The Irritable Bowel Syndrome: How Stress Can Affect the Amygdala Activity and the Brain-Gut Axis

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1. Introduction

Irritable bowel syndrome (IBS) is a functional digestive disorder characterized by abdominal pain, bloating and altered bowel habits without any organic cause (Drossman 1999b; Mulak and Bonaz 2004). Patients with IBS exhibit enhanced perception of visceral sensation to colonic distension which is associated with hypervigilance at the origin of visceral hypersensitivity (VHS) (Ritchie 1973; Bradette, et al. 1994; Elsenbruch, et al. 2010). VHS is a clinical marker of IBS considered to play a major role in its pathophysiology. The exact cause of VHS is unknown but a number of mechanisms are evoked as represented by neuroplastic changes in primary afferent terminals (peripheral sensitization) due to peripheral inflammation or infection of the gut (i.e. post-infectious IBS) but also in the spinal cord (central sensitization) and in the brain (supraspinal pain modulation) or in descending pathways that modulate spinal nociceptive transmission (Bonaz 2003; Mulak and Bonaz 2004). In addition, stress is able to increase visceral sensitivity either at the central and/or peripheral level (Mulak and Bonaz 2004; Larauche, et al. 2011).

There is a bidirectional communication between the central nervous system (CNS) and the gastrointestinal (GI) tract, i.e. the brain-gut axis, such as signals from the brain can modify the motor, sensory, secretory, and immune functions of the GI tract and, conversely, visceral messages from the GI tract can influence brain functions in a top-down and bottom-up relation. Numerous data argue for a dysfunction of this brain-gut axis in the pathophysiology of IBS (Mulak and Bonaz 2004; Bonaz and Sabate 2009; Tillisch, et al. 2011).

Stress, through the corticotrophin-releasing factor (CRF) system (CRF, urocortins and their receptors CRF1,2), is a key factor involved in the pathophysiology of IBS. Indeed, stress is able to modify visceral sensitivity as well as GI motility, permeability, intestinal microbiota, and immunity of the GI tract, all mechanisms that are involved in the pathophysiology of IBS. In addition, stress is able to modulate the hypothalamic pituitary adrenal (HPA) axis and the autonomic nervous system (ANS) which is the link between the gut and the CNS and an imbalance of the ANS is observed in IBS patients (Pellissier, et al. 2010a; Mazurak, et al. 2012). The main brain areas involved in stress are the prefrontal cortex, the limbic system

(e.g. the hippocampus and the amygdala) and the hypothalamus. Relations between the prefrontal cortex and the limbic system are important in the management of stress response.

The amygdala is a key structure involved in the stress effect on the GI tract. Indeed, the amygdala is involved in brain-gut and gut-brain interactions. *i)* The amygdala receives informations from the gut through the parabrachial (PB) nucleus, a sensitive nucleus, and the dorsal vagal complex. The latter, composed of the nucleus tractus solitaries (NTS), is the main entrance of the vagus nerve (vagal afferents) and sends projections to the amygdala. The amygdala is therefore a relay of somatic and visceral nociceptive and non-nociceptive afferents through ascending inputs from the spinal cord and the NTS to the insula which is the main cortical area involved in sensitive information processing. *ii)* The amygdala controls the ANS which is a key element in the neuro-endocrine and autonomic responses to stress of the organism to maintain homeostasis. On the one hand, the amygdala projects to the dorsal motor nucleus of the vagus nerve (DMNV) at the origin of the parasympathetic branch of the vagus nerve (vagal efferents); this makes the amygdala able to modulate the functioning of the parasympathetic system through the vagus nerve. On the other hand, the amygdala projects to the intermediolateral column cells of the spinal cord, at the origin of the sympathetic nerves, and locus coeruleus (LC) in the pons. It makes the amygdala able to modulate the sympathetic nervous system, the other branch of the ANS, and thus to modulate the sympatho-vagal balance, a marker of brain-gut interactions (Mazurak, et al. 2012). *iii)* The amygdala controls the HPA axis activation either directly or indirectly via the hippocampus (i.e. inhibition), known to inhibit the HPA axis, and thus to decrease stress response. *iv)* The amygdala is also involved in childhood psycho-traumatic experiences which are key elements in the pathophysiology of IBS. Indeed, early life stress, as represented by sexual abuse in infancy or adolescence, is present in 30 to 50% of IBS patients (Chitkara, et al. 2008; Bradford, et al. 2012). The amygdala is particularly vulnerable to stressors in early life. The amygdala contains all the elements of the CRFergic system (e.g. CRF, Ucns, CRF1,2) and early life stress induces persistent changes of the CRFergic system in the amygdala leading to an increased stress sensitivity in adulthood. This has been well modelled in the maternally separated (MS) rat model where morphological modifications of the amygdala (e.g. enlarged amygdala volumes and increases in CRF-containing neurons) are induced. *v)* The amygdala (central nucleus; CeA) and the bed nucleus of the stria terminalis (BNST) are highly interconnected with limbic regions (Bienkowski and Rinaman 2012). These two regions are frequently referred as a "central extended amygdala", which shares similar connectivity with other brain regions (e.g. hypothalamus and brainstem) that coordinate behavioural and physiological responses to interoceptive and exteroceptive stressors. It makes the amygdala able to link pain and emotional processings. Furthermore, the amygdala is sensitive to stress-induced increase in glucocorticoids since the existence of elevated glucocorticoid level in the amygdala is associated with anxiety-like behavior and visceral hypersensitivity (Myers and Greenwood-Van Meerveld 2007b; 2010). The amygdala is therefore at the cross-road of anxiety, stress, and visceral sensitivity. The role of the amygdala in IBS is therefore crucial since IBS patients reported higher score of state and trait anxiety than healthy volunteers or in inflammatory bowel disease (IBD) patients in remission with IBS symptoms (Drossman 1999b; Pellissier, et al. 2010a). *vi)* The prefrontal cortex (PFC), and particularly its medial part (mPFC), is able to modulate the functioning of the amygdala. Indeed, the mPFC involvement in fear extinction process (Sotres-Bayon, et al. 2004; Quirk, et al. 2006a) has been shown to be indirectly mediated by its inhibitory action on the amygdala output (Vidal-Gonzalez, et al. 2006). *vii)* Brain imaging techniques (fMRI, PET) have contributed to a better understanding of the pathophysiology of IBS. During rectal distention, an activation of most of the brain structures referenced above, and in particular the amygdala, have been observed

in healthy volunteers (Baciu, et al. 1999) while an abnormal brain processing (i.e. abnormal loci of cerebral activation) of pain was observed in IBS patients (Bonaz, et al. 2002; Agostini, et al. 2011). In addition, brain structural changes of the HPA axis and limbic structures have been recently reported in IBS patients (Blankstein, et al. 2010; Seminowicz, et al. 2010).

