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Corticotropin Releasing Factor in the brain and blocking spinal descending signals induce hyperalgesia in the latent sensitization model of chronic pain

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Abstract

Latent sensitization is a model of chronic pain in which an injury triggers a period of hyperalgesia followed by an apparent recovery, but in which pain sensitization persists but is suppressed by opioid and adrenergic receptors. One important characteristic of latent sensitization is that hyperalgesia can be triggered by acute stress. To determine whether the effect of stress is mimicked by the activation of corticotropin releasing factor (CRF) signaling in the brain, rats with latent sensitization induced by injecting complete Freund's adjuvant (CFA, 50 μl) in one hind paw were given an intracerebroventricular (i.c.v.) injection of CRF. The i.c.v. injection of CRF (0.6 μg, 10 μl), but not saline, induced bilateral mechanical hyperalgesia in rats with latent sensitization. In contrast, CRF i.c.v. did not induce hyperalgesia in rats without latent sensitization (injected with saline in the hind paw). To determine whether descending pain inhibition mediates the suppression of hyperalgesia in latent sensitization, rats with CFA-induced latent sensitization received an intrathecal injection of lidocaine (10%, 1 μl) at the cervical-thoracic spinal cord to produce a spinal block. Lidocaine-injected rats, but not rats injected intrathecally with saline, developed bilateral mechanical hyperalgesia. Intrathecal lidocaine did not induce hyperalgesia in rats without latent sensitization (injected with saline in the hind paw). These results show that i.c.v. CRF mimicked the hyperalgesic response triggered by stress during latent sensitization, possibly by blocking inhibitory spinal descending signals that suppress hyperalgesia.

Keywords

Complete Freund's adjuvant; corticotropin releasing hormone; lidocaine block; mechanical hyperalgesia; spinal cord; stress

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Introduction

Latent sensitization to pain is both an animal model of chronic pain and a hypothesis about the mechanisms that maintain and control chronic pain (Campillo et al., 2011; Corder et al., 2013; Marvizon et al., 2015; Walwyn et al., 2016; Romero et al., 2017). The animal model consists of a triggering injury (complete Freund's adjuvant [CFA] injection, paw incision, nerve damage, etc.) that initiates a long period of hyperalgesia; however, after the hyperalgesia has subsided it can be reinstated by an injection of an opioid receptor antagonist. This reinstatement of hyperalgesia by blocking opioid receptors can be repeated many times for months (Corder et al., 2013). Recent reports in rodents indicate that the sensitization produced by the initial injury is still present but it's being suppressed by the continuous activation of opioids receptors (μ, δ and κ) and α_{2A} adrenergic receptors (Walwyn et al., 2016). Further research revealed that, at least in the case of the μ opioid receptor, its continuous activation is not due to the release of endogenous opioids but to agonist-independent or constitutive activity (Corder et al., 2013; Walwyn et al., 2016). Clinical studies (Pereira et al., 2013; Pereira et al., 2015) have shown that latent sensitization similar to that observed in rodents can be induced in about one third of the humans subjects. Therefore, some types of chronic pain in humans may also be due to a long-lasting pain sensitization that is suppressed by opioid receptors.

A key property of latent sensitization is that hyperalgesia can also be reinstated by stress (Rivat et al., 2007; Le Roy et al., 2011). Thus, acute stressors that produce somatic analgesia in normal rats produce hyperalgesia in rats with latent sensitization. Similarly, in patients with chronic pain, pain episodes are often triggered by stress (Blanchard et al., 2008; Larauche et al., 2012). The question of why stress sometimes induces analgesia and other times hyperalgesia remains unresolved (Butler and Finn, 2009; Jennings et al., 2014). The latent sensitization model suggests that chronic pain switches stress from being analgesic to being hyperalgesic. However, the underlying mechanisms have not been investigated.

To explore these mechanisms we have used a well-established form of latent sensitization, consisting of injecting CFA into the hind paw (Corder et al., 2013; Walwyn et al., 2016), to test two hypotheses. The first is that corticotropin releasing factor (CRF) release in the brain mediates the hyperalgesic effect of stress in animals with latent sensitization. This hypothesis is based on the fact that CRF in the brain plays a pivotal role in orchestrating behavioral, endocrine and visceral response to stress (Habib et al., 2000; Bale and Vale, 2004; Stengel and Tache, 2010; Dedic et al., 2017; Tache et al., 2017). It predicts that CRF injected into the brain of rats with latent sensitization will induce hyperalgesia. The second hypothesis is that descending pain inhibition pathways (Sandkuhler, 1996; De Felice et al., 2011; Maire et al., 2015) are necessary to maintain the suppression of hyperalgesia during latent sensitization. This hypothesis predicts that blocking descending signals at the upper spinal cord in rats with latent sensitization will also produce hyperalgesia.

Methods

Animals

Animals were male adult (2–4 months old) Sprague-Dawley rats (Envigo, Indianapolis, IN). All animal procedures were approved by the Institutional Animal Care and Use Committee of the Veteran Affairs Greater Los Angeles Healthcare System, and conform to NIH guidelines. Efforts were made to minimize the number of animals used and their suffering.

