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Psychological Stress Induces Visceral Analgesic or Hyperalgesic Response in Rodents: A Role of Preconditions

Agata Mulak^{a,b}, Muriel Larauche^a, and Yvette Taché^a

^aCURE/Digestive Diseases Research Center, Oppenheimer Family Center for Neurobiology of Stress, Division of Digestive Diseases, Department of Medicine, University of California Los Angeles, and VA Greater Los Angeles Health Care System, Los Angeles, Calif., USA

^bDepartment of Gastroenterology and Hepatology, Wroclaw Medical University, Wroclaw, Poland

Abstract

A dual action of stress on pain modulation has been well characterized in the somatic pain studies, while much less is known in the visceral field. In the context of clinical observations that stress plays a critical role in the pathophysiology, symptoms presentation and clinical outcome of functional gastrointestinal disorders such as irritable bowel syndrome (IBS), a number of acute and chronic stress models have been developed in rodents. Recent data have demonstrated that the state of the animal tested (naïve vs. exposed to surgery), its social environment (group housing vs. single housing), the methods used to record visceromotor responses (EMG requiring surgery and antibiotic after surgery vs. manometry not requiring surgery/antibiotic) can significantly affect the analgesic response to exteroceptive stressors. Growing body of evidence indicates that a new noninvasive solid-state manometric method to monitor visceromotor response is valuable to unravel both analgesia and hyperalgesia without confounding factors. This is of critical importance regarding the recently recognized role of a compromised engagement of the inhibitory descending pain pathways in IBS patients. Better understanding of mechanisms of stress-related modulation of visceral pain leading to analgesia and hyperalgesia, along with the role of sex-dependent factors and complex interactions of the brain-gut-enteric microbiota axis may lead to new therapeutic targets in IBS.

A decrease in pain perception is a classic effect of a variety of acute stressors in humans and in animal models. Stress-induced analgesia (SIA) mediated by descending inhibitory pathways is a well-recognized phenomenon observed in naïve animals and extensively investigated in somatic pain field [1]. There is evidence of opiate-dependent and opiate-independent pathways recruited differentially according to the modalities of stress procedures, pain test used, genotype and hormonal status of tested animals [1, 2]. However, stress-induced hyperalgesic response in somatic as well as visceral pain studies is also commonly observed [1, 3]. This dual action of stress on pain modulation depends to a great extent on pre-existing conditions, in particular previous pain experience that might be

associated with some methodological factors of visceral pain monitoring (e.g. surgery), along with previous adverse life events [3].

The brain-gut axis is recruited by various central nervous system (CNS) and gut-brain directed stressors. An infectious event in the gut affects the cortical response to visceral stimuli, and conversely, psychological events can alter the function of the gut. Experimental and clinical data confirm CNS-directed pathogenesis (connected with 'stress memory') and gut-directed pathogenesis (connected with 'pain and immune memory') in functional gastrointestinal disorders. Bi-directional communication along the brain-gut axis and processes modulating responsiveness to stressors and pain response depend not only on neural pathways, but also immunological and endocrinological mechanisms [4].

Stressors used in animals studies are conventionally categorized into exteroceptive (psychological or neurogenic) and interoceptive (physical or systemic) classes [3, 5]. The endocrine and autonomic responses to exteroceptive stressors are dependent upon the limbic-sensitive neural network including the cortex (lateral, medial pre-frontal, ventromedial, and infragenua cingulate), bed nucleus of the stria terminalis, lateral septum, hippocampus, amygdala, hypothalamus (mainly paraventricular nucleus) and periaqueductal gray. Interoceptive stressors in contrast are considered limbic-insensitive as the cognitive processing is bypassed and sensory visceral input is received by brainstem/pontine nuclei such as the lateral parabrachial nucleus, nucleus tractus solitarius, brainstem/pontine catecholaminergic neurons in the ventrolateral medulla and the locus coeruleus. Two types of visceral pain responses have been described in rodents with exteroceptive stressors models: visceral hyperalgesia and visceral analgesia [5]. In contrast, interoceptive stressors have been most consistently associated with the development of stress-induced hyperalgesia [5]. Detailed characteristics of experimental stress models in visceral pain studies have been presented extensively in our recent reviews [3, 5].