At the present time, the only medical treatment of IBS is directed at GI motor/sensory or CNS processing. Unfortunately, this treatment is poorly effective and often associated with high placebo effects, thus revealing the importance of the overlap between pain and placebo neurobiological pathways. The therapeutic approach is essentially focused on the symptoms as represented by anti-spasmodics for pain, laxatives or bulking agents, 5-HT4 agonists and guanylate cyclase-C agonist for intestinal transit regulation and anti-depressives/anxiolytics drugs. Placebo has a ≈ 40% efficacy in IBS patients (Patel, et al. 2005) and pronounced placebo analgesia is coupled with prominent changes of brain activity in visceral pain matrix, as represented by the amygdala (Lu, et al. 2010). Non-pharmacological therapies are of special interest. Cognitive behavioral therapy is associated with reduced limbic activity (e.g. reduced neural activity in the amygdala), GI symptoms, and anxiety (Lackner, et al. 2006). Hypnosis has shown efficacy in IBS (Whorwell, et al. 1984) and is known to modify the activity of the amygdala (Drossman 1999b). All methods focused on stress reduction such as mindfulness-based stress reduction should reduce pain perception (Drossman 1999a). Repetitive transcranial magnetic stimulation of the PFC that decreases the activity of the amygdala (Baeken, et al. 2010) would also be of interest in IBS patients. In this context, vagal nerve stimulation, used for the treatment of refractory epilepsy and depression, should be of interest in the treatment of IBS by modulating the amygdala. Indeed, an inhibitory action of vagal nerve stimulation on amygdala-mPFC neurotransmission, probably due to the deactivation of the amygdala, has been described under VNS (Kraus, et al. 2007). Consequently, new methods aimed at modifying the activity of the amygdala represent a therapeutic challenge in the management of IBS patients.

2. Irritable bowel syndrome

2.1. Definition-background

The irritable bowel syndrome (IBS) is the most common disorder encountered by gastroenterologists. IBS is defined as “a functional bowel disorder in which abdominal pain is associated with defecation or a change in bowel habit with features of disordered defecation and distension”(Drossman 1999b). Classically the syndrome is considered as functional since biological as well as morphological (e.g. colonoscopy) investigations are not able to evidence any detectable organic lesions or anatomical abnormalities (colonic polyps or diverticulosis…) relative to symptomatology of the affected patients. The syndrome has been defined according to Rome III criteria (Longstreth, et al. 2006). There is a female predominance in a ratio of 2:1 (Drossman, et al. 1993). IBS affects up to 10–15% of the population with an estimated 1.7 billion dollars in annual direct cost (Talley, et al. 1991). Generally patients suffer from the absence of a real diagnostic and from the consideration that they have a psychosomatic disease. Pain is perceived by patients as the most distressing symptom and constitutes their major reason for consulting a physician (Sandler, et al. 1984). Extra-intestinal manifestations are also frequently described by the patients (e.g. headache, low back pain, chronic fatigue, interstitial cystitis…) (Whitehead, et al. 2002).

2.2. Pathophysiology

The pathophysiology of IBS is multifactorial. Altered bowel motility, sensory disorders, psychosocial factors are evoked (Drossman, et al. 1999c; Gaynes and Drossman 1999; Bonaz and Sabate 2009). Local features have also been considered as important. The role of food is often evoked by patients and a number of them are intolerant to lactose, fructose, gluten, polyols (Dapoigny, et al. 2004; Morcos, et al. 2009) with an enhancement of their symptoms following an eviction of such foods from diet. There is also good evidence for a role of the GI microbiota in its pathogenesis (Parkes, et al. 2008). Neuroimmune interactions are also involved, based on the development of IBS after infectious gastroenteritis (i.e. post-infectious IBS) (Gwee 2001) or in patients with IBD in clinical remission (i.e. post-inflammatory IBS) (Long and Drossman 2010). A low grade inflammation has been observed in IBS patients with a predominance of mastocytes in close contact with neural fibers explaining why IBS is assimilated to an IBD by some authors (Ford and Talley 2011).

Sensory disorders, and especially VHS, have also been evoked in the pathophysiology of IBS. VHS, represented by the increased sensation of pain when the pelvic colon is distended with an inflated rectal balloon, is a clinical marker of IBS which is observed in most of IBS patients. The exact location of the abnormal processing of visceral pain is unknown, and can have a peripheral origin, i.e. in the digestive tract by altered peripheral functioning of visceral afferents (i.e. bottom-up model), a spinal origin, e.g. spinal hyperalgesia by a defect of the gate control, or a defect of descending inhibitory controls or an altered central processing of afferent information from the gut, i.e. top-down model or a combination of all these hypotheses. IBS patients have an alteration in the spinal modulation of nociceptive process by the inhibitory descending pain modulation systems (Wilder-Smith, et al. 2004) in which the amygdala could be involved.

Psychosocial factors are often found in IBS patients. Among 20 to 50% of IBS patients have psychiatric disorders, such as major depression, anxiety, and somatoform disorders (Garakani, et al. 2003). Low dose of tricyclic antidepressants have shown efficacy in ameliorating the symptoms in patients (Rahimi, et al. 2009). IBS is also frequently associated with fibromyalgia in 30% to 70% of the cases. This syndrome is characterized by somatic hyperalgesia, the physiopathology of which is close to IBS (Mathieu 2009). IBS and fibromyalgia are classified by some authors as central sensitization syndromes (Woolf 2011). A majority of IBS patients associate stressful life events with initiation or exacerbation of their symptoms (Whitehead, et al. 1992) and stress is able to act at all levels of the physiopathology of IBS (see below). Globally, a concept has emerged that IBS is the result of a dysfunction of the brain-gut interplay, as conceptualized in the brain-gut axis. The ANS is, with the HPA axis, the link between the CNS and the gut and an autonomic dysfunction is observed in IBS patients which could be of top-down or bottom-up origin, as observed for VHS.

3. The brain-gut axis

3.1. Definition

The brain talks to the gut and conversely through a bidirectional communication under normal conditions and especially during perturbations of homeostasis. The CNS and the gut communicate through the ANS and the circumventricular organs and the gut contains a “little brain” as represented by the enteric nervous system which is a target of the ANS.

3.2. The enteric nervous system

The enteric nervous system can control functions of the intestine even when it is completely separated from the CNS (Bayliss and Starling 1899). The enteric nervous system contains three categories of neurons, identified as sensory, associative, and motor neurons (both excitatory and inhibitory) which are the final common pathways for the control of signals to the musculature, submucosa, mucosa, and vasculature, both blood and lymphatic. The enteric nervous system contains as many neurons as in the spinal cord (400–600 million) and confers an autonomy to the digestive tract such as the enteric nervous system can function independently of the CNS for the programming of motility and secretion (Furness 2012). Some neuropeptides and receptors are present in both the enteric nervous system and the CNS. The function of the GI tract is modulated by both the enteric nervous system and the ANS.