Chemicals

Human/rat CRF and naltrexone (NTX) were purchased from Tocris Bioscience (Minneapolis, MN). CFA and lidocaine were obtained from Sigma-Aldrich (St. Louis, MO). Other reagents were from Sigma-Aldrich. All compounds were dissolved in saline.

CFA injections

Undiluted CFA was injected subcutaneously in a volume of 50 μl into one hind paw using a 50 μl Hamilton syringe and a 26 gauge needle. The needle was inserted at an oblique angle from the heel in the middle of the paw, near the base of the third toe. It was held in place for 15 s and then gently withdrawn.

Intracerebroventricular (i.c.v.) cannulation and injections

The chronic implanting of the cerebroventricular cannula was performed as previously described (Million et al., 2013). Rats were anesthetized by an intramuscular injection of a mixture of ketamine hydrochloride (75 mg/kg, Ketanest, Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (5 mg/kg, Rompun, Mobay, Shawnee, KS). They were placed in a stereotaxic apparatus (Angle Two, Leica Biosystems Inc., Buffalo Grove, IL) and a chronic guide cannula (22 gauge, Plastic One Inc., Ronaoke, VA) was implanted into the right lateral brain ventricle according to coordinates obtained from Paxinos and Watson (2005), mm from bregma: antero-posterior, −0.8; lateral, −1.5; dorsoventral, −3.8. The guide cannula was anchored by dental cement and four stainless steel jewelry screws fixed to the skull. Thereafter rats were housed individually and allowed to recover for 7 days. The i.c.v. injections were performed using a 28 gauge cannula (Plastic One Inc.), 1 mm longer than the guide cannula, connected to a 50 μl Hamilton syringe by a PF-50 catheter (Intramedic Polyethylene Tubing, Clay Adams, Sparks, MD) filled with saline. A small air bubble (1 μl) was drawn at the distal end of the PE-50 tubing for visual monitoring of the i.c.v injection. The i.c.v injection was delivered over 30–45 s in lightly hand-restrained conscious rats. At the end of the experiment, 10 μl of dye was injected to verify the cannula's position. All the injections reached the lateral ventricle.

Intrathecal (i.th.) cannulation and injections

The i.th. injections onto the cervical-thoracic spinal cord were performed using a catheter implanted from the cisterna magna as previously detailed (Yaksh and Rudy, 1976; LoPachin et al., 1981; Chen and Marvizon, 2009). The back of the head and neck of the rat were shaved. The head was held in position using the ear bars of a stereotaxic holder (Angle-Two). A midline incision was made in the skin at the back of the neck and the muscle was

cut at its juncture with the edge of the cranium. The atlanto-occipital cisternal membrane was exposed and punctured to insert the polyethylene catheter (PE-5) into the i.th. space to end at the T1 segment. The other end of the catheter was tunneled under the skin over the cranium and externalized. The skin was sutured and the catheter was flushed with 10 μl saline and sealed. Solutions were loaded into a PE-10 tube and delivered within 1 min using a 10 μl Hamilton syringe.

Thereafter rats were housed separately and allowed to recover for 5–7 days. The presence of motor weakness or signs of paresis was established as criterion for euthanasia, but this did not occur in any of the rats. The position of the catheter was examined postmortem and the following exclusion criteria were used: 1) loss of the catheter, 2) termination of the catheter inside the spinal cord, 3) occlusion of the catheter tip. Three rats in the i.th. saline group were excluded because they lost their catheters.

Measures of mechanical hyperalgesia

Mechanical allodynia was measured using von Frey filaments (Touch-Test) by the two-out-of-three method (Kingery et al., 2000; Michot et al., 2012; Jarahi et al., 2014). Rats were habituated for periods of 30 min for 3 days to acrylic enclosures on an elevated metal grid (10 × 20 × 12 cm, IITC Life Science Inc., CA). A series of von Frey filaments were applied in ascending order (0.8, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10, 15 g) to the plantar surface of the hind paw for a maximum period of 3 s. A withdrawal response was counted only if th e hind paw was completely removed from the customized platform. Each filament was applied three times, and the minimal value that caused at least two responses was recorded as the paw withdrawal threshold (PWT). The 15 g filament was taken as cut-off threshold.

Data analysis

Data were analyzed using Prism 7.03 (GraphPad Software, San Diego, CA) and expressed as mean ± standard error of the mean. Statistical significance was set at 0.05. The target sample size was $n = 8–9$ rats. Some rats were eliminated from data collection due to loss of i.th. catheter (three rats) or death (one rat). One additional rat was eliminated because it had abnormally low baseline responses to von Frey filaments before injecting saline into the hind paw, probably because the surgery to implant the i.th. catheter caused sensitization. Rats were randomly assigned to treatment. Statistical analyses consisted of repeated measures two-way ANOVA followed by Holm-Sidak’s post-hoc tests.

