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Distinct pathways for norepinephrine- and opioid-triggered antinociception from the amygdala

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Abstract

Background—The amygdala has an important role in pain and pain modulation. We showed previously in animal studies that α_2 -adrenoreceptor activation in the central nucleus of the amygdala (CeA) mediates hypoalgesia produced by restraint stress, and that direct application of an α_2 -agonist in this region produces analgesia.

Aims—In the present animal experiments, we investigated the pathways through which α_2 -sensitive systems in the CeA produce behavioral analgesia. The CeA has dense connections to a descending pain-modulatory network, centered in the midbrain periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM), which is implicated in various forms of stress-related hypoalgesia and which mediates the antinociceptive effect of morphine applied in the basolateral amygdala. We investigated whether this circuit mediates the hypoalgesic effects of α_2 -adrenergic agonist administration into the CeA as well as the contribution of endogenous opioids and cannabinoids. We also tested the possibility that activation of α_2 -receptors in the CeA produces antinociception by recruitment of noradrenergic pathways projecting to the spinal cord.

Results—Hypoalgesia resulting from bilateral application of the α_2 -adrenergic agonist clonidine in the CeA was not reversed by chemical inactivation of the RVM or by systemic injections of naloxone (μ -opioid antagonist) or rimonabant (CB₁ antagonist). By contrast, spinal α_2 -receptor blockade (intrathecal idazoxan) completely prevented the hypoalgesic effect of clonidine in the CeA, and unmasked a small but significant hyperalgesia.

Conclusion—In rats, adrenergic actions in the CeA mediating hypoalgesia require spinal adrenergic neurotransmission but not the PAG-RVM pain modulatory network, or opiate or cannabinoid systems.

1. Introduction

The amygdala, long implicated in emotional learning and regulation, is now recognized to have an important role in pain and pain modulation, particularly in persistent pain states and

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Conflict of Interest

The authors report no conflicts of interest.

environmental hypoalgesia (Helmstetter, 1992; Neugebauer *et al.*, 2009). We recently showed that activation of α_2 -adrenoreceptors in the central nucleus of the amygdala (CeA) is needed for hypoalgesia produced by a restraint stress, and that direct application of an α_2 -agonist in the CeA itself produces hypoalgesia (Ortiz *et al.*, 2007; Ortiz *et al.*, 2008). However, the pathways and neurotransmitters mediating the hypoalgesic effect of CeA α_2 -receptor activation have not been determined.

Hypoalgesia triggered by a number of stressors depends upon activation of a brainstem nociceptive modulatory network with important nodes in the midbrain periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) (Helmstetter, 1992; Helmstetter *et al.*, 1998). The CeA is a major output nucleus of the amygdala and has dense reciprocal connections with the PAG (Beitz, 1982; Rizvi *et al.*, 1991; Sah *et al.*, 2003). The antinociceptive effects of electrical stimulation of the CeA are prevented by inactivation of the PAG (Oliveira and Prado, 2001). Mild hypoalgesia triggered by brief restraint is blocked by inactivation of the RVM (Mitchell *et al.*, 1998), as is antinociception associated with conditioned fear, also known to be mediated by the amygdala (Helmstetter *et al.*, 1998). We therefore sought to determine if the hypoalgesic effects of α_2 -adrenergic agonist administration in the CeA are mediated by the PAG-RVM nociceptive modulatory circuit. For comparison, morphine was microinjected into the basolateral nucleus of the amygdala (BLA), since hypoalgesia produced by opioid action in the BLA is known to be mediated by the PAG-RVM system (Helmstetter, 1992; Helmstetter *et al.*, 1998; McGaraughty and Heinricher, 2002).

