

Stress-induced hyperalgesia: animal models and putative mechanisms

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1. ABSTRACT

Stress has been shown to affect brain activity and promote long-term changes in multiple neural systems. A variety of environmental and/or stressful stimuli have been shown to produce analgesia, a phenomenon often referred to as stress-induced analgesia (SIA). However, acute and chronic stresses also produce hyperalgesia in various behavioral tests. There are now several animal models in which stress enhances nociceptive responses. The dysfunction of the hypothalamo-pituitary-adrenocortical axis (HPA axis) and multiple neurotransmitter systems in the central nervous system (CNS), including endogenous opioid, serotonergic and noradrenergic systems, has been reported. These stress-induced hyperalgesia models may contribute to a better understanding of chronic pain and provide a more rational basis for drug therapies in a variety of pain syndromes.

2. INTRODUCTION

Physiological pain is an essential early warning device that alerts us to the damaging stimuli in the environment. This is the pain we experience, for example, if we touch a heated steel plate. Physiological pain is initiated by specialized sensory fibers innervating peripheral tissues that are activated only by noxious stimuli. The sensory inflow generated by these nociceptors activates neurons in the spinal cord which project to the cortex via a relay in the thalamus, eliciting pain (1). While physiological pain is necessary for survival, pathological pain following inflammation and/or nervous system lesions, which often persists and passes into a chronic state, is annoying and reduces the quality of life. They are characterized by hypersensitivity at the site of damage and in the adjacent normal tissue. Allodynia is a condition in which stimuli that are normally not painful (movement,

light touch) become painful. Examples of allodynia include the pain produced by touching sunburned skin or by the movement of an arthritic joint. Hyperalgesia is exacerbated pain produced by a noxious stimulus (2). The following two mechanisms contribute to hypersensitivity in pathological pain. Peripheral and central sensitizations represent an enhanced responsiveness and reduced threshold to stimulation observed in peripheral and central sensory neurons, respectively. Furthermore, pain is a complex experience that involves not only the transduction of noxious environmental stimuli, but also cognitive and emotional processing by the brain (3).

Stress has been demonstrated to induce a nonspecific stereotyped response to the body, characterized by adrenal enlargement, gastrointestinal bleeding and dysfunction of immune system, regardless of its type. This response is called a general adaptation syndrome (4). However, the perception of the individual is an important determinant in the occurrence and characteristics of the stress response. Stress consists of three elements, stimulus input, the central processing system and the response output (5). The expression of Fos, the protein product of an immediate early gene (IEG), *c-fos*, has been widely used as a marker of neural activation. The change in the expression of IEGs induced by various stimuli may contribute to the establishment of long-term functional changes in the central nervous system (CNS) (6, 7). Previously, we demonstrated that stress induces Fos expression in several brain regions (8, 9). Some of the expression areas are common, but others are different among the different types of stressors. The expression patterns of Fos in immobilization and conditioned fear are similar to that induced by noxious stimulation, indicating that these stimuli provoke similar cognitive and emotional processing in the brain. It is therefore possible that emotional stress may influence the central pain mechanisms.

In animals, various stressors, including electrical shock, restraint, swimming and rotation, have been shown to elicit analgesia. Some of these stressors are noxious and inescapable. In these situations, analgesia would appear to be the most appropriate response for adaptation (10). Thus, it is well known that acute stress produces antinociception, whereas several studies have demonstrated that stress can induce hyperalgesia under some experimental conditions. Whether a stressor induces analgesia or hyperalgesia depends on the type of stressor used and on the method selected to measure the pain threshold (10). Even the same stressor may produce analgesia or hyperalgesia due to the difference in its intensity and duration. In the 1980s, acute exposure to emotionally arousing non-noxious stress, such as inescapable holding, novel environments or vibration, was shown to produce an immediate and transient hyperalgesia (10-13). Then, mainly after 1990, it has been demonstrated that chronic stress induced by repeated exposure to cold environment (14-18), restraint (19-22) and forced swim (23,24) produces hyperalgesia. Chronic stress-induced hyperalgesia usually lasts longer than that induced by acute stress and seems to mimic the human chronic pain condition. In these animal models, it has been revealed that the HPA axis and a variety of neurotransmitter systems in

the CNS are involved in stress-induced hyperalgesia. The peripheral mechanisms, including dysfunction of the peripheral nervous system (PNS) and immune system, are also responsible for the enhanced nociception. Even if a certain neurotransmitter system is activated in one animal model, it may not be activated in the other models. In this review, first, we introduce animal models showing stress-induced hyperalgesia. Second, we discuss the mechanisms underlying stress-induced hyperalgesia in each of these models.

3. STRESS POTENTIATES A NOCICEPTIVE RESPONSE IN HUMANS

There is anecdotal evidence for stress-induced analgesia in humans. Soldiers wounded in battle and athletes injured in games report that they feel less pain. In behavioral demands prompted by exposure to stressful situations, animals' normal response to pain could put them at a disadvantage. During stressful conditions, pain perception and reactions to pain can be suppressed in favor of adaptive behavior. Thus, psychological factors have a wide-ranging effect on the perception of pain and its effects. In humans, anxiety/fear induced by expectation of an aversive event has also been related to analgesic effects. However, other types of anxiety, generally occurring in the absence of knowledge regarding a forthcoming event, produce an overestimation of painful stimuli. In this case, a high degree of arousal and a hypersensitivity to sensory stimuli, including noxious stimuli, may appear more beneficial to the organism in the elaboration of behavior (10). The patients with neurasthenia, which is a natural "model" of chronic psychoemotional stress, showed a reduction in the pain threshold compared to the group of healthy men (25). Pain sensitivity is increased in the patients with either minor or major depression and these patients have a higher prevalence of clinical pain problems (23, 26). Stimulation of the CNS by psychological stress sensitizes the perception of a painful peripheral visceral stimulus. During the psychological stress, volume thresholds inducing the sensation of the urge to defecate were lower than those during the basal condition (27). Fibromyalgia is a long-standing multifocal pain condition combined with generalized allodynia / hyperalgesia. This syndrome has been considered a stress-related disorder due to the exacerbation of symptoms in stressful events (28,29).