3.3. The autonomic nervous system (The afferent system)

The ANS is composed of the sympathetic (i.e. the splanchnic nerves) and parasympathetic nervous system (i.e. the vagus nerves and the sacral parasympathetic nucleus represented by the pelvic nerves) which are mixed systems.

The vagus nerve contains essentially 80-90% of afferent fibers vehiculating informations from the abdominal organs to the brain (Altschuler, et al. 1989) with the exception of the pelvic viscera for which informations are vehiculated to S2-S4 levels of the spinal cord by the pelvic nerves with central projections similar to other spinal visceral afferents. The vagus nerve carries mainly mechanoreceptor and chemosensory informations from the gut. If classically vagal afferents do not encode painful stimuli, they are able to modulate nociceptive processing in the spinal cord and the brain (Randich and Gebhart 1992).

The sympathetic nerves contain 50% afferent fibers. Visceral afferents that enter via spinal nerves (i.e; splanchnic and pelvic nerves), at thoracic 5 - lumbar 2 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 Aδ fibers, which will reach conscious perception.

The afferent informations of the ANS reach the CNS at the spinal cord level, for the splanchnic nerves, the nucleus tractus solitarius (NTS) level in the dorsal medulla for the vagus nerve, and the sacral parasympathetic (S2-S4) level for the pelvic nerves. At the level of the spinal cord, sympathetic afferents are integrated at the level of laminae I, II outer, V, VII (indirectly) and X. Then the information is sent to the upper level through the spino-thalamic and spino-reticular tracts, the dorsal column with projection to the thalamus (ventral posterolateral nucleus, intralaminar nucleus) and the cerebral cortex (insular, anterior-cingulate, dorsolateral PFC…). Neurons from laminae I, IV, and V responding to visceral stimuli also receive nociceptive cutaneous inputs (Foreman 1999).

At the level of the NTS, vagal afferents are integrated in subnuclei according to visceral somatotopy (e.g. medial, commissural, gelatinosus) (Altschuler, et al. 1993) and then projections to the PB nucleus, in the pons, according to a viscerotopic organization, which in turn projects to numerous structures in the brainstem, hypothalamus, basal forebrain, thalamus, and cerebral cortex (Fulwiler and Saper 1984). In the cerebral cortex, the insular cortex acts as a visceral (e.g. GI) cortex through a NTS-PB-thalamo-cortical pathway

according to a viscerotopic map. The insular cortex is connected with the limbic system (bed nucleus of the stria terminalis and CeA) and with the lateral frontal cortical system (Saper 1982). The NTS also sends projections to the ventrolateral medulla, the hypothalamus, 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 vagus nerve; some of these afferents probably being at the origin of autonomic reflex responses. This convergence is also observed at the level of the PB and ventrolateral medulla (Saper 2002) thus arguing for a relationship of pain with visceral sensations.

At the forebrain level, the spinal visceral sensory system constitutes a postero-lateral continuation of the cranial nerve to the visceral sensory thalamus and cortex (Saper 2000). There is also a spino-PB pathway since about 80% of lamina I spinothalamic axons send collaterals to the PB (Hylden, et al. 1989) and a spino–parabrachio–amygdaloid pain pathway which implicates the transmission of nociceptive information to the amygdala. Spinal nociceptive neurons in laminae I, IV, V, VII, and X directly innervate the hypothalamus and medial prefrontal cortex (Cliffer, et al. 1991; Burstein 1996). The messages coming from the gut are integrated in the central autonomic network (see below), which, in turn, adapts the response of the digestive tract through the efferent ANS through reflex loops which are essentially unconscious or become conscious in pathological conditions such as VHS observed in IBS. There is also descending pathways that control somatic as well as visceral pain by modulating visceral informations at the spinal cord level. 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 as well as facilitatory producing hyperalgesia (rostroventral medulla and OFF and ON cells) (Tsuruoka, et al. 2010).

3.4. The circumventricular organs

The circumventricular organs are highly vascularized structures with fenestrated capillaries located around the 3rd and 4th ventricles. They are characterized by the lack of a blood–brain barrier and represent points of communication between the blood, the brain, and the cerebrospinal fluid (Benarroch 2011). They are represented by the subfornical organ, median eminence, pineal gland, area postrema, organum vasculosum of the lamina terminalis. The circumventricular organs are sensitive to the vascular content (e.g. circulating interleukins, electrolytes). They activate dendritic cells releasing prostaglandins acting on PGE2 receptor of neurons located closely to these circumventricular organs. These neurons send projections to the hypothalamus, activating the HPA axis, and to the central autonomic network represented by the DMNV and the sympathetic pre-ganglionar neurons of the intermediolateralis column. The circumventricular organs are consequently involved in the central integration of a peripheral message to maintain homeosthasis. For example, they are involved in sodium and water balance, cardiovascular regulation, metabolic and energetic balance, immune function, regulation of body temperature, vomiting, reproduction. During an immune challenge represented by systemic inflammation, cytokines released in the circulation talk to the brain through two routes i.e. neural (vagal afferents) and humoral (circumventricular organs) to activate the HPA axis.

3.5. The central autonomic nervous system

The central autonomic nervous system integrates and modulates afferent informations from the gut and sends reversible inputs to the gut. In the CNS, visceral informations are integrated

in the central autonomic nervous system via brain regions involved in the autonomic, endocrine, motor, and behavioral responses (Saper 2002). The brain network can be roughly divided into executive structures, mainly hypothalamic, coordinating structures, mainly included in the limbic system, and high level control structures, mainly the frontal cortex.

The hypothalamus e.g. paraventricular nucleus (PVN), lateral hypothalamus, arcuate nucleus and adjacent retrochiasmatic area innervate the parasympathetic and sympathetic preganglionic neurons. The principal neuromediators are oxytocin and vasopressin (Hallbeck, et al. 2001). Through the release of CRF, the neuromediator of stress, the PVN is involved in the HPA axis response to stress. The limbic system is represented by the amygdala and its nuclei, the bed nucleus of the stria terminalis, considered as the extended amygdala, the septum and the hippocampus. The limbic system modulates the endocrine system and the ANS, two major components of the brain-gut axis. Classically, the amygdala is involved in the integration of emotions and the emotional conditioning which is represented by the association of a conditioned stimulus (i.e. a sound) with an unconditioned stimulus (the reinforcement) (Henke, et al. 1991; Benarroch 2006; LeDoux 2007). The amygdala receives afferents from the NTS, PB nucleus, frontal cortex, and LC and sends projection to the ANS, the frontal cortex and the hippocampus. The amygdala inhibits the DMNV, stimulates the sympathetic nervous system and the stress response through the HPA axis. The amygdala is a CRF-containing nucleus.