We also tested whether opioid and cannabinoid neurotransmission contribute to hypoalgesia induced by α_2 -adrenergic action in the CeA, using systemic injections of the opioid antagonist, naloxone, and of the CB1-receptor antagonist, rimonabant. Amygdala lesions that include the CeA attenuate the analgesic effects of systemically administered morphine, and CeA neurons respond to μ -opioid agonists (Manning *et al.*, 2001; Zhu and Pan, 2004). Although focal administration of morphine in the CeA does not produce antinociception, the antinociceptive effects of CeA stimulation are blocked when an opioid receptor antagonist is given systemically or in the PAG (Oliveira and Prado, 1998; Oliveira and Prado, 2001; McGaraughty and Heinricher, 2002). Hypoalgesia associated with restraint stress is reported by many authors to be naloxone-reversible (Porro and Carli, 1988). Opioid mechanisms are also implicated in conditioned fear, another form of amygdala-dependent environmental hypoalgesia (Helmstetter *et al.*, 1998). Morphine and beta-endorphin analgesia elicited from the amygdala are blocked by delta-2 and μ -opioid receptor antagonists applied in the periaqueductal gray (Pavlovic *et al.*, 1996). Further, co-administration of sub-threshold doses of morphine into the amygdala and periaqueductal gray results in synergistic analgesic responses (Pavlovic *et al.*, 1998). Analgesia is also produced by amygdala administration of OFQ/nociceptin, and this effect is attenuated by administration of opioid antagonists in the periaqueductal gray (Shane *et al.*, 2001; Shane *et al.*, 2003; Rossi *et al.*, 2002). Endocannabinoids have also been linked to amygdala antinociception (Martin *et al.*, 1999; Manning *et al.*, 2003; Connell *et al.*, 2006). In the present studies we therefore used systemic administration of the opioid receptor antagonist naloxone and the CB₁ antagonist/

inverse agonist rimonabant to identify the contribution of endogenous opioids and cannabinoids in the hypoalgesic effect of α_2 -receptor activation in the CeA.

2. Experimental procedures

2.1 Animals and surgical preparation

Experimental protocols were approved by the Institutional Animal Care and Use Committee of Oregon Health & Science University and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Charlton, 1995). All efforts were made to reduce animal discomfort and minimize the number of animals used. Male Sprague-Dawley rats (Taconic, 250–350 g) were initially anesthetized with pentobarbital (60mg/kg, i.p.), and a catheter for later administration of anesthetic was placed in an external jugular vein. For animals receiving a systemic injection of experimental drug, a second catheter was placed in the contralateral external jugular vein. The rats were placed in a stereotaxic apparatus and craniotomies performed bilaterally over the amygdala, and in some experiments above the RVM, to allow placement of microinjection pipettes. A circulating water pad was used to maintain body temperature at approximately 37 °C. Between nociceptive testing trials, the tail was kept at a constant temperature of 35 °C by a feedback-controlled projector lamp. To test nociceptive responsiveness, a focused beam of light was projected on to the blackened ventral surface of the tail. Once adequately recovered from the initial dose of anesthetic to give a nociceptive response at 45 °C or below, the rat was put on a continuous infusion of sodium methohexital (35–65 mg/kg per h, i.v.) and maintained in a lightly anesthetized state. The anesthetic infusion rate was chosen to provide a stable tail-flick latency (TFL) and to prevent any signs of discomfort such as spontaneous movement, vocalizations, or a vigorous or prolonged response to nociceptive testing. A stabilization period of at least 30 min took place prior to the initiation of the experimental protocol.

2.2 Nociceptive testing

Nociceptive responsiveness was gauged using the latency to tail-flick from heat. The tail was placed over a light source at a distance of 1, 3, or 5 cm from the tip (used in rotation). Temperature was monitored using a thermocouple contacting the tail surface. The thermocouple was shielded from direct radiant heating. The temperature was ramped at approximately 1.7 °C /s from a holding temperature of 35 °C until a tail-flick occurred or the cut-off temperature was reached, 52 °C at approximately 10.2 s. Trials took place throughout the stabilization period and the experiment at 5-min intervals. Tail-flick latency and temperature were monitored and recorded for later analysis (Spike 2, Cambridge Electronic Design, Cambridge, UK).

