DESCENDING MECHANISMS OF CANNABINOID STRESS ANALGESIA

by

RICHARD L. SUPLITA II

(Under the Direction of Andrea G. Hohmann, PhD)

ABSTRACT

Stress-induced analgesia (SIA) is a state of decreased responsiveness to noxious stimulation following exposure to an environmental stressor. Inescapable foot-shock causes either opioid or non-opioid stress analgesia in rats depending on shock duration or intensity. Recent research from our laboratory indicates that non-opioid SIA elicited by brief continuous footshock is mediated by endogenous cannabinoids. The present study tested the hypothesis that pharmacological inactivation of cannabinoid receptors in brainstem nuclei modulating descending pain control attenuates non-opioid SIA. The competitive CB1 antagonist SR141716A was microinjected into the dorsolateral periaqueductal gray (dPAG), rostroventral medulla (RVM) and onto the lumbar spinal cord to evaluate the sites of action of non-opioid stress analgesia. Antagonist infusion into the dPAG and RVM, but not onto the spinal cord, was associated with decreased latency of responding to noxious stimulation. These results support the hypothesis that a descending cannabineric neural system is activated in response to environmental stressors to modulate pain sensitivity.

INDEX WORDS: Cannabinoid, Stress-induced analgesia, Periaqueductal gray, Rostroventral medulla
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CHAPTER 1

INTRODUCTION

Stress-induced analgesia (SIA) is the behavioral phenomenon in which animals are less responsive to noxious stimulation after exposure to an environmental stressor. Inescapable footshock induces either an opioid- or nonopioid-mediated analgesia in rats depending on shock duration and intensity (Lewis et al., 1980). Brief continuous footshock is associated with analgesia that is insensitive to the opioid antagonists naltrexone and naloxone (Lewis et al., 1980). Recent work in our laboratory has demonstrated that stress analgesia elicited by brief continuous footshock is mediated by a cannabinoid mechanism (Hohmann et al., 2001). However the sites-of-action underlying nonopioid stress analgesia remain unknown. Lesions of the dorsolateral funiculus (Lewis et al., 1983; Watkins et al., 1984) and the nucleus raphe magnus (Watkins et al., 1983) attenuate nonopioid stress-induced analgesia, implicating the involvement of descending mechanisms of nonopioid stress analgesia. Thus a neural system that does not rely upon the activation of endogenous opioids appears to be activated in response to certain environmental stressors and, in turn, modulates sensitivity to painful stimulation.

Cannabinoids act at the spinal level to induce antinociceptive (Yaksh, 1981; Lichtman et al., 1992), anti-hyperalgesic (Richardson et al., 1998) and anti-allodynic (Martin et al., 1999; Strangman & Walker, 1999) states. Moreover these effects are attenuated by co-administration of the competitive CB$_1$ antagonist SR141716A, implicating modulation by cannabinoid interaction with CB$_1$ receptors. Recent work in our laboratory indicates that
facilitating endocannabinoid activity at the spinal level by blocking reuptake or preventing enzymatic breakdown enhances nonopioid stress analgesia (Hohmann et al., 2001). These findings suggest that endocannabinoid activity at the spinal level can contribute to nonopioid SIA although the relative contribution of supraspinal sites of endocannabinoid analgesic action to SIA remains unknown.

The role of the periaqueductal gray (PAG) in mediating antinociceptive states has been well documented. Electrical stimulation of the PAG produces analgesia (Mayer et al., 1971), and analgesia elicited by stimulation of the dorsal PAG (dPAG) is not blocked by coadministration of the opiate antagonist naloxone (Canon et al., 1982). Later it was discovered that microinjection of the potent and selective synthetic cannabinoids into the PAG reduces sensitivity to noxious thermal stimulation (Martin et al, 1995; Lichtman et al., 1996). Moreover, Walker’s group showed that electrical stimulation of the dorsal and lateral PAG resulted in cannabinoid receptor-mediated analgesia concurrent with the increased release of the endogenous cannabinoid anandamide (Walker et al, 1999). These actions were blocked by systemic administration of SR141716A, a competitive antagonist for the cannabinoid CB₁ receptor.

