucleus, perifornical area between the fornix and the PVH, dorsal division of the medial amygdaloid nucleus, and superior paraolivary nucleus [107, 113]. Major Ucn 3 terminal fields are found in the forebrain, including the amygdala, the lateral septum, and the ventromedial hypothalamus.

CRF and Ucn 1 and Ucn 2 are localized in several stress-related nuclei involved in the modulation of GI functions and the integration of afferent signals from visceral origin, such as the PVH, SON, Barrington nucleus, and LC.

CRF ligands and receptors are also expressed in the *GI tract* in animals and humans where they can act directly on the gut in a paracrine or autocrine manner, thus supporting a role for peripheral CRF and/or Ucn in the modulation of GI functions [38, 47, 48, 143, 144, 178].

CRF is present in guinea pig cecal smooth muscle cells and in epithelial and submucosal immune cells of the GI tract as well as in epithelial cells of the colonic mucosa including endocrine (enterochromaffin) cells, in the myenteric and submucosal plexuses along the GI tract. Numerous CRF-containing nerve terminals reached the circular smooth muscle layer and submucosal arterioles. *Ucn 1* has been described in the rat duodenum and in human gastric tissue, in parietal cells and others, as well as in the human colonic mucosa and in the submucosal and myenteric plexuses along the small intestine and colon of the rat. *Ucn 2* is mainly distributed in the stomach, while low levels are observed in the small intestine and colon. In contrast, Ucn 3 is highly expressed in the gut, mainly in the stomach, small intestine, and colon, but not in the ileocecal region, cecum, and esophagus.

CRF receptors: CRF1 is present in the rat colon in goblet and stem cells of the crypts as well as on surface epithelial cells, lamina propria, and preferentially in the myenteric nervous plexus. CRF1 and CRF2 are expressed in the lamina propria of the human colon [133], particularly at the level of the colonic myenteric plexus [125]. CRF2a mRNA is expressed in human colonic epithelial HT-29 cells, with little expression of the b and c splice variants [95]. CRF2 receptors are highly expressed in the rat upper gut, including the esophagus and stomach.

Neuroanatomy of the Brain–Gut Axis (Figs. 7.2 and 7.3)

The CNS and the gut communicate bidirectionally through the *ANS* (i.e., the brain–gut axis) and the circumventricular organs. The gut contains a “little brain,” i.e., the enteric nervous system (ENS), which contains as many as neurons as in the spinal cord (400–600 million) and can function independently of the CNS for the programming of motility and secretion [66]. Some neuropeptides and receptors are present in both the ENS and the CNS. The function of the GI tract is modulated by both the ENS and the ANS which is composed of the sympathetic (i.e., the splanchnic nerves) and parasympathetic nervous system, i.e., the vagus nerves (VN), the largest visceral sensory nerve in the body, and the sacral parasympathetic nucleus represented by the

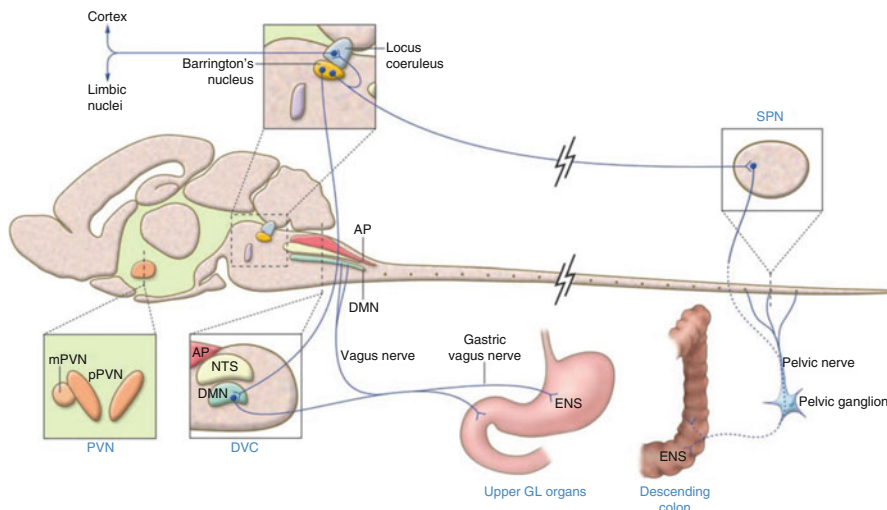


Fig. 7.3 Regions where the activation of CRF receptors influences gastric and colonic motor functions through neural pathways innervating the gut (With permission from Taché and Bonaz [185]). *AP* area postrema, *DMN* dorsal motor nucleus of the vagus nerve, *DVC* dorsal vagal complex, *ENS* enteric nervous system, *NTS* nucleus tractus solitarius, *PVN* paraventricular nucleus of the hypothalamus (*m* magnocellular, *p* parvocellular), *SPN* sacral parasympathetic nucleus

pelvic nerves, which are mixed systems. The VN contains 80–90% of afferent fibers carrying information from the abdominal organs to the brain [4] with the exception of pelvic viscera for which information is transmitted to the sacral (S2–S4) spinal cord by the pelvic nerves with central projections similar to other spinal visceral afferents. The VN carries mainly mechanoreceptor and chemosensory information of the gut. Vagal afferents do not encode painful stimuli but are able to modulate nociceptive processing in the spinal cord and the brain [149]. The VN is described as the sixth sense based on how sensory vagal information is processed in the CNS [210]. The sympathetic nerves contain 50% afferent fibers. Visceral afferents that enter via spinal nerves (i.e., splanchnic and pelvic nerves), at thoracic five–lumbar two segments of the spinal cord, carry information concerning temperature as well as nociceptive visceral inputs related to mechanical, chemical, or thermal stimulation through C and A δ afferents, which will reach conscious perception. The afferent information of the ANS reaches the CNS at both the spinal cord, for the splanchnic nerves, and the NTS in the dorsal medulla, and sacral parasympathetic (S2–S4) for the VN and pelvic nerves, respectively. At the level of the spinal cord, sympathetic afferents are integrated at the level of laminae I, II outer, V, VII (indirectly), and X, and the information is then projected to the upper level through the spinothalamic and spinoreticular tracts, the dorsal column with projection to the thalamus (ventral posterolateral nucleus, intralaminar nucleus) and the cerebral cortex (insular, anterior cingulate, dorsolateral prefrontal cortex, etc.). At the level of the NTS, vagal afferent information is integrated according to a visceral somatotopy in subnuclei (e.g., medial, commissural, gelatinosus) [3] then projecting to the parabrachial nucleus, in the pons,