The prefrontal, insular, and anterior cingulate cortices are involved in the integration of visceral informations, attention, emotions and in the regulation of humor. The anterior cingulate cortex is divided in a cognitive dorsal part and an affective ventral part i.e. the perigenual part which has been frequently activated in brain imaging by numerous emotional stimuli. Most of these structures (ANS, HPA axis, limbic system, endogenous pathways that modulate pain and discomfort…) are part of the emotional motor system that mediates the effect of emotional states on the GI function, modulates gut functions and communicates emotional changes via the ANS to the gut. The threshold for visceral perception is dependent on the individual's emotional and cognitive state (Mayer 2000; Mayer 2011).

Visceral as well as stressful informations activate the LC, a nucleus belonging to central noradrenergic system localized in the pons. The LC is the largest group of noradrenergic neurones. It is involved in emotional arousal, autonomic, and behavioural responses to stress and attention-related processes through its dense projections to most areas of the cerebral cortex and alertness-modulating nuclei (e.g. majority of the cerebral cortex, cholinergic neurones of the basal forebrain, cortically-projecting neurones of the thalamus, serotoninergic neurones of the dorsal raphe and cholinergic neurones of the pedunculopontine and laterodorsal tegmental nucleus). The LC also exerts an indirect action on autonomic activity via projections to the PVN and to the cerebral cortex and amygdala, structures which are known to influence the activity of premotor sympathetic neurones in the PVN. LC activation leads to anxiety through an activation of the amygdala (Tasan, et al. 2010).

4. Stress and the gut

4.1. Background

Stress is defined as the response of the organism to a solicitation of the challenging environment. The body engages a "fight or flight" response when exposed to an acute challenge with a sympathetic activation leading to an increase of heart rate and respiration,

increased arousal, alertness, and inhibition of acutely non adaptive vegetative functions (feeding, digestion, growth and reproduction). The time course of the reaction corresponds to the general syndrome of adaptation defined by Hans Selye in 1950 (Selye 1950). The reaction of stress is physiological but may become pathological following an unbalance between the capacities of adaptation and the requirement of the environment, thus leading to functional, metabolic, and even lesional disorders.

4.2. The CRFergic system

CRF is a 41-amino acid peptide derived from a 191-amino acid preprohormone. CRF is secreted by the paraventricular nucleus (PVN) of the hypothalamus in response to stress (Vale et al. in 1981) as well as its related peptides the urocortines (Ucn) i.e. Ucn 1, Ucn 2 (also known as stresscopin-related peptide), and Ucn 3 (also known as stresscopin). CRF and the Ucns exert their biological actions on target cells through activation of two 7–transmembrane-domain G protein–coupled receptors, known as CRF receptor type 1 (CRF1) and CRF receptor type 2 (CRF2) which are encoded by 2 distinct genes [for review (Gravanis and Margioris 2005)]. CRF and Ucn 1 have equal affinity for the CRF1 receptor, although Ucn 1 is 40 times more potent than CRF in binding CRF2. In contrast, Ucns 2 and 3 bind selectively to CRF2. The population of CRF synthetizing neurons is predominantly expressed in the parvocellular part of the PVN of the hypothalamus and projects via the external zone of the median eminence to the anterior pituitary. In addition to its role as a hypothalamic hypophysiotropic hormone, CRF acts as a neurotransmitter in several brain areas. CRF has predominantly excitatory actions on neurons in the hippocampus, cortex, LC, and hypothalamic nuclei (Siggins, et al. 1985). CRF1 mediates anxiety-like behaviors whereas CRF2 mediates anxiolytic effects in the defensive withdrawal test (Heinrichs, et al. 1997). Competitive CRF receptor antagonists have been developed to determine the functions of CRF receptors under basal and stress conditions (Bonaz and Tache 1994b). The CRF system plays a critical role in coordinating the autonomic, endocrine, and behavioural responses to stress (Dunn and Berridge 1990).

The effect of stress on the GI tract is now well characterized. Stress induces modifications of motility, secretion, visceral sensitivity, local inflammatory responses (Delvaux 1999; Mawdsley and Rampton 2006; Tache and Bonaz 2007) through a central and/or peripheral action through CRF1,2 related receptors. Alterations of this complex system in humans are linked to a variety of anxiety-related psychiatric disorders and stress-sensitive pain syndromes, including IBS. Dysfunction in the HPA axis regulation attributable to overactivation of CRF/CRF1 signaling in response to chronic stress has been implicated in the pathophysiology of IBS symptoms (Chang, et al. 2009).

4.3. Stress effect on GI functions

4.3.1. Motility and secretion

Stress is known to decrease gastric emptying, lengthen small bowel motility and increase colonic motility (Tache and Bonaz 2007). The effects of stress on gut function are mediated by the ANS represented by the sympathetic, vagal and pelvic parasympathetic innervation of the enteric nervous system (Grundy 2006). At the central level, stress inhibits the parasympathetic nervous system and activates the sympathetic nervous system through the effect of PVN projections on the DMNV and intermediolateral column cells of the spinal cord.

CRF signaling is a key component in the alterations of gut motor function in response to stress in both the brain and the gut. The CRF/CRF1 signalling pathway is involved in stress-induced anxiety/depression (Holsboer and Ising 2008) and alterations of colonic motor and visceral pain while both central and peripheral CRF2 receptor activation may exert a counteracting influence (Tache, et al. 2005; Million, et al. 2006). At the level of the GI tract, stress delays gastric emptying through CRF2 while increasing colonic motility and secretion through CRF1 (Tache and Bonaz 2007). In the small bowel, CRF-like peptides stimulate the contractile activity of the duodenum through CRF1 receptor while inhibiting phasic contractions of the ileum through CRF2 receptor (Porcher, et al. 2005).

Stress also induces an activation of the sacral parasympathetic nucleus through the projections of the Barrington nucleus through CRF activation thus stimulating recto-colonic motility (Tache and Bonaz 2007). Numerous data have established the involvement of peripheral CRF signalling in the modulation of secretory function under stress conditions via activation of both CRF1 and CRF2 receptors, activation of cholinergic enteric neurons, mast cells and possibly serotonergic pathways (Larauche, et al. 2009).

4.3.2. Intestinal permeability

An increase of intestinal permeability is observed in the colon of IBS patients, associated with visceral or somatic hypersensitivity (Zhou and Verne 2011). Stress is able to disrupt the intestinal epithelial barrier thus increasing the penetration of luminal antigens into the lamina propria, leading to nociceptors sensitization and favoring the development of visceral hypersensitivity (Ait-Belgnaoui, et al. 2005). This increase of intestinal permeability is due to an activation of peripheral CRF signaling involving both CRF2 and CRF1 (Buckinx, et al. 2011) as well as mast cell activation (Santos, et al. 2001).

4.4. Stress effect on intestinal inflammation

Stress is able to increase intestinal inflammation by increasing intestinal permeability (see above) thus activating mast cells and visceral afferents in a local loop. Stress favours intestinal inflammation by stimulating the sympathetic nervous system and inhibiting the vagus nerve thus decreasing the cholinergic anti-inflammatory pathway. Stress, through its immune-suppressive function also favours inflammation (Ghia, et al. 2006; Mawdsley, et al. 2006; Bonaz 2010).