2.3 Drug administration protocol

Glass microinfusion pipettes (50–100 μ m outer diameter, pulled on a Narishige vertical puller (model PS2), with the tip polished and beveled using a fine abrasive) connected to a 1 μ l Hamilton syringe with PE-50 tubing were placed bilaterally in the amygdala (CeA: relative to bregma, anterior/posterior (A/P) –2.8 mm, medial/lateral (M/L) $\pm$ 4.2 mm, relative to brain surface, dorsal/ventral (D/V) –8.1 mm; BLA: relative to bregma, A/P –3.0

mm, M/L $\pm$ 4.7 mm, relative to brain surface, D/V -8.8 mm) (Paxinos and Watson, 1997). For experiments in which a microinjection was given within the RVM, a microinfusion pipette was placed in the RVM (using the transverse sinus and midline as landmarks, D/V: -9.3 mm from brain surface). After a stable TFL was attained, the protocol was initiated and three baseline TFLs were recorded. The animal then received a pretreatment injection (within the RVM, intravenous (i.v.) or intrathecal (i.t.)). Two inter-injection TFLs were recorded and clonidine (CeA) or morphine (BLA) injected bilaterally in the amygdala. Clonidine (Tocris Bioscience, St. Louis, MO, 1.25 μ g/side in 200 nl saline vehicle, pH 7.4) or vehicle was microinfused within the CeA. Morphine sulfate (15 μ g/side in 400 nl in saline vehicle, pH 7.4) or vehicle was microinfused within the BLA. The doses of clonidine and morphine utilized for intra-amygdala injection were chosen based on previous work (Helmstetter *et al.*, 1998; McGaraughty and Heinricher, 2002; Ortiz *et al.*, 2007; Ortiz *et al.*, 2008). Microinjections were made over 90 s, and the micropipette was left in place for an additional 60 s and then retracted. After completion of the bilateral amygdala injections, six TFLs were recorded at 5 -min intervals.

Pretreatments—Muscimol (Tocris Bioscience, St. Louis, MO, 10 ng or 50 ng in 200 nl saline vehicle) or vehicle was injected into the RVM. The lower dose has been shown in electrophysiological experiments to be sufficient to suppress firing in all RVM neurons (Martenson *et al.*, 2009). Naloxone (Hospira, Lake Forest, IL, USA, 1 mg/kg i.v.), and rimonabant (Sanofi-Aventis, Montpellier, France, 5 mg/kg i.v., in 10% Tween 80 , 20% DMSO and 70% saline, pH 7.4) or vehicle were given systemically. The doses of naloxone and rimonabant used were chosen based on their effectiveness in reversing the analgesia resulting from intra-amygdala morphine in previous studies (McGaraughty *et al.*, 2004; Chhatwal *et al.*, 2005). Idazoxan (Tocris Bioscience, St. Louis, MO, 10 μ g in 10 μ l saline vehicle) or vehicle was given intrathecally using a Hamilton syringe connected to a 30 g needle placed in the L5-6 interspace (Pogatzki *et al.*, 2000). The injection volume was given over 30 s and the needle was held in place for an additional 60 s to help ensure the drug solution did not escape the intrathecal space.

2.4 Histology

At the conclusion of each experiment pontamine sky blue dye (2 g%, 200 nl) was infused through the microinjection canula. Intrathecal infusion sites were also marked using infusion of pontamine sky blue dye (2 g%, 10 μ l). The rat was euthanized with an overdose of methohexital and perfused transcardially with saline followed by a 10% formalin solution. Tissue was sectioned, sections were counterstained with cresyl-violet, and the centers of brain microinjections and intrathecal injections were deduced from the distribution of pontamine sky blue dye. Microinjections were and plotted on standardized sections from Paxinos and Watson (Paxinos and Watson, 1997); only microinjections whose centers were found to be within their intended targets upon histological examination were included in the results (Figs. 1 and 2).