Researchers have recently used synthetic cannabinoids targeted at other brainstem nuclei such as the rostroventral medulla (Martin et al., 1998; Vaughan et al., 1999) and the nucleus reticularis gigantocellularis (Monhemius et al., 2001) to more fully characterize cannabinoid-mediated antinociception. Furthermore, cannabinoids modulate on and off cells in the rostroventral medulla (Meng et al., 1998), demonstrating the ability of these ligands to control descending neuronal signaling. Collectively, these results
suggest that nociceptive responsiveness is, in part, modulated supraspinally through the actions of endogenous cannabinoids.

While prior research has established the role of exogenously-administered cannabinoids in regulating the transmission of pain signals, the endogenous mediators of non-opioid forms of environmental analgesia and their sites-of-action are largely unknown. The present studies were conducted to evaluate the sites-of-action of descending mechanisms of nonopioid SIA. We hypothesized that cannabinergic activity in brainstem nuclei purported to modulate stress induced analgesia would be especially susceptible to blockade by a competitive CB₁ antagonist. Thus, the present pharmacological experiments were conducted to test the hypothesis that site-specific infusion of the competitive cannabinoid antagonist SR141716A into the dPAG and RVM would attenuate stress induced analgesia.
CHAPTER 2

MATERIALS AND METHODS

Subjects. Male Sprague-Dawley rats (250-320 g) were used in these experiments. All procedures were approved by the University of Georgia Animal Care and Use Committee and followed the guidelines for the treatment of animals of the International Associations for the Study of Pain (Zimmermann, 1983).

Surgical Procedures. Rats were anesthetized with a mixture of sodium pentobarbital and ketamine (25 mg/kg and 40 mg/kg ip, respectively). Stainless steel guide cannulae (24 g, Small Parts, Miami Lakes, FL) were implanted in either the dorsolateral periaqueductal gray (dPAG) or the rostral ventral medulla (RVM) of animals receiving intracranial injections. Stereotaxic coordinates were based upon coordinates in the rat brain atlas of Paxinos & Watson (1998). Guide cannulae were implanted above dPAG (+1.0 mm AP, +0.6 mm LM, and –5.6 mm DV) and RVM (–2.6 mm AP, 0 mm LM, and –10.1 mm DV) using zero points from lambda, the midline suture and the skull surface, respectively. Cannulae were fixed to the skull with skull screws and dental acrylic. Intrathecal catheters were constructed from PE10 tubing (Yaksh & Rudy, 1979) and advanced caudally through an incision in the atlanto-occipital membrane. Catheters were implanted to a depth of 8.5 cm so that the catheter tip extended just rostral to the lumbar enlargement. Catheters were fixed to the skull with stainless steel screws and dental acrylic. Animals were allowed to recover three to six days prior to testing.
**Injections.** For supraspinal injections, SR141716A (10 µg) or DMSO vehicle was administered to the RVM or dPAG in a volume of 1 µl using a 10 µl Hamilton Syringe. Injection needles extended 2 mm beyond the tip of the cannula. Prior to injection, insect pins were removed and cannulae were reamed with stainless steel reamers to ensure unobstructed delivery of drug or vehicle. For intrathecal injections, SR141716A (1 or 10 µg) or DMSO vehicle was administered in a volume of 10 µl followed by an equivalent volume of saline to flush the catheter.

**Behavioral Testing.** Stress analgesia was quantified behaviorally using the tail-flick test (D’Amour and Smith, 1941). After establishing stable baseline responses to thermal stimulation of the tail (model 33A tail-flick unit, IITC Inc., Woodland Hills, CA), rats received a single intracranial or intrathecal injection. Tail-flick latencies were assessed three times at 2 min intervals immediately following drug administration to assess changes in tail-flick latency induced by the injection procedure. Rats were subsequently subjected to brief continuous footshock (1mA for 3 min). Tail-flick latencies were reassessed at 2-min intervals for 60 minutes.