according to a viscerotopic organization, which in turn sends projections to numerous structures in the brainstem, hypothalamus, basal forebrain, thalamus, and cerebral cortex [65]. Among the cerebral cortex, the insular cortex acts as a visceral (e.g., GI) cortex through an NTS–parabrachial–thalamocortical pathway according to a viscerotopic map. The insular cortex has connections with the limbic system (bed nucleus of the stria terminalis and CeA) and with the lateral frontal cortical system [165]. The NTS also sends projections to the ventrolateral medulla, the parvocellular part of the PVH, and the amygdala/bed nucleus of the stria terminalis contributing to visceral perception. The NTS receives convergent afferents from both the spinal cord (i.e., laminae I, V, VII, and X) and the VN; some of these afferents can be at the origin of autonomic reflex responses. This convergence is also observed at the level of the parabrachial nucleus and ventrolateral medulla [164], thus arguing for a relationship of pain with visceral sensations. The dorsal vagal complex (DVC), located in the brainstem, in the floor of the fourth ventricle, and the PVH are the best candidates for the action of stress and CRF/Ucns in the brain. The DVC is composed of the NTS, the dorsal motor nucleus of the VN (DMNV), which contains the perikarya of efferent vagal preganglionic neurons, and area postrema (AP), which is a circumventricular organ lying dorsally in the middle third of the rostrocaudal extent of the DVC. The PVH and DVC, as well as limbic structures, contain neurons bearing CRF2a [24] and are known to influence autonomic nervous outflow. The messages coming from the gut are integrated in the central autonomic nervous system which contains brain regions involved in the autonomic, endocrine, motor, and behavioral responses [164]. This brain network can be divided into executive structures, mainly hypothalamic, and coordinating structures, mainly included in the limbic system, and high level control structures, mainly the frontal cortex. The LC and the Barrington nucleus are also important parts of this network. The LC, the largest group of noradrenergic neurons located in the pons, mediates emotional arousal, autonomic, and behavioral responses to stress and attention-related processes through its dense projections to most areas of the cerebral cortex [60]. The Barrington nucleus is ventrolaterally located to the LC and projects to the sacral parasympathetic nucleus to increase the motility of the distal recto-colon through an LC-Barrington nucleus interaction [198]. The central autonomic nervous system, in turn, adapts the response of the digestive tract through the efferent ANS (the VNs, the sacral parasympathetic pelvic nerves, and the splanchnic nerves) through reflex loops which are essentially unconscious or become conscious in pathological conditions. Descending pathways that control somatic as well as visceral pain by modulating at the spinal cord-level visceral informations have been described. These pathways are both inhibitory, thus producing analgesia as represented by projections from the periaqueductal gray to the rostroventral medulla and LC descending fibers to the spinal cord, and facilitatory producing hyperalgesia (rostroventral medulla and OFF and ON cells) [195]. Many of the regions of the CNS that we have described above contain the CRFergic system. Among them, the PVH, Barrington's nucleus/LC, and amygdala are well positioned to participate in the reciprocal brain–gut interactions as it pertains to sensory information from the colon and reflex behavioral and autonomic responses to the viscera.

The circumventricular organs are highly vascularized structures with fenestrated capillaries located around the third and fourth ventricles and characterized by the

lack of a blood–brain barrier; they represent points of communication between the blood, the brain, and the cerebrospinal fluid [17]. They are represented by the subfornical organ, median eminence, pineal gland, area postrema, and organum vasculosum of the lamina terminalis. They are involved in sodium and water balance, cardiovascular regulation, metabolic and energetic balance, immune function, regulation of body temperature, vomiting, and reproduction.

Stress Effect on Gastrointestinal Functions

Motility and Secretion (Fig. 7.3)

Acute stress is currently known to delay gastric emptying and small bowel transit while increasing colonic motility in experimental animals as well as in healthy humans [185, 188]. The effects of stress on gastric emptying are reproduced by central (intracerebroventricular or intracisternal) injections of CRF or Ucn, mainly performed in rodents and dogs, and are prevented by central (intracerebroventricular or intracisternal) injection of either nonselective CRF1/CRF2 antagonists (α -helical CRF9–41, d-Phe12CRF12–41, astressin, astressin-B) or selective CRF2 antagonist (astressin2-B), while selective CRF1 antagonists (CP-154,526, antalarmin, NBI 27914) have no effect, thus arguing that CRF and Ucn act primarily through interaction with CRF2 receptors [185]. In contrast to gastric motor function, few data are available about the effects of stress on small intestinal motility, the role of brain CRF/urocortins and their receptors being often studied in parallel with those observed in the stomach. Central injection of CRF and Ucn 1 induces inhibition of duodenal, small intestinal transit, and propulsive motility in rats and dogs [39, 93]. Central injection of CRF1 preferential agonists such as ovine CRF, rat/human CRF, and Ucn 1 induces increase in colonic transit and defecation [33]. Central injection of Ucn 2 and 3, selective CRF2 agonists, is ineffective in mice at a dose similar to that of CRF [116], while this effect is blocked by central administration of either nonselective CRF1/CRF2 receptor antagonists or selective CRF1 antagonists [33, 115]. In addition, central or peripheral injection of a selective CRF1 antagonist reduces stress-induced increase of colonic transit time. CRF1 invalidated mice have significantly less defecation in an open field test than their wild-type littermates [13, 115, 188]. Central injection of a selective CRF2 antagonist (astressin2-B), at a dose which inhibits the CRF2-induced delay in gastric emptying, is not able to reverse the increase in colonic transit following central injection of CRF in rodents [188], thus confirming that CRF1 is the receptor involved in stress-induced colonic motility disturbances.

Numerous data have established the involvement of peripheral CRF signaling in the modulation of secretory function under stress conditions via activation of both CRF1 and CRF2 receptors and activation of cholinergic enteric neurons, mast cells, and possibly serotonergic pathways [101].