2.5 Data analysis

Data are presented as mean $\pm$ SEM. Percent maximal possible effect (%MPE) or percent decrease relative to baseline was used for ease of comparison among groups. Between-group

comparisons of %MPE were accomplished using a t-test or ANOVA. The effects of drug administration on TFL were analyzed using a repeated measures t-test or ANOVA followed by Dunnett's test for comparison to baseline latency when the overall F parameter was significant. $P < 0.05$ was considered significant.

3. Results

3.1 The antinociceptive effect of the α_2 -adrenergic agonist clonidine in the CeA is not mediated via the RVM

We first tested the hypothesis that inactivation of the RVM would prevent the antinociceptive effect of α_2 -adrenoreceptor activation in the CeA. Saline (200 nl) and muscimol (10 and 50 ng) were microinjected into the RVM, and by themselves did not alter TFL (Figure 3, $p > 0.05$). Subsequent bilateral CeA clonidine microinjections produced robust antinociception in control animals pretreated with saline in RVM, with an increase in TFL from 5.5 ± 0.2 s in baseline, to 9.1 ± 0.4 s following clonidine microinjections ($p < 0.01$, Fig. 4A). This antinociceptive action of clonidine was not attenuated by RVM block with muscimol, as both muscimol-treated groups showed antinociception indistinguishable from that seen in saline-treated animals (Fig. 4A).

To verify the adequacy of the RVM block we tested the ability of the lower dose of RVM muscimol to prevent the antinociceptive effect of morphine microinjected into the BLA, since the antinociceptive effect of opioids in this nucleus is known to be mediated by the RVM (Helmstetter and Bellgowan, 1994; Helmstetter *et al.*, 1998; McGaraughty and Heinricher, 2002). In this positive control, BLA morphine produced a significant antinociception in RVM saline-pretreated animals (6.1 ± 0.2 in baseline, vs. 8.0 ± 0.6 s following morphine), that was completely prevented by muscimol microinjection in the RVM ($p < 0.01$, Fig. 4B).

3.2 Opioid and CB₁ action are not required in the antinociceptive effect of α_2 -adrenergic agonist clonidine in the CeA

We next tested whether the antinociceptive effects of clonidine in the CeA are mediated by endogenous opioids or cannabinoids, using systemic administration of the opioid-receptor antagonist naloxone and the CB₁ receptor antagonist rimonabant, respectively.

Naloxone—As in the previous set of experiments, saline-pretreated animals exhibited a significant increase in TFL following CeA clonidine microinjection, from 6.2 ± 0.5 s in baseline, to 8.6 ± 0.8 s after clonidine ($p < 0.01$, Fig. 5A). This antinociception was not reduced by systemic naloxone pretreatment (Fig. 5A). TFL was not changed following saline microinjection in the CeA, with or without naloxone administration (Fig. 5A).

Rimonabant—The CB₁ antagonist rimonabant similarly had no effect on the antinociceptive action of CeA clonidine (Fig. 5B). TFL was not changed following saline microinjection in the CeA in animals pretreated with rimonabant vehicle (Fig. 5A).

3.3 The antinociceptive effects of the α_2 -adrenergic agonist clonidine in the CeA are blocked by intrathecal idazoxan

In the final set of experiments we used spinal α_2 -receptor blockade to test the possibility that activation of α_2 receptors in the CeA produces antinociception by recruitment of noradrenergic pathways projecting to the spinal cord (Fig. 6). Lumbar intrathecal administration of the α_2 -adrenergic antagonist idazoxan (10 μ g) produced a small increase in TFL (from 6.0 ± 0.3 s in baseline to 7.0 ± 0.4 s, $p < 0.05$ compared to baseline (%MPE for idazoxan: $24.9 \pm 7.7\%$, for saline: $11.9 \pm 5.8\%$, saline was not significantly different from idazoxan, $p = 0.2$). Idazoxan nevertheless completely prevented the antinociceptive effect of CeA clonidine, unmasking a small but significant hyperalgesia (TFL 5.2 ± 0.3 s following clonidine in idazoxan-treated animals, $p < 0.05$ compared to baseline, repeated-measures ANOVA followed by Dunnett's test Fig. 6).