Results

CRF i.c.v. induced hyperalgesia in rats with latent sensitization

The objective of this experiment was to determine whether CRF injected i.c.v. mimics stress-induced hyperalgesia in rats with latent sensitization. All rats were surgically implanted with i.c.v. guide cannulas. Latent sensitization was induced seven days after cannula implantation by injecting into one hind paw 50 μl of either CFA. Control rats received 50 μl saline into the hind paw, which does not induce latent sensitization (Walwyn et al., 2016). Mechanical hyperalgesia was measured at baseline and on days 1, 3, 5, 7, 14, 21 and 28 after the

injection of CFA or saline. The i.c.v. injections of CRF or saline were given on day 30. There were 3 experimental groups.

Group 1: hind paw CFA, i.c.v. CRF (n=9 rats). After the CFA injection, rats developed hyperalgesia in the ipsilateral but not the contralateral hind paw, which subsided by day 30 (Fig. 1A). Repeated measures two-way ANOVA yielded significant effects of CFA over time, side (ipsilateral vs. contralateral paw), and the interaction of these two variables (Table 1 row 1). In these rats, i.c.v. CRF (0.6 μg in 10 μl) produced hyperalgesia lasting more than 150 min in the ipsilateral paw and 120 min in the contralateral paw (Fig. 1B). Time points after 150 min were not included in the experimental design. The effect of CRF over time was significant, and there were significant differences between the hind paws and significant interaction between the two variables (Table 1 row 2). Therefore, in rats with latent sensitization i.c.v. CRF produced hyperalgesia in both hind paws, and the effect was more pronounced in the ipsilateral paw.

Group 2: hind paw CFA, i.c.v. saline (n=8 rats). After the CFA injection, rats in this group also developed hyperalgesia in the ipsilateral hind paw lasting until day 28 (Fig. 1C, Table 1 row 3), but did not develop hyperalgesia when injected i.c.v. with saline (Fig. 1D, Table 1 row 4).

Group 3: hind paw saline, i.c.v. CRF (n=8 rats). Rats in this group did not develop mechanical hyperalgesia after the injection of saline in the hind paw (Fig. 1E, Table 1 row 5) or i.c.v. injection of CRF (0.6 μg in 10 μl, Fig. 1F, Table 1 row 8) on day 30. Therefore, the hyperalgesia produced by i.c.v CRF required the previous CFA-induced inflammation.

When we compared the effect of CRF i.c.v. (group 1) with that of saline i.c.v. (group 2) using two-way ANOVA, we found significant differences in the ipsilateral paw (Table 1 row 7) but not in the contralateral paw (Table 1 row 8). Therefore, the hyperalgesia was indeed produced by CRF and not by a non-specific response to the i.c.v. injection.

Cervical spinal blockade induced hyperalgesia in rats with latent sensitization

The objective of the second experiment was to determine whether the suppression of hyperalgesia during latent sensitization is mediated by descending pain inhibition signals from the brain. To test this hypothesis, we performed a cervical-thoracic spinal block with an i.th. injection of lidocaine.

The optimal dose and volume of lidocaine to block descending pain signals at this level was determined in a previous study (Chen and Marvizon, 2009) by assessing motor deficits in the paws and by measuring substance P release (as neurokinin 1 receptor [NK1R] internalization) induced by injecting formalin in the fore paw or the hind paw. In that study we found that 1 μl of 10% lidocaine i.th. at spinal segments C8-T1 eliminated NK1R internalization in the cervical-thoracic spinal cord produced by formalin injection in the fore paw, but not NK1R internalization in the lumbar spinal cord produced by formalin injection in the hind paw. This showed that this dose of lidocaine effectively blocked action potentials in primary afferents entering the spinal cord at the cervical-thoracic level but not in primary afferents entering the spinal cord at the lumbar level. Therefore, it is likely that the same

dose of lidocaine is able to block action potential is fibers of passage in the cervical-thoracic spinal cord without affecting the processing of sensory signals from the hind paws at the lumbar spinal cord.

Rats were implanted with i.th. cannulas terminating over the C8-T1 spinal segments 3–5 days before injecting CFA or saline in the hind paw. Mechanical hyperalgesia was measured at baseline and on days 1, 3, 5, 7, 14, 21 and 28 after the injection of CFA or saline. On day 30 after CFA, rats received an i.th. injection of 1 μl of lidocaine 10% or saline, followed by a 3 μl saline flush. There were 3 experimental groups.

Group 1: hind paw CFA, i.th. lidocaine (n=8 rats). After injection of CFA, rats developed hyperalgesia in the ipsilateral but not the contralateral hind paw, which subsided by day 28 (Fig. 2A). Repeated measures two-way ANOVA showed significant effects of CFA over time, ipsilateral vs. contralateral paw, and the interaction between these two variables (Table 2 row 1). Subsequent i.th. injection of lidocaine at the cervical-thoracic level produced hyperalgesia for about 30 min in both the ipsilateral and contralateral paws (Fig. 2B). The effect of lidocaine over time was significant and there were significant differences between the hind paws (Table 2 row 2). Therefore, an upper spinal cord blockade with lidocaine reinstated hyperalgesia in both hind paws in rats with latent sensitization.