The HPA axis is not involved in stress-induced inhibition of gastric emptying. These effects are mediated by the ANS. The effect of CRF and Ucn 1 is mediated by

the VN and abolished by vagotomy [93, 103, 130]. The effect of Ucn 2 requires the integrity of the sympathetic nervous system and peripheral α -adrenergic receptors while not altered by vagotomy [54]. The effect of stress-induced CRF1-dependent activation of colonic motility is mediated by the parasympathetic nervous system, i.e., the celiac branch of the VN, which innervate the proximal colon, and the sacral parasympathetic fibers, which innervate the distal part of the colon and the rectum [128, 130]. The intrinsic nervous system of the colon, as represented by myenteric cholinergic and nitrergic neurons, is involved in the effector mechanism of parasympathetic activation, as well as the action of serotonin (5-HT), from enterochromaffin cells and/or enteric neuron origin, on 5-HT₃ and 5-HT₄ receptors. The PVN and LC/Barrington's complex are involved in central CRF1 signaling-induced increase in colonic motor function. Changes in *c-fos* and CRF gene expression in the Barrington nucleus have been reported under acute and chronic stress [86], and CRF-synthesizing neurons in the Barrington nucleus project to the noradrenergic LC as well as to the intermediolateral column of the sacral spinal cord, which contains the sacral parasympathetic nucleus innervating the descending colon [197]. There are also CRF efferent projections directly from the PVH to the LC, thus modulating the activity of LC neurons and integrating autonomic responses in brain by influencing LC neurons [151]. Water avoidance stress activates the PVN and LC/Barrington's nuclei and CRF gene transcription in the PVH, whereas icv injection of the nonselective CRF1/CRF2 antagonist α -helical CRF9–41 reduces Fos expression in these nuclei in correlation with defecation score [33]. In agreement with a role for CRF/CRF1 signaling in the PVN in stress-induced increase in colonic motility, α -helical CRF9–41 injected directly into the PVN blocks partial restraint- and water avoidance-induced stimulation of colonic transit and defecation, and various neurogenic and systemic stressors activate the transcription of the CRF1 receptor gene in the PVN ([31]; 130). CRF increases the firing rate of noradrenergic neurons in the LC and releases noradrenalin into the brain prefrontal cortex, an input of the LC, which results in arousal and anxiogenic behavior [198]. Consequently, CRF/CRF1 signaling pathways in the PVN and LC/BN complex may physiologically regulate the behavioral and autonomic responses to stress that influence colonic function as part of the brain–gut axis [129, 198]. These CRF/CRF1 signaling pathways may play a role in diarrhea-predominant IBS patients with psychic comorbidities such as anxiety and depression [115].

Intestinal Permeability

The digestive tract is characterized by an epithelial monolayer essentially composed of enterocytes and colonocytes in the small bowel and colon, respectively, and to a lesser extent mucin-secreting goblet cells, enteroendocrine cells, and others such as intraepithelial lymphocytes. Its integrity is preserved by both cell polarity and the interactions between the cell adhesion complexes, represented by tight junctions and adherens junctions, with the actin cytoskeleton [79]. In physiological

conditions, a limited quantity of antigens goes through the epithelial barrier, playing an important role in the induction of normal immune tolerance toward foreign food antigens and the resident microbiota [88]. The gut is the largest immune organ with the majority of immune cells present in the mucosa. Consequently, a mucosal barrier function is necessary to separate the luminal compartment from the body proper and control antigen-induced inflammation. Any anatomical or functional damage to the intestinal epithelium may allow access of luminal contents to the mucosa then stimulating an inflammatory response in a susceptible host, as observed in IBD, IBS, and celiac disease. This mucosal barrier function is regulated by neuroendocrine and immune factors; thus, it is not surprising that stress, and the CRFergic system, can have a major impact. In contrast to the effects of stress on GI motility, fewer studies have evaluated its effect on intestinal permeability. However, abnormal intestinal permeability has been described in IBS [40] and IBD [175], two GI disorders where stress has a role in their pathogeny [27, 132]. The influence of stress on mucosal function has been recently recognized in short acute stress, early life trauma, and chronic ongoing psychological stress in rats [174]. Indeed, restraint stress or water avoidance stress performed in Wistar–Kyoto rats, a stress-sensitive strain, induces ion secretion, a decrease in barrier function, and an enhancement of small (e.g., proinflammatory bacterial tripeptides) and large molecules (e.g., horseradish peroxidase, HRP) across the epithelium through the transcellular and paracellular pathways [94, 167]. These effects are reproduced by ip injection of CRF 1h before stress [163] and blunted by ip injection of the specific nonselective CRF receptor antagonist, alpha-helical CRF9–41 as well as by pretreatment of the animals with adrenergic and cholinergic antagonists and a mast cell stabilizer. Acute immobilization stress or iv injection of CRF increases colonic mucin release through cholinergic-, adrenergic-, and mast cell-dependent mechanisms [43]. Immobilization stress induces increase in mast cell protease II and mucine release from colonic explants; these effects are prevented by peripheral administration of alpha-helical CRF9–41 [43]. The effects of acute stress on permeability returned to normal within 24 h. These data argue for a complex mechanism of action involving peripheral CRF pathways, mast cell activation, mucine secretion, and increase of intestinal permeability. Mast cells regulate barrier physiology in normal as well as inflamed intestine in rats and humans [19]. Despite convincing evidence of intestinal expression of CRF-like peptides and receptors and its production by local immunocytes and enteroendocrine cells [47, 185], only recently a role for subepithelial CRF–mast cell loops in the regulation of colonic permeability in human biopsies has been reported [204]. Santos et al. [162] have recently shown that the stress-like peptides CRF and sauvagine stimulated ion secretion and macromolecular permeability in the distal colon of WKY rats *in vitro*. Epithelial responses were inhibited by both the nonselective CRF receptor antagonist astressin and the mast cell stabilizer doxantrazole and significantly reduced in tissues from Ws/Ws rats. These data argue for the involvement of mucosal/submucosal CRF receptors through mast cell-dependent pathways. Neonatal Sprague–Dawley rats, a strain not normally sensitive to stress in contrast to Wistar–Kyoto rats, separated from their mothers from days 2 to 21 for 4 h per day then housed normally until day 60 and then submitted at day

90 to either water avoidance stress or sham stress for 30 min, present an increase of permeability to macromolecules but not in controls [173]. More recently, CRF2 signals through Src/ERK pathway induce the alteration of cell–cell junctions and the shuttle of p120ctn and Kaiso in the nucleus. In HT-29 cells, this signaling pathway also leads to the remodeling of cell adhesion by the phosphorylation of focal adhesion kinase and a modification of actin cytoskeleton and focal adhesion complexes [58].