Psychological Stressors in Rodent Models

The two main acute stressors used in experimental models of visceral pain studies are water avoidance stress (WAS) and partial restraint stress (PRS). To induce WAS, rodents are placed on a small platform surrounded by water for 1 h [6]. The level of psychological stress is more intense in PRS, in which forelimbs of rats are taped for 2 h in order to prevent their movements [7]. Using a classical electromyographic (EMG) method to monitor visceromotor response (VMR) to colorectal distension (CRD), exposure of male Wistar rats to WAS for 1 h leads to the development of a delayed visceral hyperalgesia to CRD appearing 24 h after the end of the stress [8], while exposure to PRS induces an immediate hyperalgesia to CRD in male and female Wistar rats [7].

Chronic models of stress in rodents are also divided into two categories. The first category consists of exposing animals repeatedly (over a few days to weeks) but intermittently (once or twice per day) to one or different stressors (e.g. confrontation with a predator or aggressive conspecific animals) with the aim of mimicking the daily exposure to psychosocial stress that humans can encounter through their personal and professional interactions. The second category consists of sustained exposure to stress as for instance

single housing which mimics the effect of isolation in humans. In particular, repeated intermittent exposure to WAS is one of the first ‘chronic’ stress models that has been adapted to the study of visceral hypersensitivity and is presently widely used [9]. Due to potential habituation occurring in response to homotypic stressor exposure, heterotypic stress models using different and alternating types of stressors have been also developed including a combination of cold restraint stress, WAS or forced swimming (one stressor per day for 9 consecutive days) [10].

An experimental stress model commonly used to mimic an early stress/childhood trauma is the neonatal maternal separation in rodents. This is achieved by isolating pups from the dam for 2–3 h/day during the first 2 weeks after birth from postnatal day (PND) 1–2 to PND 14 [11]. At adulthood, rats previously subjected to neonatal maternal separation exhibit visceral hypersensitivity to CRD under basal conditions which is further exacerbated by exposure to the acute psychological stressor in the form of WAS [11].

In these models, strain- and sex-dependent differences in anxiety/depression backgrounds associated with the influence of genes may significantly affect the vulnerability of rodents to exhibit visceral hypersensitivity [3].

Monitoring Visceral Pain Response

Colorectal distension applied at nociceptive range (40–80 mm Hg) in rodents results in autonomic and behavioral pseudoaffective reflexes (changes in arterial pressure and heart rate, passive avoidance behaviors, and contraction of abdominal musculature). Monitoring contractions of abdominal muscles or VMR is the most commonly used index to assess visceral pain in rats and mice [12]. In conscious animals, it can be directly recorded from EMG signals via electrodes surgically-implanted into the external or internal abdominal muscle, and externalized through the skin or connected to radiotelemetric implants into the abdominal cavity [3]. Although there are no data in the literature to ascertain the impact of chronic EMG electrodes placed into the abdominal wall, such intervention could induce a host-tissue response with local inflammation and infiltration by neutrophils, lymphocytes and macrophages as it has been shown for other types of implants in the skin and peritoneum [5]. We have recently developed an alternative noninvasive solid-state manometric method to study visceral sensitivity to CRD in conscious rodents, using a commercially available miniaturized pressure catheter to record intraluminal colonic pressure (ICP) directly in the colonic lumen and not in the balloon inserted into the distal colon [13]. In mice experiments, a PE50 catheter was taped 2 cm below the pressure sensor of a miniaturized pressure transducer catheter (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston, Tex, USA). A custommade balloon (1 cm width, 2 cm length) prepared from a polyethylene plastic bag was tied over the catheter at 1 cm below the pressure sensor with silk 4.0. Ligation points were covered with parafilm to prevent any air leak [13]. A similar probe was used in rats, but in this case the catheter was taped 3.5 cm below the pressure sensor and the size of the balloon was larger (2 cm width, 5 cm length). Each balloon was connected to the barostat and the miniaturized pressure transducer to a preamplifier (model 600; Millar Instruments, Houston, Tex., USA) [14]. To validate the technique, we monitored the VMR to graded phasic CRD by simultaneously recording ICP

and EMG signals in the same mice chronically implanted with electrodes and found an excellent correlation between signals from ICP and EMG during consecutive ascending phasic distensions between 15 and 60 mm Hg [13]. We also showed that the colonic pain pressure threshold to CRD detected by both methods was similar (about 32 mm Hg) [13]. As colonic pressure could be altered following abdominal contractions and/or contractions of the colonic wall proper, we assessed the effects of atropine, a muscarinic blocker known to inhibit colonic motility in mice [15], on the VMR to CRD monitored by ICP in naïve mice. We found that atropine did not significantly modify the phasic CRD-associated ICP changes, while inhibiting distal colonic motility measured by ICP changes in conscious mice maintained under similar recording conditions [13, 15]. In addition, with the use of the noninvasive method, it has been confirmed that buprenorphine, a partial agonist for mu-opioid receptors, inhibits visceral sensitivity to graded phasic ascending CRD which is consistent with opioid-induced reduction of basal visceral response to CRD in both rats and mice [13]. Mice that had undergone surgery for the placement of EMG electrodes and were subsequently single-housed to avoid deterioration of the implanted electrodes by cage mates, developed visceral hyperalgesia to CRD in response to repeated WAS (1 h/day for 10 consecutive days). By contrast, mice tested for visceral pain to CRD using the noninvasive solid-state ICP recording, which were naïve and kept group housed, developed a strong visceral analgesia under otherwise similar conditions of repeated intermittent WAS [13].