4. Discussion

We showed previously that α_2 -adrenoceptor activation in the CeA mediates hypoalgesia triggered by restraint stress, and that this hypoalgesia can be mimicked by direct administration of exogenous α_2 agonists in the CeA, and not in adjacent nuclei such as the basolateral amygdala (Ortiz *et al.*, 2007; Ortiz *et al.*, 2008). The present results demonstrate that CeA α_2 -adrenoceptor mediated hypoalgesia is not prevented by inactivation of the RVM, and is also insensitive to systemic blockade of either opioid or cannabinoid receptors. By contrast, this hypoalgesia is reversed by local α_2 -adrenoceptor blockade at the level of the spinal cord, suggesting that restraint-stress taps into a system that depends on α_2 -adrenergic neurotransmission at the level of the spinal cord, as well as in the amygdala.

4.1 Pathways engaged by α_2 -adrenoceptor activation in the CeA

Many forms of supra-spinal pain modulation rely on the function of an opioid-sensitive neural system with critical links in the PAG and RVM. The PAG-RVM system represents by far the best-studied descending pain modulatory system (Fields, 2002; Heinricher *et al.*, 2009), and it mediates antinociception produced by opioid-actions in the BLA, as well as conditioned hypoalgesia that is dependent on the BLA (Helmstetter and Tershner, 1994; Helmstetter *et al.*, 1998; McGaraughty *et al.*, 2004). Direct application of opioids in the BLA activates pain-inhibiting neurons in the RVM (McGaraughty and Heinricher, 2002). By extension from the pathways mediating BLA antinociception, and because the CeA has substantial reciprocal connections with the PAG, it seemed reasonable to hypothesize that antinociception produced by adrenergic action in the CeA also acts through the PAG-RVM system. However, in the present experiments RVM blockade did not attenuate the hypoalgesic effect of CeA clonidine. This indicates that adrenoceptor action in the CeA engages a descending output distinct from that recruited by opioid-sensitive systems in the BLA.

Another descending system that is known to modulate nociceptive processing arises in pontomedullary noradrenergic cell groups (Heinricher *et al.*, 2009; Proudfit 1992; Janss *et al.*, 1987).

To test the possibility that the effects of CeA clonidine were mediated by descending noradrenergic pathways, we blocked spinal α_2 -adrenergic transmission using intrathecal administration of the α_2 -receptor antagonist idazoxan. We used a 35 °C holding temperature to maintain the temperature of the tail between tail flick trials, thus preventing any influence of spinal α_2 -receptor block on tail skin temperature. This is critical, since an increase in tail temperature could lead to reduced TFL, which could give the appearance of anti-analgesia (Hole *et al.*, 1990). Under our conditions, intrathecal α_2 -adrenoreceptor antagonism completely reversed CeA clonidine hypoalgesia, indicating that this effect is mediated by noradrenergic pathways projecting to the spinal cord. Additional studies will be needed to identify the noradrenergic cell group recruited by CeA clonidine, but one possibility is the direct connection from the CeA to the locus coeruleus (Van Bockstaele *et al.*, 1996; Viisanen and Pertovaara, 2007).