Gastrointestinal Inflammation

Numerous data argue for a direct autocrine/paracrine effect of CRF on inflammation. CRF reaches the site of inflammation either through nerve terminals or by local release of the peptide by epithelial, endothelial, or resident immune cells. CRF, Ucn, and their receptors are present in mast cells, mouse splenocytes, lymphocytes, monocytes–macrophages, and TH cells [73]. CRF has an opposite effect at the central and peripheral level. Indeed, central CRF has an anti-inflammatory role through the activation of the HPA axis and the release of glucocorticoids, while peripheral CRF acts as a proinflammatory mediator [147, 205]. CRF signals play a role in augmenting LPS-induced proinflammatory cytokine production by macrophages through CRF1 since these effects are inhibited by antalarmin [1]. Peripheral mechanisms by which CRF and Ucn are involved in the development and progression of colitis are not completely clear. Several factors seem involved: the type of inflammatory stimulus, intestinal cell phenotype, type of CRF receptor, and phase of colitis (acute versus chronic). CRF2 and its specific ligand, Ucn 2, appear to mediate proinflammatory responses in the gut. Indeed, CRF-deficient mice develop substantially reduced local inflammatory responses [68] and have dramatically reduced ileal fluid secretion, epithelial cell damage, and neutrophil transmigration 4 h after intraluminal *C. difficile* toxin A [5]. This effect is counterbalanced by administration of the selective CRF2 antagonist astressin 2B. The CRF system is also involved in the inflammatory response associated with IBD [74]. Colonic biopsies from patients with active UC show significantly increased CRF immunoreactive lamina propria mononuclear cells and macrophages [92]. In UC patients without glucocorticoid treatment, Ucn 1-positive cells and plasma cells increased in proportion to the severity of inflammation but are significantly lower in number in glucocorticoid-treated patients. Ucn 1 mRNA was expressed in lamina propria plasma cells, and both CRF1 and CRF2a mRNAs were also partially coexpressed in these cells and macrophages. Ucn 1 therefore may act as a possible local immune-inflammatory mediator in UC [166]. Ucn 2 mRNA is expressed in normal conditions in the small intestine and colon, while in a rat model of chemically induced colitis, Ucn 2 levels are increased, whereas expression levels of its only identified receptor, CRF2, are decreased. This suggests that Ucn 2 exerts its effects only in part via CRF2 [44]. The endogenous upregulation of Ucn 2 following the local inflammatory process accounts for the downregulation of the respective receptor, being either a causing or

a resulting effect. Exposure of human colonic epithelial cells HT-29 to *C. difficile* toxin A or TNF-alpha induces an increase expression of CRF2 mRNA and protein and stimulation of NCM460 colonocytes overexpressing CRF2a with Ucn 2 results in a time- and concentration-dependent increase in IL8 production as well as to an activation of NF-kappaB and MAP kinase in these cells. In addition, expression of Ucn 2 and CRF2 mRNA was increased in mucosal samples of patients with IBD (CD and UC) and after exposure of human intestinal xenografts to *C. difficile* toxin A. These data suggest that Ucn 2 has proinflammatory effects in human intestinal cells via CRF2a and may be involved in the pathophysiology of colitis in humans [131]. CRF2-deficient mice develop substantially reduced intestinal inflammation and had lower intestinal mRNA expression of the potent chemoattractant keratinocyte chemokine and MCP 1 when exposed to intraluminal *C. difficile* toxin A. This effect is mimicked by the selective CRF2 antagonist astressin 2B before toxin A exposure. Only Ucn 2, but not other Ucn, was significantly upregulated by ileal administration of toxin A at 4 h compared with buffer exposure [95]. These data argue for CRF2-mediated intestinal inflammation via the release of proinflammatory mediators at the colonocyte level. La Fleur et al. [99] have shown that, in an experimental model of toxin-induced intestinal inflammation, inhibition of CRF ablated the inflammatory response, while Ucn 2 dsRNA treatment did not modify the inflammatory response to toxin. Gonzales-Rey et al. [71] investigated the potential therapeutic effect of Ucn 1 in a murine model of TNBS-induced colitis and showed that Ucn 1 significantly improved the clinical and histopathological severity of colitis and increased the survival rate of mice with colitis. Importantly, Ucn 1 treatment was effective in established colitis and avoided recurrence of the disease. This work identifies Ucn 1 as a potent anti-inflammatory factor. Few data are available regarding the role of the CRFergic system as a pro- or anti-inflammatory system at the level of the stomach. In the upper GI, CRF2 appears to be the most prominent. Indeed, CRF1- and CRF2-positive cells are present in the oxyntic gland and the submucosal blood vessels. No specific CRF1 is observed in the antrum. CRF2 is present in the gastric glands along with immunoreactive Ucn. Thus, both CRF receptor subtypes are expressed in the upper GI tissues with a distinct pattern and regional differences suggesting a differential function. A paracrine CRF-like circuit is present in human stomach composed of Ucn and its CRF2 receptor compared to the CRF/CRF1 circuit in the human colon. Chatzaki et al. [49] examined the presence of CRF and Ucn transcripts and peptides in human gastric mucosa and the association between CRF and Ucn and *H. pylori* gastritis. They observed the presence of the Ucn transcript in biopsies obtained by gastroscopy from normal and inflamed gastric mucosa, while the CRF transcript was not detectable. Immunoreactive Ucn was localized in gastric epithelial cells and in inflammatory elements of the surrounding negative for Ucn gastric stroma. The level of immunoreactive Ucn was higher in gastric biopsies from patients with active *H. pylori* gastritis than in controls. Eradication of *H. pylori* was followed by a dramatic increase of ir-Ucn levels, while nonresponders to the eradication treatment did not show any significant change in ir-Ucn levels. These data suggest that in human gastric epithelium, Ucn is present and plays an important physiological role, while CRF is absent.

In addition, and in contrast to what has been observed for CRF in ulcerative colitis [92], a highly significant but negative correlation has been found between Ucn levels and gastric inflammation, suggesting that Ucn may exert an anti-inflammatory effect in the gastric mucosa. The Ucn/CRF2 system in the upper gastrointestinal tract plays a completely different role in gastric inflammatory response compared with that of the CRF/CRF1 system in the inflammatory response in the colon. These differences between upper and lower GI tracts, *vis-à-vis* their CRF paracrine circuits, may partially explain the lack of severe chronic inflammatory diseases in the stomach compared with the colon in IBD.

Microbiota

There are 10–100 trillion bacterial cells in our gut, ten times more than the number of somatic and germ cells in our body. The number of species is estimated between 500 and 800, and eighty percent of these species are unculturable [59]. New molecular techniques allow inventory of the gut's resident bacterial species without having to culture them. Bacterial genes outnumber our body's genes by as much as 100–1 [7]. The human microbiota is necessary to the healthy functioning of the GI tract and contributes to the intestinal development, metabolic transformations, and protections against enteric infections [75]. Bacteria in the gut have an important role in the immune response, including inflammation [106]. There is a new concept on the bidirectional communication between the nervous system and commensal, pathogenic, and probiotic organisms, i.e., the microbiota–brain–gut axis where gut microorganism can activate the VN; this activation plays a critical role in mediating effects on the brain and behavior. The VN appears to differentiate between non-pathogenic and potentially pathogenic bacteria even in the absence of overt inflammation and mediates signals that can induce both anxiogenic and anxiolytic effects, depending on the nature of the stimulus [62]. Mice treated orally with *Campylobacter jejuni* showed vagally mediated activation in the NTS in the absence of intestinal inflammation [70]. This axis is vital for maintaining homeostasis and may be involved in the etiology of several metabolic and mental dysfunctions/disorders. Commensal microbiota can affect the postnatal development of brain systems involved in the endocrine response to stress. Indeed, an exaggerated response of the HPA axis to stress (higher plasma ACTH and corticosterone elevation) was observed in germ-free mice that was reversed by reconstitution of the microbiota; germ-free mice also exhibited reduced brain-derived neurotrophic factor (BDNF) expression levels in the cortex and hippocampus [181]. The exaggerated HPA stress response by germ-free mice was reversed by reconstitution with *Bifidobacterium infantis*. Thus, commensal microbiota can affect the postnatal development of the HPA stress response in mice. Prevention of intestinal barrier impairment by a probiotic attenuates HPA response to an acute psychological stress in rats [2]. In addition, germ-free mice show reduced anxiety-like behavior in comparison to specific pathogen-free mice, a phenotype accompanied by changes in plasticity-related genes in the