Pain is a kind of stressor and chronic pain, as well as chronic stress, is frequently associated with depression. It has been reported that the prevalence of major depression in patients with chronic low back pain was 3-4 times higher than in the general population (30). Depression itself appears to intensify pain. Thus, the modification of pain induced by depression may result in a complex symptom and resistance to treatments. Depression is often accompanied by an overactivity of the HPA axis and by dysfunction of serotonergic and noradrenergic systems (31). Corticotropin-releasing factor (CRF) has been reported to enhance the abdominal pain and anxiety in patients with irritable bowel syndrome (IBS) (32). It was speculated that a stress-related reduction of dopaminergic tone within the nucleus accumbens contributes to the

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development of hyperalgesia in fibromyalgia (29). However, the mechanism by which chronic stress exerts hyperalgesic effects still remains unknown.

4. ANIMAL MODELS AND PUTATIVE MECHANISMS OF HYPERALGESIA

4.1. Acute stress-induced hyperalgesia

Previous studies demonstrated that brief exposure to emotionally arousing non-noxious stress, such as inescapable holding, novel environments or vibration, produces an immediate and transient hyperalgesia (10-13). In these experiments, the stress procedures were as follows: 1) "holding", rats were held by the nape of the neck for 5 min, allowing some movement of the head and limbs but impeding escape. 2) "novelty", rats were placed for 5 min in a novel environment (observation box). 3) "vibration", rats were restrained in the tail flick tube and were placed on a plate vibrating at 4 Hz for 15 min. A clear-cut and statistically significant hyperalgesia was observed 5 min after the exposure to inescapable holding or a novel environment, attenuated during 10 min (10,11). In the case of vibration, it was only observed for 1 min, immediately after the termination of the stress procedure (12). The rats exhibited signs of emotional activation, such as agitation, vocalization, defecation, exploration and rearing.

The role of the pituitary in pain regulation is highly controversial. Some studies have shown that hypophysectomy (HX) reduced or abolished SIA, but others have revealed the involvement of the pituitary in the hyperalgesic effects (33). It has been reported that HX may induce analgesia in patients suffering from cancer pain (34). HX potentiated the inescapable holding-induced hyperalgesia, but attenuated the novelty-induced hyperalgesia. As suggested by Vidal et al. (33), factors produced in the anterior lobe of pituitary may counteract inescapable holding-induced hyperalgesia. The intravenous (i.v.) injection of beta-endorphin induced analgesia (35). In contrast, other hypophyseal factors may be involved in novelty-induced hyperalgesia. Alpha-melanocyte stimulating hormone (MSH) antagonized the analgesic effect of beta-endorphin (36). Gamma2-MSH activates the sensory neuron-specific G protein coupled receptors (SNSRs) in small diameter dorsal root ganglion neurons, leading to hyperalgesia (37). Although not described in earlier studies (10,11,33), HX affects the expression of many neuropeptides in the hypothalamus (38). HX increases the levels of plasma and hypothalamic CRF (39, 40). Oxytocin, a neuropeptide related to the SIA, was initially decreased and then increased in the hypothalamus after HX (38, 40, 41). HX also induces a transient increase of galanin, cholecystokinin and dynorphin in the hypothalamus (38). Arginine vasopressin (AVP) is increased in the hypothalamus after HX (40). It has been reported that the plasma AVP was significantly higher in patients with chronic pain disorder (42). It is possible that the compensatory change in the CNS after HX may be responsible for the modification of stress-induced hyperalgesia.

Diazepam, an anxiolytic and positive modulator of GABA_A receptor, blocked novelty-induced hyperalgesia, but did not affect inescapable holding-induced hyperalgesia. Furthermore, a novel environment resulted in

hyperthermia, characteristic of emotional stress, which was reduced by diazepam (10). Diazepam also blocked vibration-induced hyperalgesia (13). Thus, the effect of anxiolytic on the emotional hyperalgesia depends on the types of stressors.

In addition, clonidine was shown to block vibration-induced hyperalgesia (13). Since clonidine, an alpha2-adrenoceptor agonist, inhibits the synaptic release of noradrenaline and has been shown to possess sedative effects on humans and animals, it has been speculated that the noradrenergic system is involved in this anxiety-related hyperalgesia (13).