The descending noradrenergic system has both hypoalgesic and hyperalgesic effects, which are mediated by spinal α_2 and α_1 receptors, respectively (Holden *et al.*, 1999; Nuseir and Proudfit, 2000). The unmasking of hyperalgesia following CeA clonidine by intrathecal α_2 -receptor block is consistent with this bidirectional control, and with evidence that the amygdala contributes to persistent pain states, at least in part by activating descending facilitation (Greenwood-Van Meerveld *et al.*, 2001; Qin *et al.*, 2003; Viisanen and Pertovaara, 2007; Ansah *et al.*, 2009). Further studies will be needed to determine whether the hyperalgesia revealed following α_2 -receptor block is mediated by an α_1 -receptor or by a parallel, non-adrenergic pathway, such as the pro-nociceptive output from the RVM (Porreca *et al.*, 2002; Ren and Dubner, 2002; Ansah *et al.*, 2009; Heinricher *et al.*, 2009). Whatever the descending pathway, the finding that α_2 -receptor activation in the CeA engages descending facilitation as well as inhibition raises the interesting possibility that there may be differential recruitment by physiological stimuli (various stressors, immune activation) of specific systems within the CeA that connect to antinociceptive systems (mediated by spinal α_2 -receptors) and pro-nociceptive systems (possibly mediated by spinal α_1 -receptors).

4.2 Opioids and cannabinoids do not mediate hypoalgesia from the CeA

Hypoalgesia can be provoked by a variety of stressors, including foot shock (Hayes *et al.*, 1978; Helmstetter, 1992), rotation, exposure to a predator, odors from stressed conspecifics, and restraint (Lewis *et al.*, 1980). The neural substrates of stress-induced hypoalgesia vary, depending on the nature, duration and intensity of the stressor (Hayes *et al.*, 1978; Bodnar *et al.*, 1980; Lewis *et al.*, 1980; Fox and Sorenson, 1994). Some forms of stress-induced hypoalgesia, such as that associated with conditioned fear, require activation of the PAG-RVM system and are opioid- and cannabinoid-dependent (Helmstetter and Tershner, 1994; Suplita *et al.*, 2005). By contrast, the α_2 -receptor-dependent system studied here does not recruit the PAG-RVM system, and does not require endogenous opioids or cannabinoids. This may indicate a distinction between hypoalgesia mechanisms engaged through the CeA versus the BLA. Indeed, although there is some evidence for opioid action in the CeA, opioid and endocannabinoid mechanisms of stress-induced hypoalgesia are more closely associated with the BLA than CeA (Helmstetter and Bellgowan, 1994; Nandigama and Borszcz, 2003; Connell *et al.*, 2006).

5. Conclusions

Increased noradrenergic neurotransmission under stressful environmental conditions coordinates a diverse array of forebrain functions in an adaptive manner (Robbins and Everitt, 1987; Selden *et al.*, 1991; Robbins and Arnsten, 2009). CeA α_2 -adrenoreceptors and spinal adrenergic projections may similarly participate in a coordinated response of the organism to environmental stress by mediating adaptive hypoalgesia. The present studies indicate that adrenergic mechanisms in the CeA do not engage the PAG-RVM descending pathway, and do not require endogenous opioids or cannabinoids. Hypoalgesia triggered by adrenergic activation of the CeA thus uses mechanisms distinct from those recruited by opioid-dependent systems in the BLA.

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What's already known about this topic?

- Hypoalgesia triggered by a number of stressors depends upon activation of a nociceptive modulatory network.
- Important nodes are located in the central nucleus of the amygdala, midbrain periaqueductal gray, and rostral ventromedial medulla.

What does this study add?

- α_2 -adrenoreceptor activation in the central nucleus of the amygdala mediates the hypoalgesia produced by restraint stress.
- This effect is reversed by spinal α_2 -receptor blockade but does not depend on rostral ventromedial medulla function, or on either μ -opioid or cannabinoid neurotransmission.

Figure 1.

Representative sections showing microinjector placements within the CeA, BLA and RVM. (A) CeA (thin black arrow), BLA (open arrow) and microinjector placement (bold black arrow). (B) CeA (thin black arrow), BLA (open arrow) and microinjector placement (bold black arrow). (C) RVM (box) microinjector placement (bold arrow).