4.5. Stress effect on the microbiota

Bacteria in the gut (400–1,000 different bacterial species) have an important role in the immune response, including inflammation (Lee and Mazmanian 2010). Stress is able to modify the intestinal microbiota (Bailey, et al. 2010). Alteration of the microbiota favors translocation of bacteria from the intestinal lumen to the interior of the body where they can stimulate the immune system (Clarke, et al. 2010). This can in turn have significant impact on the host and affect behavior, visceral sensitivity and inflammatory susceptibility (Collins and Bercik 2009).

4.6. Stress effect on visceral sensitivity

Stress is known to increase visceral sensitivity [(Larauche, et al. 2012) for review]. Either acting at the central and/or peripheral (e.g. digestive) level, stress is able to increase visceral

perception and emotional response to visceral events by a disturbance of the brain-gut axis at its different levels, central, gut and the ANS. Genetic model of depression or anxiety, such as the high-anxiety Wistar-Kyoto (WKY) rats or Flinders Sensitive Line rats have shown increased sensitivity to colorectal distension (Overstreet and Djuric 2001). In the same way genetic models deleting CRF1 exhibit a decrease in colonic sensitivity to colonic distension (Trimble, et al. 2007) while models overexpressing CRF1 exhibit enhanced response to colonic distension (Million, et al. 2007). These data argue for the filiation stress-anxiety-inflammation and visceral hypersensitivity.

Again, the CRF signalling, at both the central and peripheral level, is a key element involved in stress-induced visceral hypersensitivity. Recent data argue for an equally important contribution of the peripheral CRF/CRF1 signalling pathway locally expressed in the gut to the GI stress response (Larauche, et al. 2009). At the peripheral level, mast cells degranulation observed in the colon following stress and peripheral administration of CRF (Wallon, et al. 2008) induces visceral hypersensitivity via the release of mediators (histamine, tryptase, prostaglandin E2, nerve growth factor) that can stimulate or sensitize sensory afferents (van den Wijngaard, et al. 2009; 2010). Intravenous administration of CRF increases GI motility and visceral pain sensitivity in IBS patients compared with healthy controls, whereas administration of a non-selective CRF receptor antagonist improved these responses (Million, et al. 2005; Tache, et al. 2005; Tsukamoto, et al. 2006).

4.7. Gut pathologies are engineered by stress

The GI tract is a sensitive target to stress. 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 (Drossman, et al. 1996; 2000; Drossman 2011). Stress is strongly associated with symptom onset and symptom severity in IBS patients. Illness experience, health care-seeking behavior, and treatment outcome are adversely affected by stressful life events, chronic social stress, anxiety disorders, maladaptive coping style. A history of emotional, sexual, or physical abuse is often found in IBS patients [(Chitkara, et al. 2008) for review]. For example, there is a significantly higher prevalence (i.e. 44%) of sexual or physical abuse in patients with functional GI disorders than in controls with organic GI disorders (Drossman, et al. 1990). Psychiatric comorbidity, especially major depression, anxiety, and somatoform disorders, occur in 20 to 50% of IBS patients (Garakani, et al. 2003) and more likely precede the onset of the GI symptoms, thus suggesting a role for psychiatric disorders in functional GI disorder development (Sykes, et al. 2003).

Functional brain imaging studies have shown that there is a major influence of cognitive-affective processes on GI sensations and its CNS correlates in health and functional digestive disorders as IBS (Mayer, et al. 2006; Van Oudenhove, et al. 2007). Cognitive-affective processes including arousal, attention and negative emotions strongly influence visceral pain perception through modulation of its neural correlates (Mayer 2011). Feeling emotions requires the participation of brain regions, such as the somatosensory cortices and the upper brainstem nuclei that are involved in the mapping and/or regulation of internal organism states (Damasio, et al. 2000). This has led to the biopsychosocial concept of IBS (Drossman 1996b). These data are in agreement with the role of hypervigilance in the visceral hypersensitivity observed in IBS patients (Naliboff, et al. 2008). Spence et al. (Spence and Moss-Morris 2007) have characterized predictors of post-infectious IBS such as perceived stress, anxiety, somatisation and negative illness beliefs at the time of infection in favor of a cognitive-behavioural model of IBS. The importance of psychosocial factors and somatisation

compared to gastric sensorimotor function is most pronounced in hypersensitive patients with functional dyspepsia, another functional GI disorder (Van Oudenhove, et al. 2008).

5. Gut and emotional memories

Early life trauma (neglect, abuse, loss of caregiver or life threatening situation) increases susceptibility to develop later affective disorders such as depression, anxiety, and is a key factor in the development of IBS (Bradford, et al. 2012). Traumatic events, such as war, environmental disasters, physical abuse or a bad accident in adulthood can induce post-traumatic stress disorder (PTSD) with increased prevalence of GI symptoms, such as IBS (Cohen, et al. 2006).

The role of stress sensitization is also reproduced in preclinical studies. Adults rats previously subjected to neonatal maternal separation (MS) exhibit visceral hypersensitivity to colorectal distension in basal conditions (Ren, et al. 2007). This visceral hypersensitivity is exacerbated in acute stress (e.g. water avoidance stress: WAS; Avoidance to water for 1 h by standing on a small platform; Bonaz & Taché 1994b) conditions (Coutinho, et al. 2002). Chronic exposure to repeated WAS is used to study visceral hypersensitivity and is very close to clinical conditions. However, habituation of the CRFergic system is observed in chronic conditions (Bonaz and Rivest 1998) and may induce analgesia. It seems that these conflicting data are influenced by the basal state conditions of the animals before applying the repeated stressor (surgery and single housing) (Larauche, et al. 2010).

6. The amygdala in IBS pathophysiology

The amygadala is a key element in the pathogeny of IBS.

6.1. Anatomical and functional basis

6.1.1. Amygdala structures

The amygdala is divided into a primitive group of nuclei associated with the olfactory system (central, medial and cortical nuclei, and nucleus of the lateral olfactory tract), and a phylogenetically new group of nuclei (lateral and basal) (Knapska, et al. 2007). The lateral (LA), basolateral (BLA), and central nuclei (CeA) are important for sensory processing (Neugebauer 2006; LeDoux 2007). The amygdala is part of the central autonomic nervous system that is involved in the brain-gut axis. The amygdala is a key element in emotional/affective behavior (LeDoux 2007), including the emotional responses to pain such as anxiety and fear of pain (Gauriau and Bernard 2002; Neugebauer, et al. 2004; Neugebauer 2006) as well as in the reciprocal relationship between pain and affective state (Meagher, et al. 2001; Rhudy and Meagher 2003). Affective content is attached to sensory information through associative processing in the LA–BLA circuitry and is then transmitted to the CeA which is the output nucleus for major amygdala functions (Maren 2005; Phelps and LeDoux 2005). The CeA serves to attach emotional significance to afferent nociceptive transmission and coordinates appropriate autonomic, affective and motor behavioral responses through its outputs to the hypothalamus, cortex and brainstem (Neugebauer, et al. 2004).