hippocampus and amygdala [136], two key structures in the adaptation to the stress response. The intestinal microbiota influences central (i.e., hippocampal) levels of BDNF, which regulates dendritic architecture and spines, and behavior independently of the ANS, gastrointestinal-specific neurotransmitters, or inflammation [18]. Consumption of a fermented milk product with probiotic for 4 weeks by healthy women altered brain intrinsic connectivity or responses to emotional attention tasks [192]; thus, neuroimaging seems to be an interesting tool to study the microbiome–gut–brain axis. In regular functioning conditions, the intestinal barrier is able to prevent most environmental and external antigens to interact openly with the numerous and versatile elements that compose the mucosal-associated immune system. Stress is well known to induce an increase of intestinal permeability that allows bacteria to cross the epithelial barrier to activate mucosal immune response (Killiaan et al. 1998) and to translocate to secondary lymphoid organs [10], thus stimulating the innate immune system. Stress is able to modify the intestinal microbiota [9]. Indeed, exposure of mice to a social stressor affects the structure of the intestinal microbiota and increases the circulating level of cytokines; this effect is reversed by antibiotics [8]. Changes in the intestinal microbiota reduce resistance to infectious challenge with intestinal pathogens [9]. These data provide evidence for the interplay between stress, the intestinal microbiota, and the immune response. This can in turn have significant impact on the host and affect behavior, visceral sensitivity, and inflammatory susceptibility [52]. The sympathetic nervous system, through the release of catecholamines, e.g., norepinephrine, a stress mediator, stimulates growth of bacteria [111]. Stress-mediated changes may shift the microbial colonization patterns on the mucosal surface and alter the susceptibility of the host to infection; these changes in host–microbe interactions may also influence neural activity in stress-responsive brain areas [110].

Visceral Sensitivity

Stress increases visceral perception and emotional response to visceral events by a perturbation of the brain–gut axis at its different levels, central (brain and spinal cord), peripheral (gut), and the ANS [100]. Genetic models of depression and anxiety, such as the high-anxiety Wistar–Kyoto rats or Flinders Sensitive Line, rats [139] or deleting CRF1, exhibit a decrease of colonic sensitivity to colonic distension [194] while models overexpressing CRF1 exhibit enhanced response [127]. These data argue for the filiation stress–anxiety–inflammation and visceral hypersensitivity. CRF signaling, both at the central and peripheral level, is a key element involved in this effect. Data argue for an equally important contribution of the peripheral CRF/CRF1 signaling locally expressed in the gut to the GI stress response [101]. Indeed, mast cell degranulation in the colon under stress and peripheral administration of CRF induces visceral hypersensitivity through the release of their mediators (histamine, tryptase, prostaglandin E2, nerve growth factor, CRF, TNF) that can stimulate or sensitize sensory afferents [201].

Among the brain structures involved in stress-induced visceral hypersensitivity, the amygdala, a major extrahypothalamic source of CRF, is a key element. Indeed, an activation of the amygdala is observed in experimental models of somatovisceral and visceral pain [171, 172] as well as experimental models of stress-induced GI disturbances [31–33] and colitis [145] as well as in a model of visceral pain in healthy volunteers [6] and IBS patients [30]. Activation of corticosteroid receptor in the CeA is involved in the induction of anxiety and visceral hypersensitivity [135]. Implants of corticosterone micropellets in the CeA increase anxiety-like behavior as well as visceral hypersensitivity to colonic distension and increased responsiveness of viscera-sensitive lumbosacral spinal neurons that mediate visceromotor reflexes to colorectal distension [134]. Water avoidance stress performed for 7 consecutive days induced visceral hypersensitivity that is abolished by glucocorticoid receptor and mineralocorticoid receptor antagonists in the amygdala. Wistar–Kyoto rats express a greater amount of CRF and CRF1 mRNA in the CeA and PVH [36] and depict colonic hypersensitivity to luminal distension which is reversed by peripheral administration of a CRF1 antagonist as well as into the CeA, thus strengthening the role of CRF1 receptor in the amygdala in visceral hypersensitivity mechanism [90]. CRF neurons in the CeA project directly to the LC and increase the firing rate of LC neurons, thus increasing noradrenaline release in the vast terminal fields of this ascending noradrenergic system. The expression of CRF in the LC is increased in WKY rats, and a selective CRF1 receptor antagonist dampened the activation of LC neurons by colorectal distension and intracisternal CRF in rats [96]. CRF1 and CRF2 in the amygdala mediate opposing effects on nociceptive processing, i.e., pro- and antinociceptive effects of CRF, respectively [89]. Low concentrations of CRF facilitate nociceptive processing in the CeA neurons through CRF1, while higher concentrations of CRF have inhibitory effects through CRF2 receptors, in agreement with the concept that CRF2 receptors serve to dampen or reverse CRF1-initiated responses [185]. These results clarify to some extent the controversial role of CRF in pain modulation. Administration of CRF into the CeA significantly increased the number of abdominal muscle contractions in response to colorectal distension in male Wistar rats; this effect was dampened by injection of the CRF1 antagonist, CP-154526. Colorectal distension increased noradrenaline in the CeA which was further increased by CRF and inhibited by CRF1 antagonist. These data suggest that CRF in the CeA sensitizes visceral nociception via CRF1 with release of noradrenaline [180]. The insular cortex is also a key region of the pain matrix, more particularly involved in visceral pain (i.e., colorectal distension) [6, 30]. Bilateral insular cortex lesions in rats markedly inhibit visceral hypersensitivity induced by chronic stress, thus strengthening its role in the pathophysiology of stress-related visceral hypersensitivity [208]. Chronic stress increases DNA methylation and histone acetylation of genes that regulate visceral pain sensation in the peripheral nervous system of rats. These results have potential therapeutic implications when blocking epigenetic regulatory pathways in specific regions of the spinal cord [84].