4.2. Chronic stress-induced hyperalgesia

4.2.1. Repeated cold environment stress-induced hyperalgesia

When repeatedly exposed to cold environment, rodents show a facilitated response to noxious stimuli. This stress paradigm is called repeated cold stress (RCS) or specific alteration of rhythm in environmental temperature (SART) stress (14-18, 43-45). In most of these studies, rodents were exposed to a cold environment (4 or -3 degrees C.) from 16.30h to 10.00h and then alternately to room temperature (24 degrees C.) and cold temperature (4 or -3 degrees C.) at 30-min intervals from 10.30h to 16.00h. The RCS was started at 16.30h on day 0, applied for 2 days, and stopped at 10.00h on day 3. From the evening of day 3 until the morning of day 5, the rodents were exposed to cold temperatures only at night (between 16.30h to 10.00h). The exposure of rodents to RCS gradually decreased the nociceptive mechanical threshold over 2 days. The threshold on day 2 was $75.5 \pm 3.9\%$ of the pre-RCS level ($p < 0.05$). Thereafter, the threshold was kept in a decreased state for 3 days and recovered over 4 days after the last exposure to cold stress (16).

Intrathecal injections of antibody to substance P (SP) or calcitonin gene-related peptide (CGRP) have been demonstrated to inhibit RCS-induced hyperalgesia (16). And intrathecal injections of NK-1 antagonist, NMDA antagonist and non-NMDA antagonist suppressed the RCS-induced hyperalgesia (17). Furthermore, it has been suggested that RCS may increase the sensitivity of NMDA and AMPA receptors in the spinal cord and that RCS may facilitate the release of glutamate from primary afferent terminals in the spinal dorsal horn (18, 45). Thus, the enhancement of neurotransmission in the spinal cord may be one of the mechanisms underlying RCS-induced hyperalgesia.

RCS causes functional changes in the central opioid receptors. It has been demonstrated that RCS mice were hyposensitive to supraspinal mu-opioid receptor-mediated antinociception, whereas their antinociceptive activities through kappa-opioid receptors in the spinal cord were increased (15). The decrease in the antinociceptive activity of mu-opioid receptor agonist was inhibited by diazepam, an anxiolytic drug, while the enhancement of antinociceptive activity of kappa-opioid receptor agonist was not altered. Therefore, the hypofunction of supraspinal mu-opioid receptors due to anxiety may be involved in RCS-induced hyperalgesia.

SART-stress causes anxiety-like behavior (46-50). SART-stressed mice exhibited a reduction of immobility time in the forced swimming test and of time spent on the open arms of the plus-maze apparatus. The former was normalized by anxiolytic agents, diazepam, and by an antidepressant, imipramine (49). The latter was also normalized by diazepam, and by 5HT_{1A} receptor agonist, buspirone (47). Recently, it has been reported that the intracerebroventricular (i.c.v.) administration of alpha-helical CRF, a specific CRF receptor antagonist, increased the immobility time in the forced swimming test and the time spent in the open arms in SART-stressed mice (46). These data suggest that CRF plays an important role in the anxiety-like behavior caused by SART stress. The forced swimming test is a well-known screening model for antidepressants, developed by Porsolt et al. (51). At first, the immobility observed in the forced swimming test was considered as a state of “despair” in the rodent. However, a question has arisen regarding its specificity for predicting antidepressant activity, because not only antidepressants, but also many other drugs have been equally effective in the test (46). Moreover, it has been revealed that anxiolytics increase the duration of immobility time, while anxiogenic agents reduced the enhancement (50). These lines of evidence suggest that the reduction of immobility may be related to fear and/or anxiety. In the open-field test, SART-stressed rats exhibit increases in locomotor activity, rearing and defecation (44). Coincident with this, it has been reported that acute stress (15 min of vibration) produced analgesia in some rats, but hyperalgesia in others, in which analgesia was produced in quiet rats and hyperalgesia in hyperemotional rats. This vibration-induced hyperalgesia was also inhibited by diazepam (12, 13).

SART stress significantly decreased the escape latency from footshock, while the excitability of C-fiber activity, responding to mechanical and thermal stimuli, was not changed (14). It has been known that lesions of amygdala block both the hypoalgesia and fear-related behavior normally observed following footshock (52). Anatomically, the amygdala receives somatosensory information from the parabrachial nucleus and thalamus (53, 54). Inversely, the amygdala sends its fibers to the periaqueductal gray, which plays an important role in emotional behavior (55). An increase in noradrenaline levels in the hippocampus was reported in SART stressed animals (56). The microinjection of noradrenaline into the hippocampus potentiated the reaction to pain and significantly increased aversive properties of electrical shock (57). The limbic structures, the amygdala and hippocampus, may also play an important role in SART stress-induced hyperalgesia.

The autonomic nervous system is involved in SART stress-induced hyperalgesia. SART stress reduced the nociceptive threshold in vagotomized mice, but failed to bring about change in sympathectomized mice (43). Thus, SART stress-induced hyperalgesia seems to be associated with dysfunction in the sympathetic nervous system.

The hyperalgesia in SART mice was suppressed by the systemic administration of 5HTP, a precursor of 5HT, by L-DOPA, a precursor of catecholamine and by muscimol, a GABA_A receptor agonist (58). It has also been reported that the levels of both 5HT and 5HIAA in the brain are decreased in SART stressed rats (59). SART stress seemed to induce the dysfunction of serotonergic, noradrenergic and GABAergic systems. Since these agents were systemically administered (58), the sites where the agents exert their actions remain unknown. One of the brain areas consistently activated by painful stimuli is the rostral agranular insular cortex (RAIC). Recently, it has been demonstrated that the inhibition of RAIC neurons via GABA_A-receptors produced analgesia through the activation of noradrenergic neurons in the locus coeruleus (LC), projecting to the spinal cord (60). The level of GABA in the RAIC may be decreased in SART stressed rats.

