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Cross-sensitization and cross-tolerance between exogenous cannabinoid antinociception and endocannabinoid-mediated stress-induced analgesia

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Abstract

Footshock stress induces both endocannabinoid mobilization and antinociception. The present studies investigated behavioral plasticity in cannabinoid antinociceptive mechanisms following repeated activation using the tail-flick test. A secondary objective was to ascertain whether blockade of stress antinociception by the CB_1 antagonist rimonabant could be attributed to changes in locomotor activity. The cannabinoid agonist WIN55,212-2 induced hypoactivity in the open field relative to vehicle-treated controls. By contrast, rimonabant, administered at a dose that virtually eliminated endocannabinoid-mediated stress antinociception, failed to alter locomotor behavior (i.e. time resting, ambulatory counts, distance traveled) in rats subjected to the same stressor. Rats exposed acutely to footshock were hypersensitive to the antinociceptive effects of WIN55,212-2 and Δ^9-tetrahydrocannabinol (Δ^9-THC). The converse was also true; acute Δ^9-THC and WIN55,212-2 administration potentiated stress antinociception, suggesting a bidirectional sensitization between endocannabinoid-mediated stress antinociception and exogenous cannabinoid antinociception. Stress antinociception was also attenuated following chronic relative to acute treatment with WIN55,212-2 or Δ^9-THC. Repeated exposure to footshock (3 min/day for 15 days), however, failed to attenuate antinociception induced by either footshock stress or WIN55,212-2. Our results demonstrate that endocannabinoid-mediated stress antinociception cannot be attributed to motor suppression. Our results further identify a functional plasticity of the cannabinoid system in response to repeated activation. The existence of cross-sensitization between endocannabinoid-mediated stress antinociception and exogenous cannabinoid antinociception suggests that these phenomena are mediated by a common mechanism. The observation of stress-induced hypersensitivity to effects of exogenous cannabinoids may have clinical implications for understanding marijuana abuse liability in humans.

Keywords: Endocannabinoid; Sensitization; Stress antinociception; Rimonabant; Tolerance; Footshock; Anxiety; THC

1. Introduction

Stress antinociception is a behavioral phenomenon in which animals are less responsive to noxious stimulation following exposure to an environmental stressor. Different parameters and durations of stress activate either opioid-dependent or opioid-independent analgesic mechanisms (Lewis et al., 1980; Terman et al., 1986). Previous work from our laboratories demonstrated that the coordinated release of the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide mediates opioid-independent stress antinociception by engaging cannabinoid CB_1 receptors (Hohmann et al., 2005; Suplita et al., 2005, 2006). This discovery is consistent with the hypothesis that endocannabinoids, released under physiological conditions, produce adaptive changes in pain responses. However, the functional significance of the endocannabinoid signaling system to behavior remains incompletely understood.

Exogenous cannabinoids induce motor deficits (e.g. immobility, catalepsy) that may confound interpretation of behavioral studies of antinociception that largely measure motor responses to noxious stimulation (Martin et al., 1991). Electrophysiological studies demonstrate that analgesic effects of exogenous cannabinoids are independent of motor deficits induced by these compounds (Martin et al., 1996; Meng et al.,

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1998). Nonetheless, due to the potential for such confounds, it is necessary to demonstrate that apparent antinociceptive effects observed in behavioral studies are not experimental artifacts attributable to motor suppression. Here we examine the effects of the stressor used in our previous studies to induce stress antinociception (Connell et al., 2006; Hohmann et al., 2005; Suplita et al., 2005, 2006) on the ambulatory behavior of rats. These studies demonstrate that the ability of rimonabant to attenuate stress antinociception cannot be attributed to changes in basal locomotor activity.

Tolerance and dependence develop in laboratory animals as well as humans following chronic exposure to synthetic cannabinoids (for review see Lichtman and Martin, 2005). Repeated once-daily exposure to intermittent footshock stress for two weeks results in tolerance to an opioid-dependent, but not an opioid-independent, form of stress antinociception (Lewis et al., 1981; Terman et al., 1986). Similarly, we showed that chronic treatment with the cannabinoid agonist WIN55,212-2 attenuated endocannabinoid-mediated stress antinociception (Hohmann et al., 2005). The present studies were conducted to further examine the functional plasticity of the endocannabinoid system in response to repeated activation.

We tested the hypothesis that a cross-sensitization and cross-tolerance would be observed between endogenous and exogenous cannabinoid antinociception. First, we examined the impact of exposure to footshock stress (using parameters known to induce endocannabinoid-mediated stress-induced analgesia) on antinociception induced by exogenous cannabinoids. Second, we evaluated the reverse contingency to determine if sensitization and tolerance between stress-induced and pharmacologically-induced antinociception was bidirectional. Third, we examined the impact of acute and chronic exposure to exogenous cannabinoids on endocannabinoid-mediated stress antinociception. Finally, we used repeated exposure to footshock stress to determine whether repetitive activation of the endocannabinoid system would induce tolerance to endogenous and exogenous cannabinoid antinociception. Preliminary results have been reported (Hohmann et al., 2005).