Group 2: hind paw CFA, i.th. saline (n=9 rats). After injection of CFA, rats also developed hyperalgesia in the ipsilateral hind paw lasting until day 21 (Fig. 2C, Table 2 row 3). However, these rats did not develop hyperalgesia after an i.th. injection of saline at the cervical-thoracic level (Fig. 1D, Table 2 row 4)..

Group 3: hind paw saline, i.th. lidocaine (n=6 rats). Rats in this group did not develop mechanical hyperalgesia after the injection of saline in the hind paw (Fig. 2E, Table 2 row 5). Subsequent i.th. injection of lidocaine at the cervical-thoracic level had no effect (Fig. 2F, Table 2 row 6). Therefore, the hyperalgesia produced by i.th. lidocaine required the previous CFA-induced inflammation.

When we compared the effect of i.th. lidocaine (group 1) with that of i.th. saline (group 2) using two-way ANOVA, we found significant effects of the variable ‘lidocaine’ in the ipsilateral paw (Table 2 row 7) but not in the contralateral paw (Table 2 row 8). Therefore, the hyperalgesia was indeed produced by the lidocaine block and was not an artifact of the i.th. injection

Discussion

This study shows that an injection of CRF into the lateral brain ventricle produces hyperalgesia in rats with latent sensitization induced by injecting CFA into the hind paw (Walwyn et al., 2016). Furthermore, it shows that interrupting descending signals at the upper spinal cord also induces hyperalgesia in these rats, indicating that during latent sensitization descending signals from the brain are necessary to maintain the suppression of hyperalgesia by opioids receptors.

CRF induces hyperalgesia in rats with latent sensitization

CRF injected into the rat brain has been reported to modulate visceral and somatic pain (Lariviere and Melzack, 2000; Tache, 2015; Yarushkina et al., 2016; Andreoli et al., 2017) through its action on CRF_1 and CRF_2 receptors (Ji and Neugebauer, 2008; Rouwette et al., 2012; Romero et al., 2017). In the present study, we found that an i.c.v. injection of CRF induced bilateral hyperalgesia in the hind paws of rats that had undergone CFA-induced unilateral hyperalgesia, but not in control rats injected in the hind paw with saline instead of CFA. Therefore, it is unlikely that the effect of CRF was due to alterations in motor activity, anxiety or other effects unrelated to pain (Koob and Thatcher-Britton, 1985; Strome et al., 2002; Pryce et al., 2011). We have previously shown (Walwyn et al., 2016) that rats similarly injected with CFA in the hind paw are in a state known as latent sensitization (Marvizon et al., 2015), characterized by a pain hypersensitivity that is continuously suppressed by the activation of μ, δ and κ opioid receptors and α_{2A} adrenergic receptors. Another control group showed that an i.c.v. injection of saline in rats with CFA-induced latent sensitization does not produce hyperalgesia. Hence, the hyperalgesia was not a non-specific consequence of the i.c.v. injection per se in rats sensitized by CFA.

These results extend previous studies (Rivat et al., 2007; Le Roy et al., 2011) showing that two acute stressors, forced swim and novel environment, produced hyperalgesia in rats with latent sensitization induced by the injection of carrageenan in the hind paw or by multiple doses of fentanyl. These stressors have been shown to release CRF in the brain (Ramos et al., 2006). A recent study (Romero et al., 2017) found that CRF_1 receptor knock-out mice with latent sensitization induced by a hind paw incision and remifentanil had stronger hyperalgesic responses to naloxone than wild type mice. This would be consistent with an anti-hyperalgesic action of CRF at CRF_1 receptors during latent sensitization, an effect opposite to what we found in rats. Other studies indicate that CRF can have opposite effects in amygdala neurons by acting on CRF_1 and CRF_2 receptors (Fu and Neugebauer, 2008; Ji and Neugebauer, 2008), and it has been proposed that in the amygdala CRF_1 receptors mediate hyperalgesia whereas CRF_2 receptors mediate analgesia (Rouwette et al., 2012). Therefore, the pro-algesic effect of CRF that we found could be mediated by CRF_2 receptors in the amygdala. Whether the hyperalgesic effect of CRF that we found in rats with latent sensitization is mediated by CRF_1 receptors in the amygdala needs to be further investigated.