SART stressed animals have been reported to induce several physiological symptoms, such as continuous hypotension, decreased blood flow, thymic and splenic atrophy and decreased mature T and B lymphocytes in the spleen (14, 61, 62). As immune conditions and blood pressure also influence the pain threshold (63, 64), the broad and perspective studies focusing not only on the nervous system, but also on many other systems, will be needed to clarify the mechanisms underlying SART stress-induced hyperalgesia.

4.2.2. Repeated restraint stress-induced hyperalgesia

Chronic restraint stress induces long-lasting hyperalgesia (19-21). In these studies, the animals were stressed by restraint for 1h daily, 5 days per week for 40 days. Restraint was carried out by placing each animal in a 25 x 7 cm plastic tube, and adjusting it with plaster tape on the outside. This procedure induces hyperalgesia in male rats, but not in female rats (19). The hyperalgesic effect in the tail flick latency (TFL) was observed immediately after the last restraint session and lasted for at least 28 days after chronic stress interruption (20).

Adenosine is the main neuromodulator in the CNS. Extracellular adenosine is released through the adenosine transporter or originates from the extracellular catabolism of released ATP through the ecto-nucleotidase pathway (65, 66). It has been proposed that extracellular adenosine is involved in physiological pain control at the spinal cord level and in opioid antinociception (67). Repeated restraint stress reduced ADP hydrolysis and increased 5'-nucleotidase activity in the spinal cord (68). The antinociceptive effect of adenosine A₁ receptor agonist was not observed in the repeatedly restrained rats, suggesting the decreased effectiveness of adenosine A₁ receptors or the augmentation of extracellular adenosine that saturate adenosine A₁ receptors in these rats (21). Repeated restraint stress induces hyperalgesia in male rats, but not in female rats. Estradiol restored the repeated restraint stress-induced reduction of AMP hydrolysis in the spinal cord in OVX rats. However, this replacement did not restore the hyperalgesic responses in those rats. The alteration in the cascade of ATP hydrolysis in the spinal cord does not seem to be a sufficient explanation for the

sex difference in the nociceptive response seen in rats subjected to repeated restraint stress (69).

Although the control rats presented novelty-induced antinociception, which has been attributed to opioid activation, repeatedly restrained rats showed no significant difference between the pre- and post-novelty tail flick latencies (TFL) (70). The repeatedly restrained rats displayed decreased morphine effects on nociception, compared to the unstressed controls (20). The density of opioid receptors in the repeatedly restrained rats decreased significantly in the CNS structures, such as the spinal cord, frontal cortex and hippocampus (71). In addition, the repeated restraint stress has been shown to increase glutamate uptake and release, and to induce oxidative damage in the hippocampus, one of the limbic structures that mediate nociceptive behaviors (72, 73).

We have also shown that the chronic restraint stress (2-3 weeks) induced thermal hyperalgesia in rats (22). Restraint was carried out by wrapping each animal with soft wire mesh and adhesive tape for 6h daily. This chronic stress model has been reported by McEwen's group (74). Indeed, rather extended restraint is used as a stressor and such a restraint is repeated daily for as long as 3 weeks in this model. This stress model is well established and numerous experimental data have been accumulated. The influences of chronic stress on the nervous system, such as the dendritic atrophy of hippocampal CA3 neurons and impaired memory and learning behavior, have been demonstrated and discussed in this stress model.

The rostral ventromedial medulla (RVM), including the nucleus raphe magnus (NRM) and nucleus reticularis gigantocellularis pars alpha (GiA), and the LC play crucial roles in descending pain modulation system (75-77). Electrical or chemical stimulation of the RVM produces biphasic (facilitatory and inhibitory) modulation of spinal nociceptive transmission (78). The p44 and p42 mitogen-activated protein kinases (MAPK) / extracellular signal-regulated kinases (ERK1 and ERK2) are members of the serine / threonine protein kinases implicated in the transduction of neurotrophic and neurochemical signals from the cell surface to the nucleus (79). It is now well established that MAPK cascades play a crucial role in neuronal functions, including synaptic plasticity, learning and memory (80-82). We have found that repeated restraint stress, but not acute restraint stress, induces a significant increase in phosphorylated ERK (p-ERK) in RVM neurons and its decrease in LC neurons (Figure 1). It was demonstrated that in response to extracellular stimuli, the phosphorylation of MAP kinase, such as ERK and p38, activates transcription of TPH, the rate-limiting enzyme in serotonin biosynthesis, in the serotonergic neuron-like cell line (83). The repeated restraint stress significantly increased the level of TPH in the RVM compared to that in the control rats (22). 5HT released from the descending bulbospinal neurons seem to exert dual effects on spinal nociceptive processing. Several studies (84-87) reported an inhibitory effect of intrathecally administered 5HT on neuronal responses to noxious stimuli, but others (88-

90) reported a facilitatory effect. Oyama et al. (91) suggested the inhibition via 5HT1A receptors and the facilitation via 5HT3 receptors. The descending serotonergic pathways exert the bi-directional control of nociception (77). Moreover, it has been reported that the activated descending serotonergic facilitatory pathways resulted in the central sensitization of deeper dorsal horn neurons via 5HT3 receptors (92). The chronic stress-induced reduction in the activated ERK in LC neurons may underlie the impairment of the noradrenergic descending inhibitory pathway. Thus, the altered p-ERK levels in the RVM and LC may be involved in the modulation of the pain threshold in chronically stressed rats.