2. Methods

2.1. Animals

Two hundred and seven adult male Sprague–Dawley rats (275–350 g; Harlan, Indianapolis, IN) 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 Association for the Study of Pain (Zimmermann, 1983). Rats were individually housed upon arrival at the animal facility and thus were not tested in the presence of known cagemates (Langford et al., 2006). All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs

The CB_1 antagonist/inverse agonist SR141716A (rimonabant) and the CB_2 antagonist SR144528 were gifts from NIDA. Δ^9-THC, naltrexone, morphine sulfate and WIN55,212-2 were purchased from Sigma-Aldrich (St. Louis, MO). Drugs were dissolved in emulphor:ethanol:saline (1:1:8 or 1:1:0) vehicle solution and administered via intraperitoneal (i.p.) injection in a volume of 1 ml/kg body weight. Morphine sulfate was dissolved in the same vehicle (1:1:8 emulphor:ethanol:saline) and administered subcutaneously (1 ml/kg bodyweight s.c.).

2.3. Behavioral testing

Stress antinociception was induced by exposure to continuous footshock (0.9 mA, AC current for 3 min (Lewis et al., 1980)), as described in our previously published work (Hohmann et al., 2005; Suplita et al., 2005, 2006). Stress antinociception was quantified using the tail-flick test (D'Amour and Smith, 1941). The latency for rats to withdraw their tails from a radiant heat source was quantified before and after pharmacological manipulations and before and after exposure to footshock or no shock treatment. Animals were allowed to habituate to restraining tubes prior to assessment of tail-flick latencies. Withdrawal latencies to thermal stimulation of the tail were measured at 2-min intervals before and after footshock and before and after pharmacological manipulations. To assess stress antinociception, tail-flick latencies were calculated for each subject in 2-trial blocks. The ceiling latency was 10 s in all studies, except where noted.

2.3.1. Experiment 1: Evaluation of the receptor mechanism underlying non-opioid stress-induced analgesia

This experiment was designed to test the hypothesis that non-opioid stress-induced analgesia was mediated by a cannabinoid CB_1 mechanism (see Hohmann et al., 2005). Rats received either the CB_1 antagonist rimonabant (5 mg/kg i.p.), the CB_2 antagonist SR144528 (5 mg/kg i.p.), the opiate antagonist naltrexone (14 mg/kg i.p.) or vehicle following determination of baseline tail-flick latencies. Twenty-five minutes following injection, rats were exposed to the footshock stressor (0.9 mA AC current, 3 min). Post-shock tail-flick latencies were monitored over 60 min. To evaluate the effects of rimonabant on basal nociceptive thresholds, separate groups received either rimonabant (5 mg/kg i.p.) or vehicle but were not exposed to the stressor. Tail-flick latencies were measured before injections (baseline) and over the same interval used to assess stress antinociception.

2.3.2. Experiment 2: Effects of rimonabant and footshock stress on locomotor activity

This experiment was designed to test the alternative hypothesis that endocannabinoid-mediated stress antinociception could be attributable to footshock-induced changes in locomotor activity that were blocked by rimonabant. Groups received either rimonabant (5 mg/kg i.p.) or emulphor: ethanol: saline (1:1:8) vehicle 30 min prior to exposure to the footshock stressor (3 min 0.9 mA) used to elicit stress antinociception. Separate groups received either WIN55,212-2 (5 mg/kg i.p.) or vehicle but were not subjected to footshock. All rats were placed in an automated open field arena (Med Associates, St. Albans, VT) 30 min following pharmacological manipulations and were free to explore the arena for fifteen minutes. During this time, behavior was automatically recorded by computerized analysis of photobeam interrupts (Med Associates). The Plexiglas arena was 43.2 × 43.2 × 30.5 cm and had a Plexiglas floor. Total time resting, ambulatory counts, and total distance traveled were monitored and recorded automatically. On day 1, baseline locomotor measurements were assessed in all rats. On day 2, rats were again placed in the open field arena 30 min following drug or vehicle administration (i.e. 24 h following baseline assessments of locomotor activity). The same behaviors were monitored and quantified (in a 15 min interval) on both day 1 and day 2. Animals were removed from the arena 15 min following introduction into the open field. The interval evaluated corresponded to the maximal change in stress antinociception induced by footshock stress.

2.3.3. Experiment 3: Evaluation of cross-sensitization between endocannabinoid-mediated stress-induced analgesia and exogenous cannabinoid antinociception

This experiment was designed to test the hypothesis that prior activation of the endocannabinoid system by footshock stress would induce behavioral sensitization to the antinociceptive effects of synthetic cannabinoids. Experiment 3 treatments are summarized in Table 1. Tail-flick latencies were initially measured before and after exposure to footshock stress on day 1. Δ^9-THC or