The central amygdala (CeA) can modulate pain through its connections to the periaqueductal grey and the rostral ventromedial medulla (RVM) (Rizvi et al., 1991; Helmstetter et al., 1998; McGaraughty and Heinricher, 2002; Neugebauer, 2015). CeA neurons that project to the RVM contain CRF and are innervated by calcitonin gene-related peptide-containing fibers from the parabrachial nucleus, which in turn receives nociceptive signals from the spinal cord. Therefore, this would form a loop that would allow the CeA to control nociceptive signals (Neugebauer, 2007, 2015). The CeA also send CRF-containing axons to the locus coeruleus (Van Bockstaele et al., 1996) which suppresses hyperalgesia by releasing noradrenaline in the dorsal horn (Yaksh, 1985; Kwiat and Basbaum, 1990, 1992; Pertovaara, 2006; Maeda et al., 2009) and activating α_{2A} adrenergic receptors (Nazarian et al., 2008; Maire et al., 2015; Walwyn et al., 2016). The CeA also contributes to chronic pain by inducing descending pain facilitation (Greenwood-Van Meerveld et al., 2001; Qin et al.,

2003; Viisanen and Pertovaara, 2007; Ansah et al., 2009). A recent study (Andreoli et al., 2017) used the Designer Receptor Exclusively Activated by Designer Drugs (DREADD) technique to demonstrate that inhibiting CRF neurons in the CeA restored stress-induced analgesia and decreased allodynia in mice with neuropathic pain.

Since CRF injected i.c.v. would likely spread to the entire brain (Proescholdt et al., 2000), it could have reached its receptors in the pituitary gland to activate the hypothalamic-pituitary-adrenal (HPA) axis (Perusini et al., 2016). Corticosteroids released by the adrenal glands into the blood can enter the dorsal horn, where they increase NMDA receptor function (Lim et al., 2005; Wang et al., 2005) and ERK phosphorylation (Alexander et al., 2009), two mechanisms involved in the maintenance of hyperalgesia in latent sensitization (Corder et al., 2013). CRF can also act on the dorsomedial nucleus of the hypothalamus (DMH) to modulate a pathway linking it to the RVM (Mason, 2001; Martenson et al., 2009; Wagner et al., 2013).

Lidocaine spinal block induces hyperalgesia in rats with latent sensitization

If stress and CRF produce hyperalgesia in animals with latent sensitization through a neuronal pathway and not the HPA axis, then descending signals from the brain must regulate the mechanisms of latent sensitization in the spinal cord. Latent sensitization consists of a component that maintain pain hypersensitivity, which include activation of NMDA receptors and adenylyl cyclase (Corder et al., 2013), and an opposing component that suppress this hyperalgesia through the activation of μ, δ and κ opioid receptors and α_{2A} adrenergic receptors (Walwyn et al., 2016). Furthermore, extensive evidence shows that the μ opioid receptors are not activated by the continuous release of endogenous opioids but by becoming constitutively active (Corder et al., 2013; Walwyn et al., 2016). It is possible that this constitutive activity of the μ opioid receptors is maintained by cross-talk with another receptor (Selbie and Hill, 1998; Vazquez-Prado et al., 2003) which may respond to a descending signal. Further evidence that descending signals are involved in latent sensitization is that the reinstatement of hyperalgesia produced by opioid and α_{2A} adrenergic antagonists is bilateral, suggesting that brain signals induce both the contralateral hyperalgesia and its suppression.

Reinstatement of hyperalgesia by stress or CRF in animals with latent sensitization could be explained by two mechanisms: 1) activation of a descending pain facilitatory pathway (Porreca et al., 2002; Vera-Portocarrero et al., 2006), or 2) inhibition of a descending pain inhibitory pathway (Sandkuhler, 1996; De Felice et al., 2011; Cleary and Heinricher, 2013; Maire et al., 2015). Our finding that a lidocaine block of the upper spinal cord induces hyperalgesia in rats with latent sensitization is consistent with the second mechanism. Furthermore, the fact that lidocaine induced hyperalgesia bilaterally, as observed with i.c.v. CRF, supports the idea that the suppression of hyperalgesia involves a bilateral activation of opioid receptors driven by descending signals.

Conclusion

These two experiments considered together suggest that stress-induced hyperalgesia in animals with latent sensitization is mediated by CRF release in the brain, which then inhibits

a descending signal that maintains the opioid receptors in a constitutively active state. Future studies will investigate the areas of the brain responsible for the induction of hyperalgesia by CRF in animals with latent sensitization, as well as the pathways linking these areas with the spinal cord.

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Abbreviations

ANOVA	analysis of variance
CeA	central amygdala
CFA	complete Freund’s adjuvant
CRF	corticotropin releasing factor
HPA	hypothalamic-pituitary-adrenal
i.c.v	intracerebroventricular
i.th	intrathecal
NTX	naltrexone
NK1R	neurokinin 1 receptor
NMDA	N-methyl-D-aspartate
PWT	paw withdrawal threshold
RVM	rostral ventromedial medulla
SEM	standard error of the mean

Glossary

Constitutive activity
Activation of a receptor in the absence of agonist binding.

Descending pathways

Neural pathways connecting brain regions like the rostral ventromedial medulla, locus coeruleus, and other adrenergic (A5, A7) and dopaminergic (A11) nuclei to the dorsal horn of the spinal cord, resulting in pain inhibition or facilitation.

Hyperalgesia

An increase in responses to noxious stimuli.