**Histology.** Placement of intracranial and intrathecal cannulae were verified histologically (Figure 1). Rats receiving intracranial injections were perfused intracardially with 10% formalin. Following injection of 1 µl fast green dye, brains were dissected, cryoprotected, and stored overnight in 30% sucrose. Brain sections (40 µl) were cut with a cryostat at various levels throughout the PAG and RVM. Sections were mounted onto gelatin subbed slides and stained with cresyl violet. Placement of cannulae was confirmed microscopically. Appropriate placement of intrathecal catheters was confirmed by microinjection of fast green dye followed by exposure of the spinal cord.
Figure 1. Placement of intracranial cannulae into the dPAG (A) and RVM (B) was verified histologically. White circles denote placement of cannulae in vehicle-treated animals. Dark circles denote placement of cannulae in rats treated with the cannabinoid antagonist SR141716A. Maps represent coronal sections posterior from bregma –5.80 mm (left panel) and –6.04 mm (right panel) through the PAG and –10.80 mm (left panel) and -11.30 mm (right panel) through the RVM.
Figure 1. Site maps indicating placement of intracranial cannulae in (A) the dPAG and (B) the RVM.
Statistical Analyses.

Data were analyzed by repeated measures analysis of variance. Post hoc comparisons were performed using the Tukey test to correct for inflated familywise error, with $P < .05$ considered significant.
CHAPTER 3

RESULTS

Baseline tail-flick latencies did not differ between groups prior to administration of drug or vehicle. Moreover, latencies recorded just prior to footshock, following injection of drug or vehicle, did not differ between groups. Tail-flick latencies assessed after drug injection, just prior to footshock, were similar to baseline levels, indicating that any stress associated with the injection itself was insufficient to produce analgesia. Additionally, post-shock latencies significantly differed from baseline measures for both dPAG ($F_{1,17} = 19.70, P < 0.0001$) and RVM ($F_{1,12} = 2.78, P < 0.05$) manipulations, demonstrating that brief continuous footshock was effective in producing analgesia.

**Pharmacological Inactivation of dPAG and RVM Cannabinoid Receptors**

Attenuates Stress Analgesia. Microinjections of the cannabinoid antagonist SR141716A (1 µg) into the dorsolateral PAG (Figure 2) decreased stress analgesia relative to control conditions ($F_{1,17} = 28.69, P < 0.0001$). Stress analgesia was suppressed by SR141716A for more than 25 minutes following footshock.

Infusion of SR141716A (1 µg) into the RVM (Figure 3) decreased SIA in the tailflick test ($F_{1,12} = 3.57, P < 0.0001$). Rats receiving microinjections of SR141716A into the RVM were significantly less analgesic than controls for 37 minutes post-stress.
Effects of spinally-administered SR141716A. Intrathecal administration of SR141716A (1 or 10 µg) did not alter stress analgesia in rats receiving SR141716A relative to controls (Figure 4).
Fig 2. Microinjection of the selective cannabinoid CB\textsubscript{1} antagonist SR141716A (1 $\mu$g/1$\mu$l) into the dorsolateral PAG attenuates footshock induced elevations in tail-flick latencies relative to the control condition. *$P<0.05$, **$P<0.01$ for all comparisons by ANOVA and Tukey-Kramer post hoc test. $N = 10$ rats receiving SR141716A. $N = 9$ rats receiving vehicle.
Figure 2
Microinjection of SR141716A (1\mu g/\mu l) into the dPAG Attenuates Non-Opioid Stress Analgesia

Analgesia Index (Tail-flick Latency in s)

Time Post Stress (min)

- 100% DMSO (1\mu l)
- SR141716A (1\mu g)
Fig 3. Microinjection of the selective cannabinoid CB₁ antagonist SR141716A (1 μg/μl) into the RVM attenuates footshock induced elevations in tail-flick latencies relative to the control condition. *P<0.05, **P<0.01 for all comparisons by ANOVA and Tukey-Kramer post hoc test. N = 7 rats per group.
Figure 3
Microinjection of SR141716A (1µg/µl) into the RVM Attenuates Non-Opioid Stress Analgesia

Analgesia Index (Tail-flick Latency in s)

Time Post Stress (min)

100% DMSO (1µl)
SR141716A (1µg)
Figure 4. Intrathecal administration of the CB₁ antagonist SR141716A (1 or 10 µg/10 µl) fails to alter non-opioid stress induced analgesia.
Figure 4
Intrathecal Administration of SR141716A (1 or 10 µg/10 µl) Fails to Alter Non-Opioid Stress Analgesia