6.1.2. Amygdala inputs

The CeA receives numerous sensory informations from descending cortical, thalamic (perigeniculate, paraventricular) and brainstem inputs (Whalen and Kapp 1991), as well as from the olfactory system, medial PFC, insula, brainstem viscerosensory and nociceptive centers (NTS, PB), and from all parts of the amygdala. The amygdala increases the excitability of CNS sites regulating behavioral, neuroendocrine, and autonomic responses to stress (LeDoux, et al. 1988) and thus is able to modify GI functions. The amygdala is involved in the affective processing of sensory information and in the generation of anxiety and fear (Davis 1997), elements which are involved in the pathogeny of IBS.

6.1.3. CRF as a key mediator in amygdala

The amygdala, and particularly the CeA, is a major site of extrahypothalamic CRF, in cell bodies and terminals as well as CRF1 and, to a lesser extent, CRF2 receptors. The amygdala is a key element of the extrahypothalamic circuits through which CRF contributes to anxiety-like behavior and affective disorders (Aguilera, et al. 1987; Sajdyk, et al. 1999; Reul and Holsboer 2002; Fu and Neugebauer 2008). Excepting the hypothalamus, the amygdala is the major site of urocortin III (the endogenous ligands for CRF2 receptors) expression (Li, et al. 2002). In particular, activation of CRF neurons in the CeA that project to the LC increase its firing thus resulting in a noradrenaline release in the structures it is projecting to (Bouret, et al. 2003). LC activation leads to anxiety through the activation of the amygdala and, conversely, anxiety producing stimuli (stressful and fear-inducing stimuli) that increase the activity of the amygdala lead to LC activation (Samuels and Szabadi 2008).

6.1.4. Amygdala output to gut

The CEA is involved in the modulation of the ANS because of its brainstem projections to the DMNV, NTS, PB and the periaqueductal gray (Rizvi, et al. 1991), known to modulate the spinal cord processing of noxious information through descending inhibitory controls (Le Bars, et al. 1992). The CEA innervates hypothalamic nuclei, modulating the HPA axis (Rodrigues, et al. 2009). The CeA also projects to the medial peri-LC dendritic region, resulting in increased norepinephrine release and other monoamine systems in the brainstem and forebrain (Gray 1993; Fudge and Emiliano 2003; Pare 2003) which are involved in arousal and hypervigilance.

6.1.5. Modulators of amygdala

The LC has an inhibitory effect on the BLA and the activation of this pathway leads to a disinhibition of the CeA, since the BLA has a predominantly inhibitory influence over the CeA (Rosenkranz, et al. 2006). The LC is involved in the stress response through CRF1 receptors as well as CRF afferent fibers from the Barrington nucleus which is ventro-laterally located to the LC. The Barrington nucleus projects to the sacral parasympathetic nucleus to increase the motility of the distal recto-colon (Valentino, et al. 1993). Colorectal distension increases the firing of the LC through CRF1 through a LC-Barrington nucleus pathway (Rouzade-Dominguez, et al. 2001). In addition, the LC is involved in the brain noradrenergic modulation of the GI tract motility (Bonaz, et al. 1992a; 1992b; 1995). Consequently, the Barrington-LC-amygdalo complex is ideally positioned to bidirectionally coordinate brain-gut interactions.

6.2. Amygdala and the pathophysiology of IBS

6.2.1. Amygdala and visceral hyperalgesia

The use of C-Fos expression as a marker of neuronal activation has shown that somato-visceral (Bonaz and Fournet 2000; Sinniger, et al. 2004; 2005), and visceral (Wang, et al. 2009) pain as well as stress- or abdominal surgery-induced GI disturbances (Bonaz and Tache 1994a; 1994b; 1997; Bonaz and Rivest 1998) and colitis (Porcher, et al. 2004) induced the activation of the amygdala. In addition, the amygdala is one of the central areas from where digestive sensations are elicited in epileptic patients (Mulak, et al. 2008) during intracerebral electrical stimulations. In a model of visceral pain induction such as inflating a balloon into the rectum, an activation of the amygdala is observed in healthy volunteers (Baciu, et al. 1999) while aberrant functional responses (e.g. deactivation of the amygdala) to noxious rectal stimulation was observed in areas of the brain involved in emotional sensory processing, particularly the amygdala, insula, and prefrontal cortex in IBS patients (Bonaz, et al. 2002; Elsenbruch, et al. 2010; Tillisch, et al. 2011) thus arguing for an abnormal brain processing of visceral pain following rectal distension.

Activation of corticosteroid receptor (both glucocorticoid and mineralocorticoid receptors) in the CeA is involved in the induction of anxiety and visceral hypersensitivity (Myers and Greenwood-Van Meerveld 2007b). High levels of glucocorticoids result in CRF mRNA level increases in the amygdala (Makino, et al. 1994). The group of Greenwood-Van Meerveld) have shown that 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 colo-rectal distension (Greenwood-Van Meerveld, et al. 2001; Myers, et al. 2005; Greenwood-van Meerveld, et al. 2006; Myers and Greenwood-Van Meerveld 2007a). Indeed, exposure of the amygdala to corticosterone-releasing micropellets caused an increase in action potential frequency in the dorsal horn neurons in the L6-S1 spinal segments suggesting that a descending neuronal pathway, originating in the amygdala, could be triggered by continuous activation by corticosterone. The neurons responding with excitation to colorectal distension were short-lasting and long-lasting excitatory neurons based on the duration of the reponse (Venkova et al. 2009). Mineralocorticoid receptors but not glucocorticoid receptors in the amygdala trigger descending pathways facilitating viscero-nociceptive processing in the spinal cord (Venkova, et al. 2009). In addition, a WAS known to activate the amygdala (Bonaz and Tache 1994b), performed during 7 consecutive days induced VHS that was abolished by glucocorticoid receptor and mineralocorticoid receptor antagonists in the amygdala. These results argue for a role of amygdaloid glucocorticoid receptor and mineralocorticoid receptor in IBS.