4.2.3. Repeated swim stress-induced hyperalgesia.

Repeated swim stress induces a thermal and chemical hyperalgesia (23, 24). The rats were subjected to a forced swim procedure for 10 min by placing them in a plastic cylinder (diameter 30 cm, height 50 cm) containing 24-26 degrees C. water at a depth of 20 cm on day 1 and were subjected to a similar swim stress for 20 min on days 2-3. The hyperalgesia to thermal and chemical stimulants (formalin injection) was still present 8 and 9 days after the last swim session. The nociceptive behavior to the subcutaneous injection of formalin was negatively correlated with the swim effort of struggle times during the last swim session (23). Since the escape behavioral deficit induced by inescapable swim stress is thought to be a surrogate model of human depression (51) and patients with depression have increased pain sensitivity (26), the authors suggest that this model may well reflect the clinical pain problem of patients with depression. However, there is controversy about the deficit in escape behavior in this model, as described above.

It has been suggested that the repeated swim stress-induced hyperalgesia in this model may result from a deficit in the central serotonergic transmission, because the serotonin-selective reuptake inhibitor (SSRI) and serotonin precursor tryptophan blocked the development of both the thermal and chemical hyperalgesia compared to vehicle-treated rats (23). Since the SSRI and tryptophan were systemically administered, the sites where the agents exert their actions remain to be elucidated. Microdialysis studies in rats have shown that the 5-15 min of forced swim increased serotonin release in some brain regions, including the median raphe nucleus, amygdala and striatum. However, the same swim stress decreased serotonin release in other brain regions, such as the ventral hippocampus, medial prefrontal cortex and lateral septum (93, 94). Re-exposure to the same swim stress on the next day produced no effect on extracellular 5HT in the brain, suggesting the rapid adaptation to the effects of repeated swim stress on the response of extracellular 5HT (94). CRF mediates the alteration of 5HT levels in the brain evoked by swim stress and is involved in the mechanism of adaptation (95). It has been reported that SSRI treatment at first inhibits the 5HT transporters in the brain, resulting in increased extracellular