A temporary disruption of the gut microbiota in early life induces specific and long-lasting changes in visceral sensitivity in male rats, a hallmark of stress-related

functional disorders of the brain–gut axis such as IBS [138]. Early life adversity is known to induce visceral hypersensitivity through ovarian hormones, specifically estradiol, and signaling within the HPA axis, either through reduced negative feedback or increased facilitation, with specific changes in amygdala-mediated mechanisms. Stress-induced visceral hypersensitivity following maternal separation is transferred across generations, this transfer depending on maternal care [200].

Implication in Functional Digestive Disorders and Inflammatory Bowel Diseases

Functional Digestive Disorders: Irritable Bowel Syndrome (Fig. 7.4)

IBS is the most common functional digestive disorders, with an estimated prevalence rate in the general population of 10–15 % in industrialized countries. IBS is characterized by abdominal pain, bloating, and altered bowel habits without any organic cause [132]. Women have a higher prevalence of symptoms than men. IBS accounts for up to 12% of visits to primary care doctors and 28% of visits to

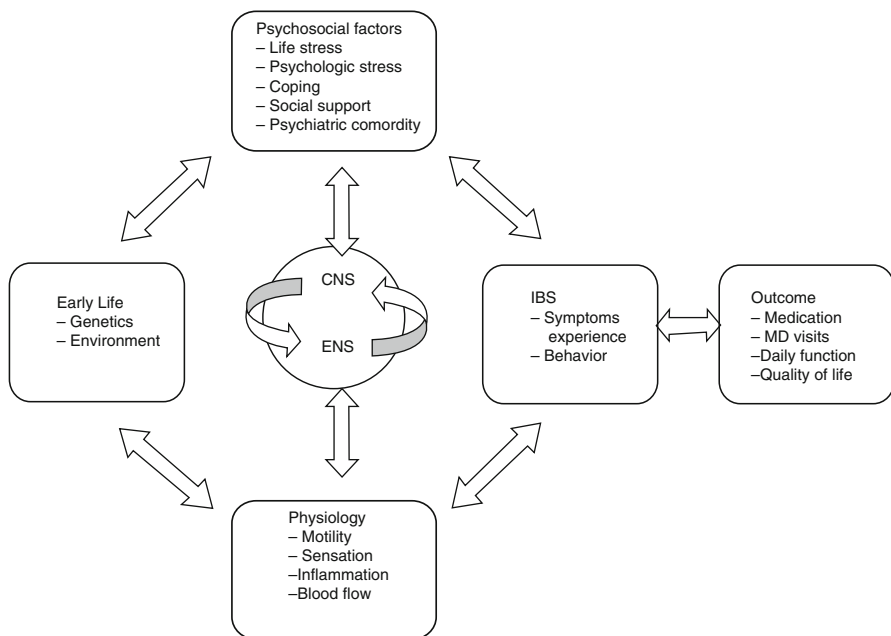


Fig. 7.4 The relationship between early life, psychological factors, physiology, subjective experience of symptoms, behavior, and outcome in irritable bowel syndrome (With permission from Mulak and Bonaz [132])). *CNS* central nervous system, *ENS* enteric nervous system, *IBS* irritable bowel syndrome, *MD* medical doctor

gastroenterologists [41]. IBS is associated with a significant impairment in quality of life, a high rate of absence from work, and a significant increase in healthcare costs. Extraintestinal manifestations are frequently associated to digestive symptoms such as headache, arthralgia, urinary manifestations, insomnia, and fatigue. Fibromyalgia is often observed in IBS and conversely [46]. Psychiatric comorbidity, mainly major depression, anxiety, and somatoform disorders, is observed in 20–50% of IBS patients [67]; stress is involved in such disorders and psychiatric disorders precede the onset of the GI symptoms [184]. Numerous data argue for a role of stress in the pathophysiology of IBS. Patients with IBS report more stressful life events than medical comparison groups or healthy subjects. Stress is strongly associated with symptom onset and symptom severity in IBS patients. Illness experience, healthcare-seeking behavior, and treatment outcome are adversely affected by stressful life events, chronic social stress, anxiety disorders, and maladaptive coping style. A history of emotional, sexual, or physical abuse is found in 30–50% of IBS patients [35]. A majority of patients with IBS have a visceral hypersensitivity as represented by lower pain thresholds to intestinal distension compared to healthy controls [153]. Among the peripheral mechanisms of this visceral hypersensitivity, low-grade inflammation in the GI tract could favor modifications of neuronal plasticity [37, 119] and mast cells could also be involved by sensitizing visceral afferent terminals [76]. A postinfectious IBS has been observed in 4–30% following bacterial gastroenteritis [78]; perceived stress, anxiety, somatization, and negative illness beliefs at the time of infection in favor of a cognitive-behavioral model of IBS were predictors of postinfectious IBS [176]. Modifications in central sensory processing are described in IBS [30]. A spinal hypersensitivity has been evoked in IBS patients as well as a supraspinal cause where stress is of primary importance [132]. A defect of descending pain inhibition pathways is also evoked [206], as described in fibromyalgia patients [102]. Globally, IBS is assimilated to a central sensitization syndrome as observed for chronic fatigue syndrome, fibromyalgia, posttraumatic stress disorders, headaches, restless legs syndrome, and others [209].

Early life traumas, known to increase the risk of developing IBS later in life, play a major role in the development of mood and anxiety disorders and increased CRF signaling [81]. Neonatal maternal separation (MS), as an early life trauma, is a model of IBS in rodents, leading to chronic dysregulation in the limbic-HPA axis [150]. This has led to the biopsychosocial model of IBS [189] and the concept that IBS is due to a brain-gut axis dysfunction consistent with an upregulation in neural processing between the gut and the brain. Globally, there is a hypervigilance state that explains the visceral hypersensitivity observed in these patients most likely through a central (and peripheral) hyper-CRFergic state. In addition, pain is a stress per se that could amplify such disturbances, contributing to its chronicity. Since the ANS is the link between the gut and the brain, it is not surprising in this context of an abnormal brain-gut axis to observe an important dysautonomia, with a high sympathetic and a low parasympathetic tone, whatever the positive or negative affective adjustment [142].