The CRF signaling is also involved in pain processing. WKY is a rat strain for studying anxiety and IBS. WKY express a greater amount of CRF and CRF1 mRNA in the CeA and the PVN (Bravo, et al. 2011). In this model, it has been shown that colonic hypersensitivity to luminal distension is reversed by peripheral administration of a CRF1 antagonist (O'Malley, et al. 2011). Infusion of CRF1 antagonist into the CeA attenuates the hypersensitivity to colonic distension in the WKY rats, thus confirming the role of CRF1 receptor in the amygdala in VHS mechanism (Johnson, et al. 2012). The basal expression of CRF in the LC is increased in WKY rats and a selective CRF1 receptor antagonist abolished the activation of LC neurons by colorectal distension and intracisternal CRF in rats (Kosoyan, et al. 2005). These data strengthen the role of the CeA and LC in VHS through CRF1 which is in agreement with the interactions between both nuclei involved in emotional-arousal circuit. Indeed, 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. In humans, oral administration of a selective CRF1 antagonist (GW876008) is followed by a significant BOLD signal reductions within the amygdala during pain expectation in IBS patients (Hubbard, et al. 2011). CRF1 receptors in the amygdala contribute to pain-related sensitization, whereas the normally inhibitory function of CRF2 receptors is suppressed in the arthritis pain model. Thus, due to the opposing effect of CRF1 and CRF2 receptors, CRF can induce a dual effect in the amygdala. The differential effects of CRF1 and CRF2 receptor antagonists on pain-related processing in the amygdala have reciprocal opposing influences on anxiety-like behaviors. CRF1 and CRF2 receptors in the amygdala mediate opposing effects on nociceptive processing (Ji and Neugebauer 2007). Numerous data argue for a role of CRF1 and CRF2 to mediate pro- and anti-nociceptive effects of CRF respectively. It has been shown that low concentrations of CRF facilitate nociceptive processing in the CeA neurons through CRF1 while higher concentrations of CRF have inhibitory effects through CRF2 receptors. This is in agreement with the concept that CRF2 receptors serve to dampen or reverse CRF1-initiated responses (Tache and Bonaz 2007). These results clarify the controversial role of CRF in pain modulation and show that the CRFergic system in the amygdala may be a key link between pain and affective states and disorders.

6.3. Amygdala and stress conditioning

6.3.1. The synchronic stress engineering

Systemic cortisol is a classical marker of the HPA axis activation. The amygdala and hippocampus have numerous receptors for cortisol and are consequently highly susceptible to the products of the HPA axis. Glucocorticoid occupation of hippocampal receptors has a suppressive effect on the HPA axis (van Haarst, et al. 1997) whereas glucocorticoid occupation of amygdala receptors have a facilitating effect on the HPA axis, often increasing CRF expression within the amygdala (Makino, et al. 1994). CRF receptors are greatly expressed in the amygdala and hippocampus early in development (Baram and Hatalski 1998), thus explaining why young animals are especially vulnerable to threat. In agreement, early-life stress induces a decrease of hippocampal volume and functional alterations when measured in adulthood (Nemeroff, et al. 2006). Structural changes have also been observed in IBS patients using brain imaging (Blankstein, et al. 2010; Seminowicz, et al. 2010). Also, circulating glucocorticoids can have contrasting effects in the amygdala and hippocampus, and these two structures can play contrasting roles in the activity of the HPA axis. In the context of an overactivity of the HPA axis due to an enhanced stress responsiveness, greater basal levels of systemic cortisol have been reported in IBS patients (Chang, et al. 2009). Circulating cortisol regulates the HPA axis and is also able to act within the amygdala by binding to selective glucocorticoid and mineralocorticoid receptors, highly expressed in the amygdala (Sapolsky, et al. 1983) to facilitate behavioral and psychological stress responses including GI motility.

6.3.2. Amygdala and stress memorisation

Functional imaging studies indicate that the mPFC is engaged in fear extinction process in relation with the amygdala (Phelps, et al. 2004). The amygdala is an important region involved in the acquisition of fear conditioning, a learning that corresponds to the association between a conditioned stimulus andan unconditioned stimulus. The infralimbic region of the mPFC participates in the mechanism of fear extinction (Rosenkranz, et al. 2003; Quirk and

Vidal-Gonzalez 2006b) and also in the recall of fear extinction with an active inhibition of the previous fear condition responses. This is mediated by a down regulation of amygdala outputs with mPFC neurons exciting (glutamate) inhibitory neurons (GABA) within the BLA or in the intercalated region inhibiting in turn amygdala outputs from the CeA (Vidal-Gonzalez, et al. 2006). The activity of intentional regulation of treat related-cues by the PFC is decreased in anxious patients and the conditioned fear extinction is also less active, in PTSD-anxious patients and this is associated with symptoms provocations (Bradette, et al. 1994). The amygdala is also activated by uncertainty and the capacity of the PFC to regulate attention, (re) interpretation of the situation will modulate the level of the response of amygdala to uncertainty. In IBS, uncertainty plays an important role in the perception of pain. Therefore it seems important to study the fronto-amygdalar relations in IBS patients. The inhibitory control of the mPFC on CeA would maintain an homeostatic state with an equilibrated sympatho-vagal balance and low glucocorticoids circulating levels. In the case of a deficit in PFC activity with a lack of inhibitory regulatory communications with the amygdala, a chronic imbalance of the ANS with an increase sympathetic activity should appear as we have observed in IBS patients exhibiting a low heart rate variability and a high score of anxiety (Pellissier, et al. 2010a). Moreover, there is a strong relation between the activity of the ANS and the immune system as recently shown by the cholinergic anti-inflammatory pathway (Huston and Tracey 2011). Hence, when the parasympathetic system is hypoactive as a consequence of anxiety for instance, it could facilitate inflammation which could be deleterious for health and well-being (Bonaz 2003). The hypoactivity of the PFC and the enhancement of amygdala (re)-activity are strongly influenced by stress as demonstrated by a number of studies. It has recently been shown an increase in the dendritic arborization, and synaptic connectivity in the LA/B neurons under chronic stress conditions (Vyas, et al. 2002; Vyas, et al. 2006). LA/B neurons from stressed animals display increased firing rates and greater responsiveness (Kavushansky and Richter-Levin 2006) since the mediators of stress i.e. norepinephrine, and glucocorticoids decrease GABA inhibition (Rodriguez Manzanares, et al. 2005), thereby allowing for increased excitability in LA/B. In the meantime, an atrophy and spine loss of neurons in the mPFC following stress and glucocorticoid exposition is observed (Czeh, et al. 2008) allowing an over-activation of amygdala under chronic stress exposition.

6.3.3. Amygdala and early stress

Environmental events during early postnatal life can influence the formation of neural circuits that provide limbic and cortical control over autonomic emotional motor output since a differential timing of hypothalamic and limbic forebrain synaptic inputs to autonomic neurons has been observed during the first 1–2 weeks postnatal (Rinaman, et al. 2011). This provides a potential structural correlate for early experience-dependent effects on later responsiveness to emotionally evocative stimuli and an enhanced risk for the development of psychopathologies such as mood and aggressive disorders. MS is classically used as a model of brain-gut axis dysfunction (O'Mahony, et al. 2011) and early life trauma are often observed in IBS patients (Bradford, et al. 2012). The amygdala is functionally active early in life and demonstrates continued refinement, through increased cortical connections, throughout childhood and adolescence. The amygdala is particularly vulnerable to stressors early in life. Reduced hippocampal volumes (Woon, et al. 2010) and increased amygdala volumes (Tottenham, et al. 2010) have been associated with early life stress.