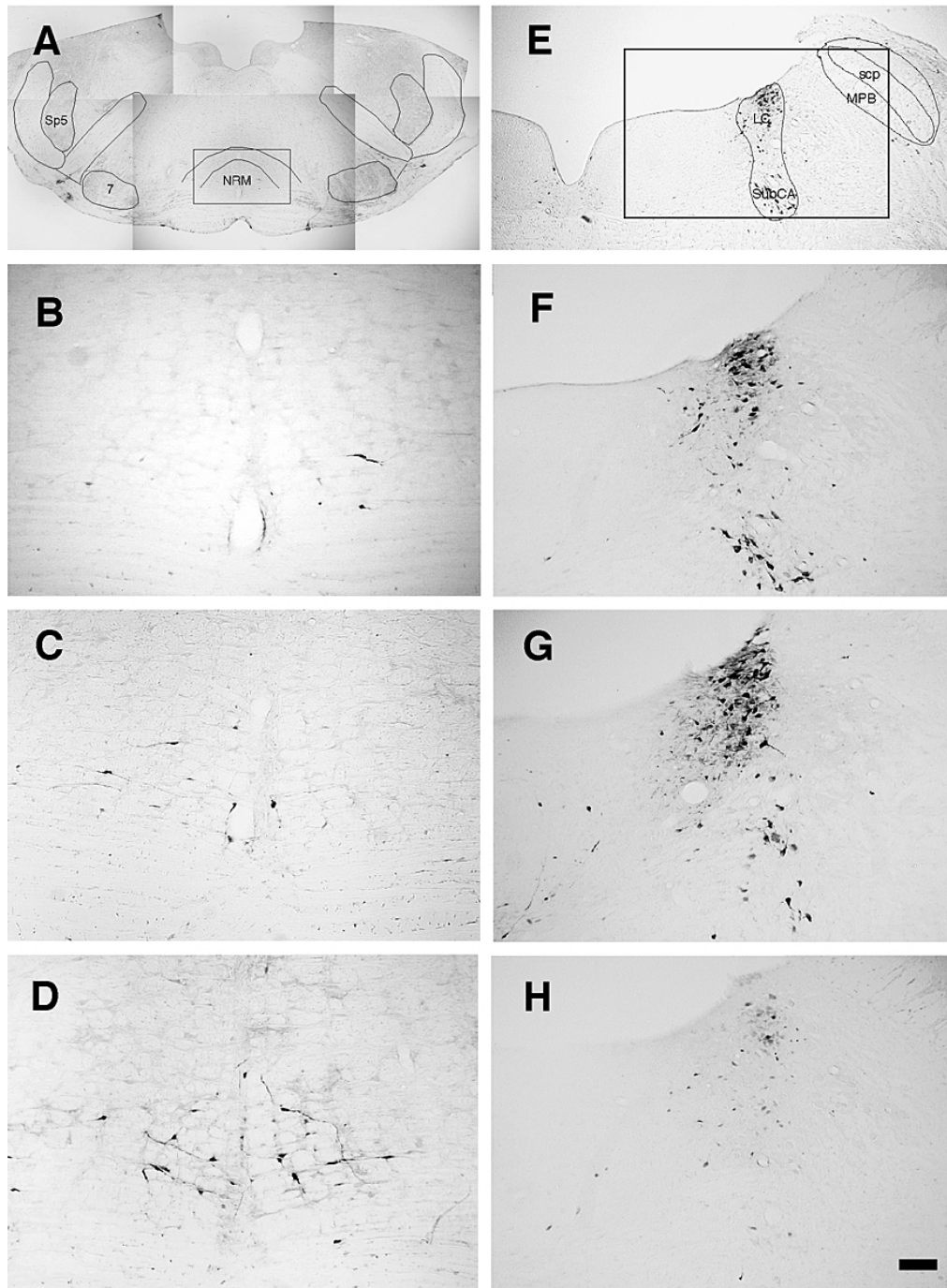


Figure 1. Photomicrographs showing p-ERK-IR in the RVM (Bregma -10.30 mm: A-D) and the LC (Bregma -9.68 mm: E-H) following acute and chronic restraint stress. A,B,E,F: control. C,G: acute restraint (1 day). D,H: chronic restraint (D: 3 weeks, H: 2 weeks). B-D and F-H correspond approximately to the areas outlined in box in A and E, respectively. 7, facial nucleus; LC, locus coeruleus; MPB, medial parabrachial nucleus; NRM, nucleus raphe magnus; scp, superior cerebellar peduncle; Sp5, spinal trigeminal nucleus; SubCA, subcoeruleus nucleus alpha. Scale bar (B-D, F-H) = 100 micro-meter. In the medulla of control and stressed rats, almost all the p-ERK-IR neurons were distributed in the NRM, the GiA and the LC. The number of p-ERK-IR neurons was increased in the NRM after chronic restraint stress, while it was decreased in the LC. There was no change in the number of p-ERK-IR neurons in the NRM and the LC after acute restraint stress. There were few p-ERK-IR neurons in other areas of the medulla. No p-ERK-IR neurons were found in other sensory and motor areas of medulla sections, such as the spinal trigeminal nucleus and facial nucleus. A few p-ERK-IR neurons were scattered in the pontine and medullary reticular formations.

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5HT, while sustained SSRI treatments decrease intracellular levels of central 5HT. However, it is known that following 2 weeks treatment with SSRI, 5HT autoreceptors are desensitized and serotonin synthesis is restored (96). As the effect of swim stress on serotonin release in the brain is regionally-specific and bidirectional, and the effect of SSRI on serotonin biosynthesis seems to depend on the length of the treatment period, further studies are needed to elucidate the functional changes in the central serotonergic system that underlie the repeated swim stress-induced hyperalgesia.

Repeated swim stress produced a significant increase in Fos protein expression in the rat spinal cord following the injection of formalin into the hindpaw (24). It has been suggested that the injection of formalin induced higher activities in dorsal horn neurons in the rats subjected to repeated swim stress than in the control rats. However, the mechanism by which repeated swim stress enhances the activity of dorsal horn neurons (ex. increased afferent input, enhancement of descending pain facilitation, reduction of descending pain inhibition) still remains unknown.

4.3. Stress-induced visceral hyperalgesia

Visceral hyperalgesia is related to an enhanced perception of sensations originating from the gut and is commonly observed in patients with irritable bowel syndrome (IBS). Psychological stress is widely believed to play a major role in the IBS (27, 97). Acute and chronic stresses facilitate visceral sensitivity to colorectal distention in rats (97-100). Partial restraint stress (PRS) was used in these experiments. Rats were lightly anesthetized with diethyl ether, and their foreshoulders, upper forelegs and thoracic trunk were wrapped in a confining harness of paper tape to restrict, but not to prevent, body movement. They were then placed in their home cage for 2h. The rats recovered from diethyl ether anesthesia within 2-3 min and immediately moved about in their cages and ate and drank freely, but the mobility of their forelegs was restricted, thus preventing grooming of the face, upper head and neck. Acute PRS enhanced the response of the abdominal muscle to rectal distension of all the volumes tested (0.4, 0.8, 1.2ml). In contrast, chronic PRS induced a hyperalgesic response only to the higher volume of distension (0.8, 1.2ml) (99).

Various neurotransmitters are involved in visceral nociception (27, 101, 102). Among these neurotransmitters, CRF is reported to play an important role in stress-induced visceral hyperalgesia (103). CRF is a 41-amino-acid hypothalamic peptide that stimulates the synthesis and release of ACTH and beta-endorphin from the pituitary. Three novel mammalian CRF-related peptides, urocortin 1, urocortin 2 and urocortin 3, were added in the CRF family. CRF ligands interact with CRF receptors, subtype1 (CRF1 receptor) and/or subtype 2 (CRF2 receptor) cloned from two distinct genes (103). It has been demonstrated that the i.c.v. injection of CRF induced the same visceral hyperalgesic response as PRS and that the hyperalgesic effect by RCS was blocked by i.c.v. injection of alpha-helical CRF₉₋₄₁ (104). In addition, the selective CRF1 receptor antagonist abolished the stress-

induced visceral hyperalgesia (105). A recent study in humans has also shown that alpha-helical CRF significantly reduced the abdominal pain and anxiety evoked by electrical stimulation in IBS patients (32). It has been reported that distinct areas of the brain can be activated by rectal distension in IBS patients and in controls (106). Several brain structures are involved in the integration of stress and visceral perception. One of these structures is the LC. Norepinephrine (NE) -neurons in the LC respond to non-noxious rectal distension (107). And it has been suggested that CRF released from paraventricular hypothalamic nucleus (PVH) alters the activity of LC neurons (108, 109).

