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Anxiety does not affect the antinociceptive effect of Δ^9-THC in mice: participation of cannabinoid and opioid receptors

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Abstract

Cannabinoid receptor agonists significantly inhibit nociceptive responses in a large number of animal models. The present study examined whether mice displaying different basal levels of anxiety in the plus-maze test of anxiety might differ in terms of responsiveness to the antinociceptive effects of Δ^9-tetrahydrocannabinol (Δ^9-THC). Further, the involvement of the cannabinoid and/or opioid receptors in Δ^9-THC-induced antinociception was investigated by using SR 141716A and naloxone, respectively, cannabinoid and opioid receptor antagonists. Δ^9-THC-induced antinociception was evaluated in the formalin test that involves a biphasic response with an early and a late phase of high paw-licking activity. This characteristic biphasic response was observed in all control animals selected as ''anxious'' and ''nonanxious.'' Δ^9-THC (0.5–5 mg/kg ip) caused a dose-dependent antinociceptive effect in both groups of mice during the early and late phases. This response was fully reversed by SR 141716A (1 mg/kg ip) and partially reversed by naloxone (2 mg/kg ip). These findings suggest that mice selected for differences in anxiety-related behavior show similar responses to the antinociceptive action of Δ^9-THC and that this action involves predominantly cannabinoid mechanisms.

Keywords: Cannabinoid; Opioid; Anxiety; Antinociception; Mice

1. Introduction

Cannabis derivatives have been used medicinally and recreationally for thousand of years (Mechoulam, 1986). In addition, it is known that cannabis preparations can cause several behavioral and pharmacological effects in laboratory animals, among which is a notable antinociceptive effect. Cannabinoids have been shown to produce antinociception in a variety of animal models, such as the formalin (Moss and Johnson, 1980; Strangman et al., 1998), tail-flick (Buxbaum, 1972; Martin et al., 1999), and hot-plate tests (Dewey, 1986; Martin, 1985; Reche et al., 1996). In addition, several studies indicate that cannabinoids produce antinociception by acting at spinal and supraspinal sites (Lichman et al., 1996; Martin et al., 1995). Indeed, recently a novel system to modulate pain sensitivity based upon the existence of cannabinoid receptors and their endogenous agonists has emerged (Calignano et al., 1998; Martin et al., 1999; Fuentes et al., 1999).

On the other hand, it is known that several physiological and psychological processes play important roles in the influence of anxiety on pain sensation. Some of the hypothesized mechanisms suggest that anxiety increases pain, while others imply a reduction in pain (Janssen and Arntz, 1996; Rhudy and Meagher, 2000). Indeed, animal studies suggest that fear, an immediate alarm reaction to present threat, inhibits pain whereas anxiety, a future-oriented emotion characterized by negative affect and apprehensive anticipation of potential threats, enhances it (Rhudy and Meagher, 2000). One of the most widely used animal models of anxiety is the elevated plus-maze that has been pharmacologically and ethologically validated (Pellow et al., 1985; Rodgers et al., 1997; Lister, 1987). Using this procedure, recent studies have shown a large range of responses of inbred rats (Ramos et al., 1997), as well as normal Wistar rats (Blatt and Takahashi, 1999; Rogério and Takahashi, 1991). Moreover, it is important to note that current knowledge of the mechanism of the antinociceptive action of cannabinoids is largely derived from animal

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experiments that do not provide information on the emotional aspects of pain. Indeed, most of the animal models currently in use assess the effects of drugs in an unselected population of animals in which no attempt has been made to induce, for example, an anxious state.

In the present study, it was of interest to investigate whether any individual basal behavior of mouse on the elevated plus-maze might predict different reactivity for Δ^9-tetrahydrocannabinol (Δ^9-THC)-induced antinociceptive effects. For this purpose, drug-naive albino mice were tested in the elevated plus-maze for their initial level of anxiety. Using different criteria for anxious behavior, mice were then classified as "anxious" and "nonanxious" and subsequently evaluated in the formalin test. Further, the possible contribution of cannabinoid and/or opioid receptors to cannabinoid antinociception in mice was examined.

2. Materials and methods

2.1. Animals

Male Swiss adult albino mice weighing 30–40 g from our own colony were used. All animals were kept in cages, in groups of 15–20, with free access to laboratory food and water. They were maintained in a temperature-controlled room (23 ± 1 °C) under a 12-h light cycle (lights on 07:00 h). All procedures used in the present study complied with the Local Committee on Animal Care and Use (protocol number 140/CEUA) that operates under accepted guidelines such as *Guiding Principles in the Care and Use of Animals* (DHEW Publication, NIH).