Functional brain imaging studies have allowed a better understanding of the pathophysiology of IBS. Indeed, an abnormal brain processing to visceral pain has been described in IBS patients [30, 193] particularly in brain loci of the pain matrix such as the somatosensory, insular, prefrontal, and cingular cortices as well as sub-

cortical loci such as the thalamus, the amygdala, and the periaqueductal gray. In addition there is a major influence of cognitive–affective processes, including arousal, attention, conditioning and negative affect, and coping strategies, on GI sensations, and its central correlates in health and IBS [118, 142, 202]. The role of the central and/or peripheral CRF system is gaining clinical recognition as part of the neurobiological common denominator of IBS symptoms susceptible to stress and anxiety/depression [63, 186]. Elevated concentrations of CRF in the CSF are observed in patients with anxiety and vulnerability to stress as well as in those suffering from obsessive–compulsive disorders, posttraumatic stress disorders, or childhood trauma, and CRF in the CSF is a predictor of perceived aversive early life experiences [105]. In patients suffering from fibromyalgia, known to have comorbidity with IBS, CSF levels of CRF are associated with both pain symptoms and autonomic dysfunction [122]. An overactivity of the HPA axis and enhanced plasma CRF response to mental stress has been described in IBS patients [146]. IBS patients have previously been proposed to have an exaggerated brain–gut response to CRF [64]. Basal levels of noradrenaline are higher in IBS patients than in controls [82] indicating enhanced activity of the sympathetic nervous system, known to selectively increase visceral sensitivity [87]. An alteration in central noradrenergic signaling is observed in IBS; early life trauma may be one mediator of these abnormalities [20]. In healthy controls, CRF iv decreases rectal pain threshold to distension and mimics an IBS-specific visceral response [137]. Peripheral injection of α -helical CRF9–41 prevents rectal electrical stimulation-induced enhanced sigmoid colonic motility, visceral perception, and anxiety in IBS patients compared to controls without altering the HPA axis [161] and improves decreased alpha power spectra and increases beta power spectra of electroencephalogram in IBS patients [190]. The induction of IBS-like symptoms in healthy subjects and heightened sensitivity in IBS patients are alleviated by a peptide CRF antagonist targeting CRF1 receptor [63] that may provide a new therapeutic avenue in the treatment of IBS [115]. However, in women with diarrhea-predominant IBS, a CRF1 antagonist did not significantly alter colonic or other regional transit or bowel function, thus requiring further study [183].

The medical treatment of IBS is disappointing, often targeting symptoms. In the meantime, because of the complexity of the pathophysiology of IBS, one might wonder that such an ideal treatment would not exist. Nonmedical treatments, as represented by cognitive–behavioral therapy or hypnosis, are of interest [61, 104].

Inflammatory Bowel Diseases (Fig. 7.5)

IBDs are organic diseases classically divided in CD and UC involving the digestive tract, and particularly the small bowel and colon, starting early in life (between 15 and 30 years), and evolving by flares alternating with periods of remissions of variable duration. Symptoms are characterized by abdominal pain, diarrhea, fever, weight loss, and extraintestinal manifestations. The rising incidence of IBD in Western countries supports the hypothesis that “Westernization” of our lifestyle has led to the

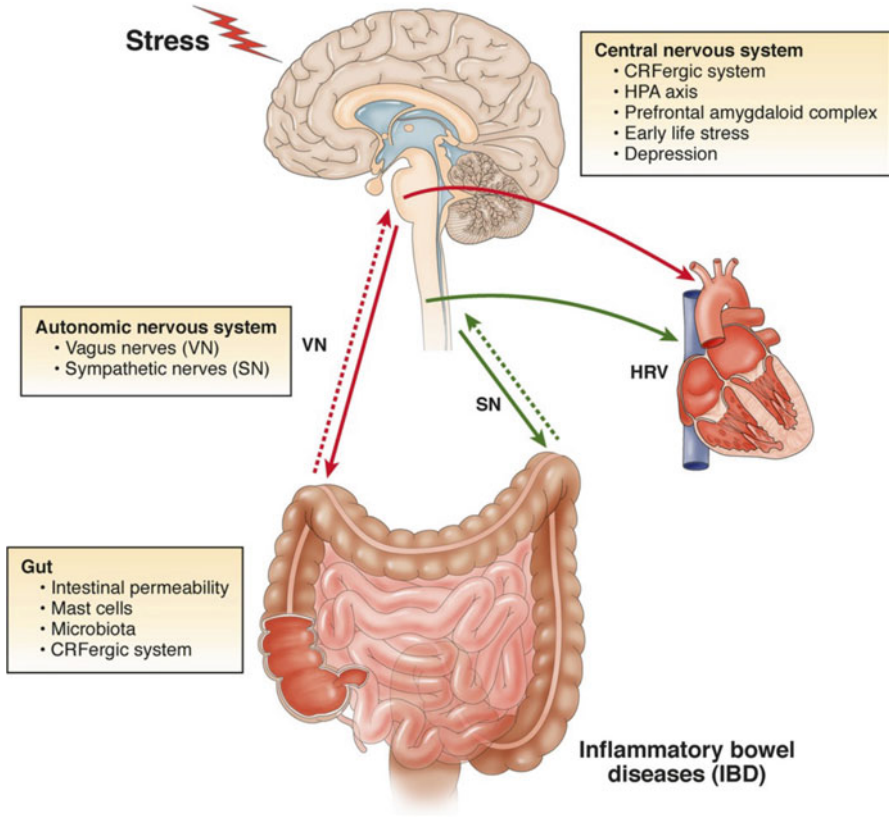


Fig. 7.5 Actors and pathways through which stress may play a role in the pathophysiology of inflammatory bowel disease (With permission from Bonaz and Bernstein [27]). *HRV* heart rate variability, *VN* vagus nerves, *SN* sympathetic nerves

increased incidence of IBD. Today, there is no medical treatment susceptible to cure definitively IBD; the treatment is only suspensive. The pathophysiology of IBD is multifactorial involving immunological, genetic, infectious, and environmental factors [85]. Among the latter, the role of stress is evoked based on experimental and clinical data [27]. IBDs are models of “brain–gut” interactions; they represent an interoceptive (immunogenic) and exteroceptive (psychological) stress involving the neuroendocrine–immune axis. The ANS has a key role in the relation between stress and digestive inflammation. A dysautonomia is reported in IBD, as represented by a sympathetic dysfunction in CD [109] and a vagal dysfunction in UC [108]. This dysautonomia could explain the differential effect of tobacco, nicotine being a parasympathetic activator, which is protective in UC and deleterious in CD. Stress may play a deleterious role in IBD through different pathways close to the ones described for IBS [27] (Fig. 7.5): (1) Activation of mast cells in the intestinal mucosa, in close contact with sympathetic and VN terminals, induces the release of their mediators