Likewise, in rats surgically equipped for EMG monitoring of the VMR to CRD as classically performed, this combined stress paradigm (surgery, postsurgical housing and repeated WAS 1 h daily for 10 days) induces visceral hypersensitivity in 82–86% of the animals starting 24 h after the first exposure, which is maintained up to 40 days after the last stress session [8, 16]. In other studies paw incision has been associated with long-lasting enhanced visceral hyperalgesia to CRD in male rats monitored by EMG recording [17]. By contrast in naïve animals, with the use of a novel noninvasive manometric method of visceral sensitivity monitoring to CRD that bypassed the surgery, the majority of rats (66.7–85.7%) exposed to repeated WAS exhibited a consistent visceral analgesic response [14].

These data demonstrate that the state of the animal tested (naïve vs. exposed to surgery), its social environment (group housing vs. single housing), the methods used to record visceromotor responses (EMG requiring surgery and antibiotic after surgery vs manometry not requiring surgery/antibiotic) can significantly affect the analgesic response to exteroceptive stressors.

Mechanisms of Stress-Induced Visceral Hypersensitivity

Stress-related visceral hypersensitivity is primarily mediated by the activation of brain corticotropin-releasing factor (CRF)/CRF₁ receptor signaling pathways. Acute exposure to WAS is a well-characterized psychological stressor that induces transcription of the CRF gene in the paraventricular nucleus of the hypothalamus and consequently activates the hypothalamic-pituitary-adrenal (HPA) axis as the efferent endocrine response [18]. In addition, CRF acts a neurotransmitter in specific brain area to induce CRF₁ receptor-mediated stimulation of colonic motor function assessed at the end of the first hour of WAS [6]. An equally important contribution in the pathophysiology of visceral hypersensitivity

has also been assigned to the peripheral CRF₁ signaling within the gut. Using the noninvasive ICP monitoring, a hyperalgesic response in Wistar rats can be induced by a peripheral injection of the selective CRF₁ receptor agonist, cortagine, known to act directly on CRF₁ receptor subtype expressed in the colon [19]. The role of peripheral CRF signaling in the modulation of stress-induced visceral sensitivity is also associated with an increase in paracellular and transcellular permeability in the colon and mast cell activation. The effect of both acute (restraint, WAS) and chronic stress (WAS 4–10 days, maternal separation) related to an increase in colonic permeability can be abolished by pretreatment of rat with the peripheral administration of the nonselective CRF antagonist, astressin or selective CRF₁ antagonists [20]. In a recent study, it has been demonstrated that the mast cell-dependent visceral hypersensitivity observed in maternally separated rats after an acute exposure to a psychological stress can be prevented but not reversed by the peripherally restricted CRF receptor antagonist, α -helical CRF_{9–41} [21]. Furthermore, the preventive effect of the CRF receptor antagonist was linked to the stabilization of mast cells and maintenance of the epithelial barrier at the colonic level [21].

Interestingly, it has been also shown in the somatic pain field that in animals sensitized by previous pain experience (e.g. surgery, inflammation) opioids can contribute to stress-related hyperalgesia involving opioid-induced NMDA-dependent pronociceptive systems [22].