Figure 2.

Histologically verified infusion sites within the CeA, BLA and RVM. Section drawings were adapted from Paxinos and Watson (Paxinos and Watson, 1997). (A) Infusion sites within the CeA ($n = 102$) and BLA ($n = 19$). Distance from bregma indicated for each section. (B) Infusion sites within the RVM ($n = 43$). Distance from interaural indicated for each section.

Figure 3. Microinjection of saline or muscimol did not significantly alter response latency ($F_{2,22} = 0.4$, $p = 0.67$).

Figure 4.

Effect of RVM block with muscimol on antinociception evoked by infusion of clonidine within the CeA (**A**) and by infusion of morphine within the BLA (**B**). **A:** CeA clonidine microinjection produced a significant increase in TFL that was not significantly attenuated when the RVM was blocked prior to the clonidine microinjections. Analysis of %MPE following CeA microinjections revealed no difference among the three groups ($F_{2,22} = 0.51$, $p = 0.61$). 6–10 animals/group, baseline TFL 5.6 ± 0.2 s, with no difference among groups in baseline. **B.** By contrast, the antinociceptive effect of BLA morphine microinjection was completely prevented by RVM block using the lower muscimol dose (10 ng) that had been tested against clonidine ($p = 0.007$, t -test for independent means). $n = 10$ and 9 in saline and muscimol pretreated groups, respectively. Baseline TFL 6.0 ± 0.2 s. # $p < 0.05$ difference in TFL compared to baseline, ** $p < 0.01$ difference in %MPE compared to RVM saline-pretreated controls.

Figure 5.

Effects of systemic administration of the opioid-receptor antagonist naloxone (**A**) and CB₁ receptor antagonist rimonabant (**B**) on antinociception produced by infusions of clonidine within the CeA. **A:** CeA clonidine microinjection produced similar antinociception in saline- and naloxone-pretreated groups. CeA saline had no effect on TFL with or without naloxone pretreatment. Analysis of %MPE following CeA microinjections showed a significant difference among groups, with Saline/Clonidine and Naloxone/Clonidine groups significantly different from Saline/Saline and Naloxone/Saline groups ($F_{3,26} = 9.0$, $p = 0.0003$, followed by Fisher's PLSD for post-hoc comparisons). 6–9 animals/group, baseline TFL 5.7 ± 0.3 s. **B:** CeA clonidine microinjections produced similar antinociception in vehicle- and rimonabant-treated groups. Analysis of %MPE following CeA microinjections revealed a significant difference among groups, with Vehicle/Clonidine and Rimonabant/Clonidine significantly greater than Vehicle/Saline group ($F_{2,22} = 12.4$, $p = 0.0003$ followed by Fisher's PLSD for post-hoc comparisons). 6–13 animals/group, baseline TFL 5.6 ± 0.1 s. # $p < 0.05$ difference in TFL compared to baseline, * $p < 0.05$ difference in %MPE compared to CeA saline controls.

Figure 6.

Effect of intrathecal idazoxan administration on the antinociception produced by bilateral infusions of clonidine within the CeA. Animals with spinal α_2 -receptor block exhibited hyperalgesia following CeA clonidine microinjection. Analysis of %MPE following CeA microinjections revealed a significant difference between groups ($t_{20} = 5.1$, $p < 0.0001$). Analysis of TFL showed a significant hypoalgesia in the saline-pretreated group, and hyperalgesia in the idazoxan-treated group following clonidine in CeA. 11 animals/group, baseline TFL 5.8 ± 0.2 s. # $p < 0.05$ difference in TFL compared to baseline using repeated measures ANOVA followed by Dunnett's test, * $p < 0.05$ difference in %MPE compared to intrathecal saline controls using t-test for independent means.