(see above) that increase intestinal permeability and activate the mucosal immune function [23, 187]; (2) Catecholamines, acting through α - and β -adrenergic receptors, mediate stress-induced increases in peripheral and central inflammatory cytokines and activation of the inflammatory nuclear factor- κ B signaling pathway [91]. Classically, the SNS has a proinflammatory role [120], (3) The VN has a dual anti-inflammatory effect both through its afferents, activating the anti-inflammatory HPA axis, and efferents through the cholinergic anti-inflammatory pathway [28]. Indeed, acetylcholine (ACh) released at the distal end of VN efferents decreases the production of proinflammatory cytokines such as TNF by human macrophages through α 7 nicotinic ACh receptor (α 7nAChR) expressed by macrophages [140]. VN stimulation (VNS) has been shown to reduce the systemic inflammatory response to endotoxin [34] and intestinal inflammation [57, 124] and could be a nonpharmacological treatment of IBD [28, 51]. The VN also modulates the immune activity of the spleen either directly through connections with the splenic sympathetic nerve [159] or indirectly [114]. Stress has a proinflammatory effect based on its activation of the SNS and adrenomedullary activity while inhibiting the VN [185, 207]. (4) The activity of the sympathovagal balance and the HPA axis, monitored by heart rate variability and cortisol, is linked to the activity of the prefrontal cortex (PFC) and amygdala, respectively [191]. The hypoactivity of the PFC and the enhancement of amygdala activity are strongly influenced by stress [53]. A dysregulation of the amygdala–PFC equilibrium induces an imbalance between the HPA axis and the ANS, as observed in IBD [179], and thus a proinflammatory condition. There is an imbalance between the HPA axis and the vagal tone in CD patients with an inverse association between vagal tone and TNF- α level [141], (5) Habituation of the hypothalamic CRFergic system is observed in chronic stress conditions [31]. Chronic colitis suppresses CRF gene activation in the hypothalamus and plasma corticosterone level and dampens the counter-regulatory anti-inflammatory mechanisms during water avoidance stress, thus contributing to the stress-related worsening of colitis [98]. A predisposition to a hyporeactive HPA axis and an inhibition of the central response to a chronic interoceptive stress may favor inflammation in IBD, (6) As described above, the CRFergic system is present in the GI tract and may play an anti- or proinflammatory role. The peripheral CRFergic system forms an interacting and balanced system, and the differences observed among studies depend on the model of inflammation and the receptors activated and the ligands; an imbalance of this system could favor GI inflammation, (7) There is a microbial basis of IBD [55]. Stress-induced increased intestinal permeability allows bacteria to cross the epithelial barrier to activate mucosal immune response [94] and to translocate to secondary lymphoid organs [10] to stimulate the innate immune system. The sympathetic nervous system, through the release of catecholamines (e.g., norepinephrine), stimulates growth of bacteria [111]. The intestinal microbiota may act as a mediator in the communication between the gut and the brain (i.e., the microbiota brain–gut axis), (8) Modification of the stress axis early in development, as observed in early life traumas, could induce a maladaptive control of neuroendocrine immune axis. Indeed, the HPA axis is programmed by early life events, and neonatal inflammatory stimuli exert long-term changes in HPA activity and immune regulation in adult animals [169]. Experimental colitis induces a

significantly higher inflammatory reaction in MS animals [14]. Deficient maternal care in rats increases glucocorticoid receptor promoter methylation leading to decreased expression in the hippocampus, a recognized target for glucocorticoid feedback [123]. There is a link between early life stress and depression that may predispose to increased inflammation both under baseline conditions and following stress [56]. MS mice develop a behavioral pattern reminiscent of depression and are more susceptible to inflammation; this vulnerability is reversed by tricyclic antidepressants [203]. Experimental depression in mice is followed by impaired parasympathetic function and increased susceptibility to an experimental colitis that was reduced by desmethylimipramine, through a vagally dependent enhanced parasympathetic function [69]. Most of the data presented above are described in experimental conditions. However, there are now increasing data arguing for a role of stress in IBD patients. There is consistent evidence that psychological factors play a role both in the pathophysiology and course of IBD and in how patients deal with IBD. In a population-based cohort of IBD patients, significantly more people in the persistently inactive disease group indicated they had experienced no stressful events compared to those in the persistently active disease group. Only the psychological factors, including occurrence of a major life event, high perceived stress, and high negative mood during a previous 3-month period, were significantly associated with the occurrence of a flare [21]. On multivariate logistic regression analyses of these variables, only high perceived stress was associated with increased risk of flare. Perception of stress is a key factor, which incorporates the individual's appraisal of the demands created by stress in general and resources to cope with stress. The interaction between perceived stress and avoidance coping was predictive of earlier relapse in quiescent CD [26]. Chronic stress, including caregiving and marital discord, and perceived stress are associated with increases in CRP and other inflammatory mediators [126]. Stress increased LPS-stimulated cytokines, leukocyte and NK cell counts, platelet activation, and reactive oxygen metabolites production and reduced rectal mucosal blood flow in a study of rectal mucosa of UC patients compared to healthy controls [117]. Sympathetic nerve fibers and neurotransmitters are lost in inflamed areas of the colon in both CD but not in noninflamed ileum [112]; thus, stress may generate symptoms from the uninflamed areas where sympathetic nerve fibers are intact. Perhaps, then, stress may contribute to spreading of the inflammatory lesion. Recent reviews have concluded that stress has an impact on the course of disease, but the jury is still out as to whether cognitive therapies or psychotropic medications can positively influence the course of IBD [72, 148].

At least 33–57% of IBD patients are able, during the course of their disease, to switch from IBD to IBS [170] most likely through modifications of neuroplasticity induced by chronic inflammation [37]. Proinflammatory cytokines (IL1, IL6, and TNF- α) could favor visceral hyperalgesia via an activation of spinal immune-like glial cells inducing release of proinflammatory substances (IL1, IL6, TNF- α , prostaglandins, nitric oxide) triggering the amplification of pain by modulating the excitability of spinal neurons. However, if visceral hypersensitivity is classically described in IBS, we [160] and others [22, 45] found a visceral hyposensitivity in IBD patients in remission.

Conclusions

Major advances have been made in the domain of stress and CRF signaling pathways in the brain and, more recently, in the gut. The CRFergic system mediates the effects of psychological, physical, and immunological stressors on hormonal responses, anxiety, mood, feeding behavior, and GI functions. Conclusive experimental data show that activation of brain and colonic CRF1 pathways mimics the features of stress-induced stimulation of colonic motility, defecation/watery diarrhea, intestinal permeability, and visceral hypersensitivity described in pathological conditions such as IBS. In contrast, in the upper gut, the brain and gastric CRF2 signaling systems are more prominently involved in CRF ligands- and stress-related suppression of gastric motor function. CRF2 signaling has proinflammatory properties in the lower GI tract but anti-inflammatory properties in the upper GI tract. Targeting these CRF1/CRF2 signaling pathways by selective antagonists/agonists should be of clinical interest in the domain of IBS and IBD.

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