6.3.4. The maternal separation model (MS)

Numerous studies have shown that the HPA axis of MS rodents shows hyperactivity in the PVN and amygdala (Plotsky and Meaney 1993; Coutinho, et al. 2002; Plotsky, et al. 2005; Schwetz, et al. 2005). Offspring of mothers that exhibit more licking and grooming of pups show reduced plasma ACTH and corticosterone responses to acute stress and decreased levels of hypothalamic CRF mRNA in correlation with the frequency of maternal licking and grooming during the first 10 days of life (Plotsky, et al. 2005). Thus, it is likely that a major part of the alterations associated with early life stress are related to CRF hyperproduction that account for amygdala hyperactivity. Maternal care during the first week of life is associated with increased GABAergic inhibition of amygdala activity (Diorio and Meaney 2007). These data reflect the importance of early environmental factors in regulating the development of the hypothalamic CRF system in relation with amygdala activity and the vulnerability to stress. Moreover, there is a sex-specific difference in the effects of early life stress on HPA axis activity consistent with the higher prevalence of major depression with hypercortisolism in women than in men. Moreover, women who experienced early life stress are more likely to develop depression as well as IBS (Bradford, et al. 2012). Sex-hormones influence amygdala development in human populations (Rose, et al. 2004). An alteration in the central CRF system has been evidenced in two different rat models of comorbid depression and functional GI disorders (e.g. IBS) represented by neonatal MS and the WKY rat, a genetically stress-sensitive rat strain, that display increased visceral hypersensitivity and alterations in the HPA axis. These rat strains express a greater amount of CRF and CRF1 mRNA in the amygdala (CeA) as well as in the PVN (Bravo, et al. 2011). They also present a positive correlation between increased central CRF and CRF1 receptor expression, with elevated anxiety-like behavior and colonic hypersensitivity (Gunter, et al. 2000; Shepard and Myers 2008). An increase of CRF1 mRNA was observed in the PVN and amygdala while CRF2 mRNA, classically counteracting CRF1 in the CNS, was lower in the amygdala of MS rats. Such modifications, by affecting the HPA axis regulation, may contribute to behavioral changes associated with stress-related disorders, and alter the affective component of visceral pain modulation, which is enhanced in IBS patients (Bravo, et al. 2011).

6.4. The alteration of amygdala control in IBS

The amygdala has interconnections with the anterior cingulate cortex, the PFC, the hippocampus, the hypothalamus (e.g. PVN), the bed nucleus of the stria terminalis, the lateral septum, the thalamus, the periacqueductal gray, the PB, the LC, the raphe nuclei, and the dorsal vagal complex (area postrema, nucleus tractus solitarius and DMNV) (Knapska, et al. 2007). All these regions have been shown to be activated in experimental models of stress, inflammation, and pain as represented by c-fos expression and/or CRF receptor mRNA induction (Bonaz and Tache 1994a; Bonaz and Rivest 1998; Bonaz, et al. 2000; Porcher, et al. 2004; Sinniger, et al. 2004; 2005) or electrical stimulations (Mulak, et al. 2008). In addition, brain imaging techniques (fMRI, PET), have contributed to the better understanding of IBS. An activation of most of the brain structures referenced above, and particularly the amygdala, has been observed in healthy volunteers following rectal pain while an abnormal brain processing of pain was observed in IBS and IBD patients (Baciu, et al. 1999; Bonaz, et al. 2002; Agostini, et al. 2011). In addition, brain structural changes of the HPA axis and limbic structures have been recently reported in IBS patients (Blankstein, et al. 2010; Seminowicz, et al. 2010). Because psycho- or pharmacotherapy tends to result in normalization of activity of key structures such as the PFC including anterior cingulate cortex, hippocampus, or amygdala, either through a top-down or bottom-up effect (Quide, et al. 2012), the determination of psycho-physiological vulnerability in IBS patients should be a flag to

consider the psychological needs in the follow-up of such patients in the prevention of relapses of such diseases (Pellissier, et al. 2010b).

7. Therapeutic implications-treatment targeting amygdala activity reduction in IBS

The effect of stress on amygdala functioning has therapeutic implications both with non-pharmacological and pharmacological treatment to reduce stress perception. Psychological mind-body interventions including psychotherapy, cognitive behavioral therapy, hypnotherapy, relaxation exercises or mindfulness mediation have been shown to improve symptoms of IBS patients (Kearney and Brown-Chang 2008; Ford 2009; Whorwell 2009). Repetitive transcranial magnetic stimulation of the PFC, based on the central role of the mPFC in cognitive theory of mind, can cause changes in acute pain perception and has been used in a model of central sensitization syndrome such as fibromyalgia (Mhalla, et al. 2011; Short, et al. 2011) but no data have been currently published in IBS patients. Modulation of the ANS by restoring the sympatho-vagal balance (DeBenedittis, et al. 1994; Nishith, et al. 2003; Gemignani, et al. 2006) as well as modifying coping strategies vigilance state and globally the restoration of a functional brain-gut axis, are at the origin of the efficacy of these treatments. Brain imaging techniques have shown modulation of brain activation, as for example in the amygdala, by such treatments (Goldin and Gross 2010; Lawrence, et al. 2011). Conventional treatment as represented by anti-depressives, anxiolytics, drug targeting the central sensitization syndrome [α2δ ligand (pregabalin, gabapentin); tachykinin receptor antagonists] either directly and/or indirectly are supposed to target the hyperfunctioning of the amygdala (Ghaith, et al. 2010; Gale and Houghton 2011; Trinkley and Nahata 2011; Larauche, et al. 2012). In the context of the microbiota-brain-gut axis, probiotics, prebiotics, antibiotics such as rifaximin, an antibacterial agent that is virtually unabsorbed after oral administration and is devoid of systemic side effects, are of interest (Bercik, et al. 2011; Fukudo, et al. 2011; Fukudo and Kanazawa 2011). If targeting CRF signaling with CRF1 receptor antagonists, based on pre-clinical and/or clinical data (brain imaging) has been used successfully in humans to treat depression and anxiety (Kunzel, et al. 2003) their efficacy is still matter of debate in the treatment of IBS patients (Sweetser, et al. 2009).

8. Conclusion

A growing body of evidence argues for an important role of stress, through the HPA axis, limbic system activity (e.g. the amygdala), and the ANS, i.e. the sympathetic and the parasympathetic (e.g. the vagus nerve) nervous system, in the initiation and perpetuation of IBS. Stress, pain, and immune activation are common risk factors involved in the pathogenesis of IBS which are able to act through this neuro-endocrine-immune axis. The amygdala, through its connections with the PFC, LC, hippocampus, HPA axis, and ANS is a key structure involved in the pathogeny of IBS. Animal models of activation of the CRFergic system in the amygdala, as represented by maternal separation stress or WKY rats, developed VHS as observed in most of IBS patients. Thereofore, a therapeutic targeting of the amygdala either through pharmacological or non-pharmacological approach should be of interest for the treatment of IBS.