Latent sensitization

Model of chronic pain in which an injury triggers a period of hyperalgesia followed by a return of responses to noxious stimuli to baseline; however, the sensitivity to noxious stimuli persists but is suppressed by the continuous activation of opioid and adrenergic receptors.

Naltrexone

Inverse agonist of the mu-opioid receptor able to block its constitutive activity.

Spinal block

Intervention that decreases signals in the descending pathways from the brain to the spinal cord.

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Highlights

- The latent sensitization model of chronic pain was induced in rats by injecting Complete Freund's adjuvant in one hind paw.
- Corticotropin releasing factor in the brain induced hyperalgesia in rats with latent sensitization.
- A spinal block with lidocaine also induced hyperalgesia in rats with latent sensitization.
- Therefore, suppression of hyperalgesia in latent sensitization requires descending signals
- These descending signals can be interrupted by corticotropin releasing factor and stress.

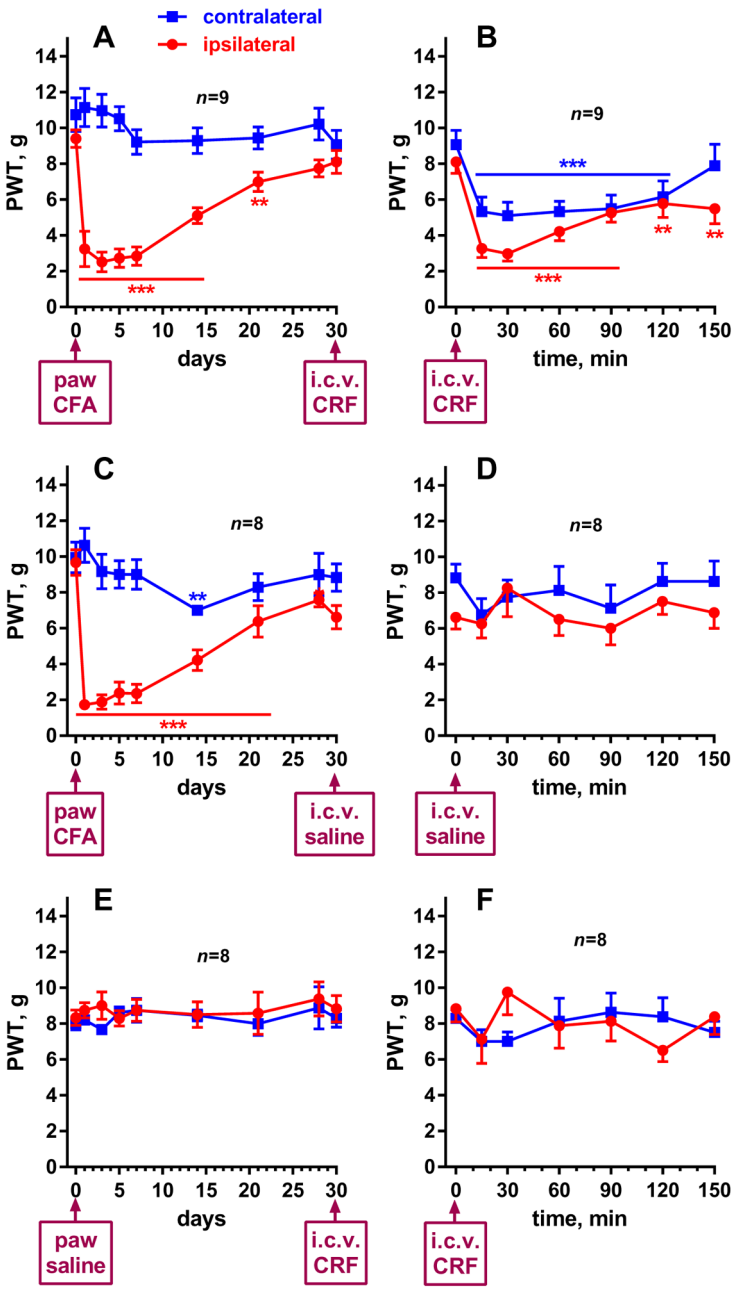

Figure 1. CRF i.c.v. induced hyperalgesia in rats with latent sensitization

Rats were implanted with intracerebroventricular (i.c.v.) cannulas. **Left panels:** Seven days later they were injected s.c. in one hind paw with 50 μl CFA (**A**, **C**) or saline (**E**). The number of rats per group (*n*) were 8 or 9, as indicated inside the panels. Rats injected with CFA, but not saline, developed hyperalgesia in the injected paw (ipsilateral), which resolved by day 28. **Right panels:** At day 30, the rats from the corresponding left panel were injected i.c.v. with 10 μl CRF (0.6 μg, **B**, **F**) or saline (**D**). CRF induced hyperalgesia in rats previously injected with CFA (**B**), but not in those injected with saline in the paw (**F**). Saline i.c.v. produced no effect in rats injected with CFA in the paw (**D**). Repeated measures 2-way

ANOVAs are given in Table 1. Holm-Sidak's post-hoc tests: ** $p<0.01$, *** $p<0.001$ compared with time 0.