A peripheral effect of stress is responsible, at least partly, for the stress-induced visceral hyperalgesia. PRS increases the release of histamine from mast cells and a mast cell stabilizer, doxantrazole, inhibits PRS-induced visceral hyperalgesia (104). PRS sensitizes mast cells to activate T lymphocytes. INF-gamma released from T lymphocytes induces phosphorylation of the myosin light chain and subsequent contractions of the epithelial cell cytoskeleton, which increases colonic permeability. The uptake of intraluminal bacterial products activates submucosal immune cells. Inflammatory mediators released from the activated submucosal immune cells sensitize sensory terminals to mechanical stimuli (100). CRF acts at distinct central and peripheral sites. Acute exposure to a variety of psychological and physical stressors stimulates colonic motility, which is known to influence the perception of luminal distension (27, 103). Peripheral injection of alpha-helical CRF₉₋₄₁ blocked the colonic mucosal mast cell activation and an increase of motility by restraint stress (103).

It has been shown that stressful life events, including a history of major traumatic events in childhood, are important risk factors for IBS and influence the onset and severity of symptoms (103). In the rat, neonatal maternal separation alters stress-induced response to viscerosomatic nociceptive stimuli (110). Maternal separation (MS) was performed daily for 180 min on postnatal days 2-14. The dams were removed from the maternity cage to separate cages. Then, the litters were removed from the cage and placed in an isolation cage in an adjacent room. These isolation cages were placed in a nursing incubator. At the end of separation period, the pups were returned to their maternity cage, reunited with their dams. At 2 months of age, the MS rats showed an increased visceromotor response to colorectal distension at the baseline. Although water avoidance stress (WAS) has no effect on visceromotor response in control rats, WAS induced visceral hyperalgesia in the MS rats. The procedure of WAS used is as follows: The test apparatus consisted of a Plexiglas tank with a block affixed to the center of the floor. The tank was filled with water (25 degrees C.) to a level 1cm lower than the top of the block. The animals were placed on the block for a period of 1h.

Maternal separation of newborn rats induced permanent changes in the CNS, including hyperactivity of CRF neurons in the hypothalamus (111, 112), a regional

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Table 1. Animal models and putative mechanisms of hyperalgesia

		HPA axis (CRF)	Central Mechanism							Peripheral Mechanism
			Neurotransmitter system							
			Endogenous opioid	GABA	Noradrenaline	Serotonin	Tachykinins /CGRP	Glutamate	Adenosine	
Acute stress-induced hyperalgesia	Inescapable holding -induced hyperalgesia	Hypophysectomy (10) UP		Diazepam s.c. (10) NO						
	Novelty-induced hyperalgesia	Hypophysectomy (10) DOWN		Diazepam s. c. (10) DOWN						
	Vibration-induced hyperalgesia			Diazepam i.p. (13) DOWN	Clonidine i.p. (13) DOWN					
Chronic stress-induced hyperalgesia	Repeated cold environment stress-induced hyperalgesia	<i>alpha-Helical CRF i.c.v. decreases anxiety-like behavior. (46)</i>	<i>Decreased supraspinal mu-opioid receptor-mediated and increased kappa-opioid receptor-mediated antinociception in the spinal cord (15)</i>	Diazepam s.c. (15) DOWN Muscimol s.c. (58) DOWN Diazepam p.o. decreases anxiety-like behavior. (47)	<i>Increased noradrenaline levels in the brain (56)</i> L-DOPA s.c. (58) DOWN	5HTP p.o. (58) DOWN Decreased serotonin levels in the brain (59) Buspirone i.p. decreases anxiety-like behavior. (47)	Anti-SP, Anti-CGRP antibody i.t. (16) DOWN CP-96345 i.t. (17) DOWN	APV i.t. (17) DOWN <i>Increase of the sensitivity of NMDA and AMPA receptors in the spinal cord (18)</i>		<i>Increased glutamate release from primary afferent terminals in the spinal dorsal horn (45)</i> Sympathectomy mimicks SART stress-induced hyperalgesia (43)
	Repeated restraint stress-induced hyperalgesia		<i>Decreased antinociceptive effect of morphine i.p. (20)</i> <i>Decreased opioid receptors in the CNS (71)</i>		<i>Decrease of p-ERK in LC (22)</i>	<i>Increase in p-ERK in RVM (22)</i>			Reduced ADP hydrolysis and increased nucleotidase activity in the spinal cord (68) <i>Decreased antinociceptive effect of CPA i.p. (21)</i>	
	Repeated swim stress-induced hyperalgesia						Clomipramine i.p. or Tryptophan i.p. (23) DOWN			
Stress-induced visceral hyperalgesia	Partial restraint stress-induced visceral hyperalgesia	alpha-helical CRF i.c.v. (104) DOWN					MEN 11420 i.v. (98) DOWN			Doxantrazole i.p. (104) DOWN TAP intracolonicly infusion or ML-7 i.p. (100) DOWN
	Neonatal maternal separation + water avoidance stress- induced visceral hyperalgesia	NBI 35965 s.c. (105) DOWN <i>Decreased reactive feedback in HPA axis (113)</i>								