![Graph showing analgesia index (tail-flick latency in s) over time post stress (min). The graph compares 100% DMSO, SR141716A (1 µg), and SR141716A (10 µg).]
CHAPTER 4
DISCUSSION

The present studies demonstrate that microinjection of the cannabinoid antagonist SR141716A into brainstem nuclei implicated in descending pain modulation markedly attenuate stress-induced analgesia. This effect was observed following infusion of a cannabinoid antagonist into both the dPAG and the RVM. By contrast, an equivalent or ten-fold higher dose of SR141716A delivered spinally was insufficient to block cannabinoid SIA. These data provide further evidence for a descending cannabinergic neural system activated in response to environmental stressors that serves naturally to modulate pain sensitivity. Moreover, the present findings underscore the importance of midbrain and medullary nuclei in mediating the nonopioid stress analgesia effect.

Our data suggest that the dorsolateral periaqueductal gray represents an important site of endocannabinoid analgesic action. These data are consistent with the ability of tonic pain induced by intraplantar formalin injection to induce release of anandamide in the PAG (Walker et al, 1999). Moreover, electrical stimulation of the dorsal and lateral PAG produced increases in anandamide in this brain region and induced behavioral analgesia—a phenomenon termed stimulation produced analgesia (SPA). The present studies suggests that, in addition to noxious stimulation and SPA, brief continuous stressors can enhance cannabinergic activity in brainstem nuclei implicated in descending pain modulation. To investigate the common role endogenous cannabinoids play in regulating SPA and SIA, future studies should examine cross-tolerance between these
two modes of analgesia. Important similarities and differences characterize opioid- and cannabinoid-mediated SIA systems. Microdialysis and pharmacological studies have shown that different subregions of the same anatomical structures are involved in opioid and cannabinoid mechanisms of pain suppression. For example, researchers have implicated involvement of different subregions of the PAG in both opioid- (Cannon et al., 1982) and cannabinoid- (Martin et al., 1995) mediated antinociception. Furthermore, a brainstem circuit that includes the RVM—a structure important in descending opioid inhibitory systems (Basbaum & Fields, 1978)—is also activated by cannabinoid antinociceptive mechanisms (Martin et al., 1997; Meng et al., 1998). Furthermore, common pharmacodynamic mechanisms characterize opioid- and cannabinoid-mediated analgesia. Like opioids (Feldman et al., 1997), cannabinoids enhance $K^+$ conductance and inhibit $Ca^{2+}$ conductance (Mackie & Hille, 1992), decreasing the probability of presynaptic transmitter release. For example, cannabinoids have been shown to inhibit GABAergic interneurons to suppress inhibitory transmission (Vaughan et al., 2000). Third, the observation of cannabinoid-opioid antinociceptive synergism (Welch & Stevens, 1992) and bi-directional cross-tolerance (Hine, 1985) strengthens the hypothesis that opioid and cannabinoid systems share common downstream effects.

Similarities between the cannabinoid- and opioid-mediated SIA systems should not be taken to suggest that the systems are merely redundant. Importantly, different environmental conditions activate endocannabinoid vs. opioid analgesic systems. In the rat footshock model of SIA, for example, brief continuous footshock is associated with cannabinoid-mediated SIA whereas intermittent shock elicits opioid SIA. Future
investigations should examine differential activation of cannabinoid and opioid circuits by varying temporal and intensity dimensions of stimuli other than footshock.

A large body of research demonstrates the effectiveness of cannabinoids as antinociceptive and anti-inflammatory agents. While these studies underscore the promise of developing novel treatments for pain, future research should also explore other promising applications for cannabinergic compounds. For instance, the finding that cannabinoids are released naturally in response to environmental stressors suggests the possibility of developing endocannabinoid pharmacotherapies for the treatment of stress-related disorders. The observation that patients suffering from PTSD are more likely to use marijuana (Calhoun et al., 2000) is consistent with a stress disorder self-medication model. Recent research has demonstrated the ability to manufacture and deliver cannabinoid agents with greater potency, greater efficacy, fewer unwanted side effects, and fewer contaminants than smoking marijuana provides. Therefore, future research should examine the effectiveness and feasibility of developing cannabinergic pharmacological interventions to prevent or treat stress-related pathologies.
REFERENCES