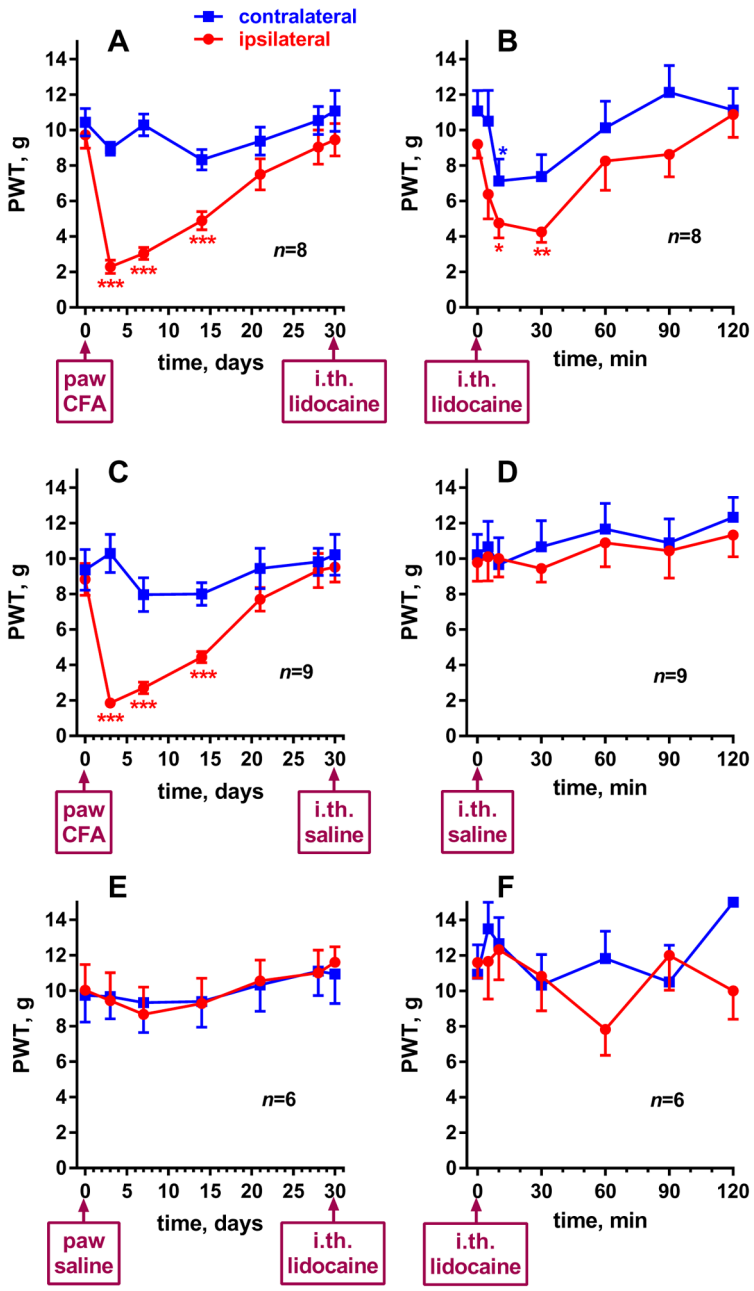

Figure 2. Cervical lidocaine induced hyperalgesia in rats with latent sensitization

Rats were implanted with intrathecal (i.th.) cannulas terminating over the C8-T1 spinal segments. **Left panels:** Three to five days later the rats were injected s.c. in one hind paw with 50 μl CFA (**A**, **C**) or saline (**E**). The number of rats per group (*n*) are indicated inside the panels. Rats injected with CFA, but not saline, developed hyperalgesia in the injected paw (ipsilateral), which resolved by day 28. **Right panels:** At day 30, the rats from the corresponding left panel were injected i.th. with 1 μl lidocaine (10%, **B**, **F**) or saline (**D**). Lidocaine induced hyperalgesia in rats previously injected with CFA (**B**), but not in those injected with saline in the paw (**F**). Saline i.th. produced no effect in rats injected with CFA

in the paw (**D**). Repeated measures 2-way ANOVAs are given in Table 2. Holm-Sidak's post-hoc tests: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with time 0.