DOWN, **UP** and **NO** indicate that treatment attenuates (**DOWN**), potentiates (**UP**), does not modify (**NO**) the stress-induced hyperalgesia. *Italics* indicate the reported dysfunctions in the animal model. 5HT1A receptor agonist, buspirone. Clonidine, an α_2 -adrenoceptor agonist. L-DOPA, a precursor of catecholamine. TAP, a tight junction blocker. 5HTP, a precursor of 5HT. CP-96345, NK-1 receptor antagonist. MEN 11420, NK2 receptor antagonist. Tryptophan, serotonin precursor. APV, NMDA receptor antagonist. CPA, adenosine A1 receptors agonist. ML-7, myosin light chain kinase inhibitor. *alpha*-Helical CRF, a specific CRF receptor antagonist. Diazepam, an anxiolytic and positive modulator of GABAA receptor. Muscimol, GABAA receptor agonist. Clomipramine, serotonin-selective reuptake inhibitor (SSRI). Doxantrazole, a mast cell stabilizer. NBI35965, CRF1 receptor antagonist

decrease in the expression of glucocorticoid receptors (113), an increase in regional norepinephrine release (114). Interestingly, the baseline TFL in MS rats was prolonged compared to control rats and MS attenuated the WAS-induced cutaneous analgesia, suggesting the functional change in endogenous pain control system (110). Coincident with this, in the IBS patients without fibromyalgia, visceral hypersensitivity is associated with normal or diminished somatic pain sensitivity to noxious stimuli (115). Psychotropic agents, including tricyclic antidepressants, reduced abdominal pain and bowel symptoms. Antidepressants reduce CRF gene expression in

similar brain sites that elicit anxiety and colonic motor responses in rats (103).

5. CONCLUSION AND PERSPECTIVES

There are now several animal models available in the literature and suitable for examining the mechanisms underlying stress-induced hyperalgesia. These animal models provide some benefits to the study of complex chronic pain. Dysfunctions of the HPA axis and multiple neurotransmitter systems, including endogenous opioids, serotonergic and noradrenergic systems, which may induce

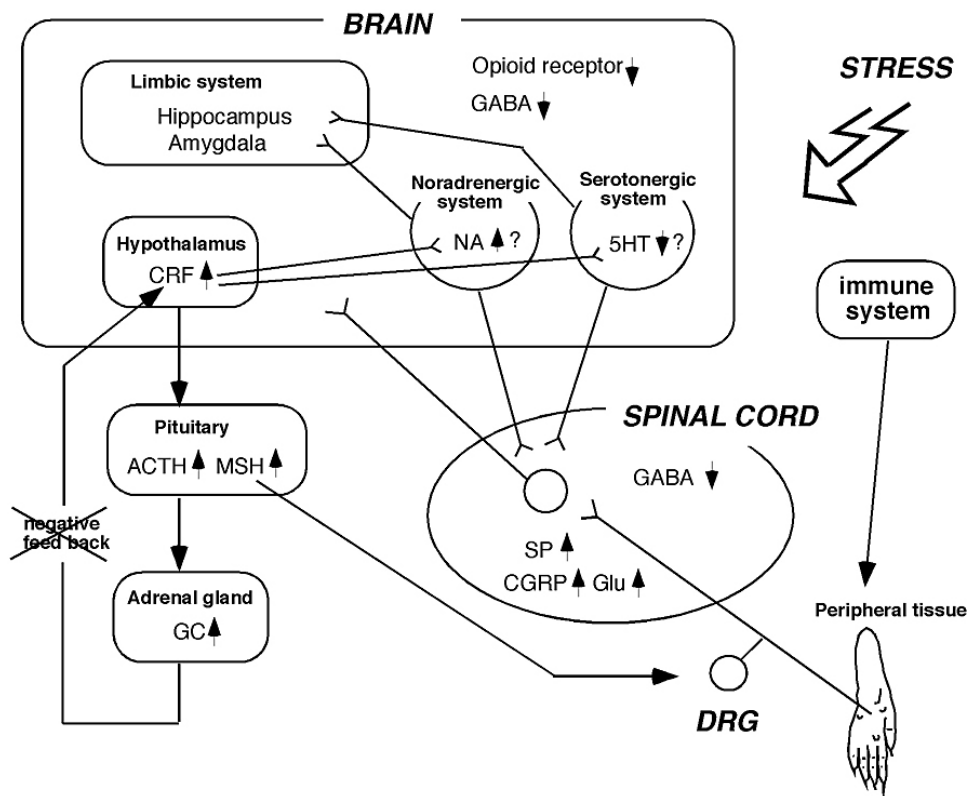


Figure 2. Schematic drawing of the putative mechanism of stress-induced hyperalgesia. CRF, corticotropin releasing factor ; ACTH, adrenocorticotrophic hormone ; MSH, melanocyte-stimulating hormone; GC, glucocorticoid ; GABA, gamma-aminobutyric acid ; NA, noradrenaline ; 5HT, serotonin ; SP, substanceP ; CGRP, calcitonin gene related peptide ; Glu, glutamate ; DRG, dorsal root ganglion. upward arrow, increase ; downward arrow, decrease ; ? site-specific change and ambiguous.

hyperalgesia, have been revealed in these models (Figure 2, Table 1).

It has been demonstrated that a neuropeptide, nociceptin, reverses stress-induced analgesia after central administration. Nociceptin-deficient mice were demonstrated to show impaired adaptation to stress (116, 117). A low dose of beta-endorphin has been shown to reduce stress-induced analgesia through the spinal cholecystinin receptors (118). In the models of stress-induced hyperalgesia, the alterations of these neuropeptides have not yet been studied.

Stress has a deep impact on the immune system. CRF produced by local inflammatory cells activates its receptors on leukocytes, leading to the release of opioid peptides. Therefore, immunosuppression abolishes stress-induced analgesia (119-121). On the other hand, an immune stimulator has been reported to produce hyperalgesia (63). Thus, we must pay much attention to the immune system in the models of stress-induced hyperalgesia.

The unraveling of mechanisms underlying stress-induced hyperalgesia may provide a more rational basis for drug therapies in a variety of pain syndromes.

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