Sexual Dimorphism in Mechanisms of Stress-Induced Visceral Analgesia

The analgesic response observed in response to psychological stress is likely to recruit stress-related brain inhibitory mechanisms. In the field of somatic pain, the underlying mechanisms of stress-induced analgesia can be mediated by opioid-dependent or opioid-independent inhibitory systems differently activated according to the type of stressors and their parameters (e.g. duration, intensity) [1, 2]. Our recent results suggest that exposure to acute or repeated mild psychological stress in the form of 1 h WAS alters visceral sensitivity to CRD in a time and sex-dependent manner [23]. Immediately after exposure to 1 or 4 sessions of WAS, both male and female rats exhibited visceral analgesia that was more robust and largely naloxone-sensitive in females-but naloxone-independent in male rats. Twenty four hours after the last session of repeated WAS, females, but not males, exhibited a naloxone-dependent visceral hyperalgesia, revealing sex differences in stress-induced immediate and delayed alterations of visceral sensitivity, which appear to be mediated by the endogenous opioidergic system [23]. A common feature of many environmental changes that induces antinociceptive response is that they are aversive or fearful [1]. Therefore, it can be speculated that females engage more antinociceptive circuits due to greater fearfulness/anxiety to WAS exposure than males. Recent evidence has confirmed that the locus coeruleus arousal system is sexually dimorphic at the molecular and neuronal structural levels with females compared to males displaying increased dendritic structures allowing for increased receipt and processing of limbic information which is linked with the CRF signaling system [24]. Therefore, the environmental stress-related higher activation of the locus coeruleus in females than males may enhance the recruitment of descending noradrenergic analgesic pathways [1]. A number of neuromodulatory mechanisms have been associated with the mediation of nonopioid stress-induced somatic analgesia including adrenergic, serotonergic, or cannabinoidergic systems, as well as other neurotransmitters [1].

Regarding stress-induced visceral analgesia, only one study has shown a nonopioid, neurotensin-mediated analgesia in rodents in response to a combined forced swimming in cold water and WAS [25].

Modulation of Stress Influence by Probiotics

We have recently shown that psychological stress-induced visceral analgesia in rats is enhanced by prebiotics. The reduction in cecal organic acids (i.e. isobutyrate and total butyrate) associated with prebiotic diet was correlated with lower visceral pain response [14].

Stress-related alterations of gut function can induce secondary changes in intestinal microbiota composition via changes in gastrointestinal motility, secretion, and intestinal permeability [26]. Prebiotics alter the composition and balance of microflora, both in the colonic lumen and on the mucosal surface, among them beneficial bacteria such as *Bifidobacteria* and *Lactobacilli* become of great importance. These microbiota can enhance visceral analgesia by preventing stress-induced subtle immune and structural histological changes in the colonic mucosa [14]. However, the interaction within the brain-gut-microbiota axis exceeds far beyond the modulation of immune and barrier function, as it affects also gut sensory-motor function and even stress-related behavioral changes (e.g. *Bifidobacterium longum* influences hippocampal brain-derived neurotrophic factor mRNA expression) [27]. Furthermore, colonization with common commensals can modify the expression of a variety of genes, and can act as a potential epigenetic factor programming the HPA axis response to stress during the postnatal period. Experimental studies showed that early developmental trauma possibly associated with dysbiosis decreases glucocorticoid receptor expression by hypermethylation of a key regulatory component and consequently affects the feedback regulation the HPA axis with impact on the endocrine/behavioral adaptation and the susceptibility to stress-related disorders [28]. Probiotics may reverse stress-induced abnormalities on the intestinal barrier thereby reducing uptake of luminal factors that sensitize sensory afferents [26]. Moreover, spinal modulation may be secondary to a direct effect of microbiota on the enteric nervous system and normalization in sensory neurotransmitters in the myenteric and submucosal plexus [26]. Communication from enteric microflora may also occur via receptor-mediated signaling as *Lactobacilli* have also been found to exert antinociceptive effects on stress-induced visceral hypersensitivity in rodents by increasing the expression of μ -opioid and cannabinoid receptors in intestinal epithelial cells [26].

Clinical Significance: Implications in Irritable Bowel Syndrome

A predominant role of stress in the pathophysiology, symptoms presentation and treatment outcome in functional gastrointestinal disorders such as IBS has been well documented [29]. Psychosocial stressors activate stress circuits within the emotional motor system and induce neuroendocrine response (CRF, cortisol) and autonomic response (norepinephrine and epinephrine) that result in modulation of gut sensory, motor and immune function as well as intestinal permeability typically resulting in visceral hypersensitivity. Only recently the role of alterations in descending pain modulatory pathways in the IBS pathophysiology of IBS

has been recognized [3, 30]. A deficit in stress-related pain inhibition in IBS may lay at the origin of both visceral and somatic hypersensitivity. Female predominance in IBS patients and significant differences in treatment effectiveness between male and female patients emphasize a key role of sex-dependent differences in stress-induced alterations of visceral sensitivity. Recent developments showing critical interactions within the brain-gut-enteric microbiota axis and the interdependence between the composition and stability of microbiota and gut sensorimotor function indicate a novel approach to IBS treatment with a use of probiotics, prebiotics, and targeted antibiotics.

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