Table 1

Repeated measures two-way ANOVA of data in Fig. 1 (CRF i.c.v.)

row	Fig. 1 panel	group	*n*	Data analyzed	variable 1	variable 2	interaction
1	A	1	9	CFA in the paw	time (CFA) $p<0.0001$ $F_{8, 64} = 10$	side $p<0.0001$ $F_{1, 8} = 236$	time x side $p<0.0001$ $F_{8,64} = 15$
2	B	1	9	CRF i.c.v. after paw CFA	time (CRF) $p<0.0001$ $F_{6, 48} = 13$	side $p=0.0062$ $F_{1, 8} = 14$	time x side $p=0.025$ $F_{6, 48} = 2.7$
3	C	2	8	CFA in the paw	time (CFA) $p<0.0001$ $F_{8, 56} = 11$	side $p<0.0001$ $F_{1, 7} = 85$	time x side $p<0.0001$ $F_{8,56} = 15$
4	D	2	8	saline i.c.v. after paw CFA	time (saline) $p=0.40$ $F_{6, 42} = 1.3$	side $p=0.089$ $F_{1, 7} = 3.9$	time x side $p=0.525$ $F_{6, 42} = 0.86$
5	E	3	8	saline in the paw	time (saline) $p=0.84$ $F_{8, 56} = 0.48$	side $p=0.34$ $F_{1, 7} = 1.04$	time x side $p=0.87$ $F_{8, 56} = 0.477$
6	F	3	8	CRF i.c.v. after paw saline	time (CRF) $p=0.64$ $F_{6, 42} = 0.70$	side $p=0.66$ $F_{1, 7} = 0.20$	time x side $p=0.11$ $F_{6, 42} = 1.8$
7	B, D	1, 2	9, 8	CRF i.c.v. vs. saline i.c.v., ipsilateral paw	time $p=0.0018$ $F_{6, 90} = 3.8$	CRF vs. saline $p=0.0316$ $F_{1, 15} = 5.6$	time x CRF $p<0.0001$ $F_{6, 90} = 5.5$
8	B, D	1, 2	9, 8	CRF i.c.v. vs. saline i.c.v., contralateral paw	time $p=0.0002$ $F_{6, 90} = 5.1$	CRF vs. saline $p=0.13$ $F_{1, 15} = 2.6$	time x CRF $p=0.26$ $F_{6, 90} = 1.3$

Rows 1–6: Data in each of the panels of Fig. 1 were analyzed by repetitive measures two-way ANOVA matching both factors, time and side (ipsilateral vs. contralateral paw), because they corresponded to the same rats. Rows 7–8: Data in panels B and D of Fig. 1 were analyzed by repetitive measures two-way ANOVA matching time only, in order to compare the effects of CRF i.c.v. with saline i.c.v. between rats. Sample sizes (n) are number of rats in each experiment.

Table 2

Repeated measures two-way ANOVA of data in Fig. 2 (lidocaine block)

row	Fig. 2 panel	group	*n*	Data analyzed	variable 1	variable 2	interaction
1	A	1	8	CFA in the paw	time (CFA) $p<0.0001$ $F_{6, 42} = 10$	side $p=0.0001$ $F_{1, 7} = 62$	time x side $p<0.0001$ $F_{6,42} = 13$
2	B	1	8	lidocaine i.th. after paw CFA	time (lidocaine) $p<0.0001$ $F_{6, 42} = 6.9$	side $p=0.045$ $F_{1, 7} = 5.9$	time x side $p=0.362$ $F_{6, 42} = 1.1$
3	C	2	9	CFA in the paw	time (CFA) $p<0.0001$ $F_{6, 48} = 11$	side $p=0.0002$ $F_{1, 8} = 41$	time x side $p<0.0001$ $F_{6,48} = 12$
4	D	2	9	saline i.th. after paw CFA	time (saline) $p=0.37$ $F_{6, 48} = 1.1$	side $p=0.54$ $F_{1, 8} = 0.4$	time x side $p=0.98$ $F_{6, 48} = 0.22$
5	E	3	6	saline in the paw	time (saline) $p=0.20$ $F_{6, 30} = 1.5$	side $p=0.996$ $F_{1, 5} = 0.0017$	time x side $p=0.989$ $F_{6, 30} = 0.34$
6	F	3	6	lidocaine i.th. after paw saline	time (lidocaine) $p=0.53$ $F_{6, 30} = 0.87$	side $p=0.055$ $F_{1, 5} = 6.2$	time x side $p=0.16$ $F_{6, 30} = 1.7$
7	B, D	1, 2	8, 9	lidocaine i.th. vs. saline i.th., ipsilateral paw	time $p<0.0001$ $F_{6, 90} = 5.4$	lidocaine vs. saline $p=0.037$ $F_{1, 15} = 5.2$	time x lidocaine $p=0.027$ $F_{6, 90} = 2.5$
8	B, D	1, 2	8, 9	lidocaine i.th. vs. saline i.th., contralateral paw	time $p=0.0065$ $F_{6, 90} = 3.2$	lidocaine vs. saline $p=0.53$ $F_{1, 15} = 0.41$	time x lidocaine $p=0.19$ $F_{6, 90} = 1.5$

Rows 1–6: Data in each of the panels of Fig. 2 were analyzed by repetitive measures two-way ANOVA matching both factors, time and side (ipsilateral vs. contralateral paw), because they corresponded to the same rats. Rows 7–8: Data in panels B and D of Fig. 2 were analyzed by repetitive measures two-way ANOVA matching time only, in order to compare the effects of lidocaine i.th. with saline i.th. between rats. Sample sizes (n) are number of rats in each experiment.