2.2. Drugs

Δ^9-THC was provided by the National Institute on Drug Abuse (USA) and SR 141716A was a gift from Sanofi-Synthelabo (France). Naloxone was purchased from RBI (USA). The appropriate concentration of Δ^9-THC was prepared by evaporating the alcohol and emulsifying the residue in Tween-80 (Takahashi and Singer, 1979). One drop of Tween-80 was added to 10 ml for the preparation of the SR 141716A suspension. Control solution was prepared with the corresponding vehicle. All solutions were administered by intraperitoneal route in a volume of 0.1 ml/10g.

2.3. Apparatus and procedure

2.3.1. Elevated plus-maze test

The wooden plus-maze consisted of two opposing open arms, 30 × 5 cm, and two enclosed arms, 30 × 5 × 15 cm, and was elevated 38.5 cm from the floor. A video camera was mounted vertically over the plus-maze and a trained observer scored behavior from a monitor in an adjacent room. Each mouse was placed in the center of the maze and the number of entries and the time spent in the open and closed arms were recorded over a 5-min period. Using a procedure adapted from Spanagel et al. (1995), which was based on the percentage of open arm entries (open entries/total entries × 100) and the percentage of time spent in open arms (open time/total time × 100), the mice were selected into groups of "anxious" and "nonanxious" animals. To consider an animal as "anxious," the two parameters had to correlate. Thus, animals with levels below 20% for entries and below 15% for the time spent were considered as "anxious." The "nonanxious" group consisted of mice with levels above 30% for the entries and above 25% for the time spent in open arms. Mice with measures between these two main groups formed the "intermediate" group, which were discarded. One week after the plus-maze test, the selected animals went through the mouse formalin test.

2.3.2. Formalin test

The formalin test is a well-established model of persistent pain consisting of two temporally distinct phases (Dubuison and Dennis, 1977), an early phase involving acute activation of nociceptors and the late phase of sustained pain behavior involving inflammation and central sensitization (Coderre et al., 1990). The formalin test was carried out in an open glass cylinder, 17 cm in diameter, with a mirror placed under the floor to allow an unobstructed view of the paws. Δ^9-THC (0.5, 1.25, 2.5, or 5 mg/kg) or control solution was injected intraperitoneally 15 min before the formalin injection. Pretreatment with SR 141716A (1 mg/kg) or naloxone (2 mg/kg) was given 15 min before drug treatment. As described in a previous work (Bittencourt and Takahashi, 1997), each animal was injected with 20 μl of 2.5% formalin into the intraplantar region of the right hind-paw. Mice were then observed for 30 min after formalin injection and the amount of time spent licking the injected paw was timed with a stopwatch.

2.4. Statistical analysis

A one-way analysis of variance (ANOVA) was conducted for the selection, and a two-way ANOVA followed by Duncan's test was used for the treatment with Δ^9-THC and the formalin test. A three-way ANOVA was conducted for the pretreatment with the antagonists, SR 141716A and naloxone. The accepted level of significance for all tests was $P < .05$.

3. Results

Table 1 summarizes the results of the selection procedure for drug-naive mice, which involved measuring their basal level of anxiety in the plus-maze test. The subsequent division into "anxious" and "nonanxious" mice resulted in statistically well-differentiated groups for time spent [$F(1,308) = 1629.51$, $P < .001$] and number of entries into the open arms [$F(1,308) = 1616.99$, $P < .001$]. Thus, mice

Table 1
Selection experiment according to the exploratory activity of undrugged mice in an elevated plus-maze

	Percentage of time spent on open arms	Percentage of entrances on open arms	Number of mice
"Anxious"	2 ± 0.2	4 ± 0.4	204
"Nonanxious"	34 ± 1*	38 ± 0.7*	104

Data are reported as mean ± S.E.M.
* $P < .05$ compared to the anxious group (one-way ANOVA and Duncan's test).

exhibiting a higher number of entries into, and overall time spent in, open arms were selected as "nonanxious." The opposite was true for mice assigned to the "anxious" group.

Fig. 1 shows the results of Δ^9-THC-induced antinociception in "anxious" and "nonanxious" groups of mice during the two phases of the formalin test. In the vehicle-treated "anxious" and "nonanxious" mice, the subcutaneous injection of formalin resulted in a reliable biphasic display of paw-licking behavior. A separate two-way ANOVA of these data showed significant antinociceptive effects of Δ^9-THC on both the early and late phases [$F(2,77) = 12.53$, $P < .001$; $F(2,77) = 7.21$, $P = .00005$, respectively]. However, no difference was found between "anxious" and "nonanxious" groups in the early and late phases [$F(1,77) = 0.25$, $P < .87$; $F(1,77) = 0.28$, $P < .60$, respectively]. Subsequent post hoc tests of the data revealed that all doses of Δ^9-THC (0.5–5.0 mg/kg) induced a significant antinociception in the "nonanxious" group during the early phase, while the higher doses of the drug (2.5–5.0 mg/kg) significantly attenuated the time of paw licking of the two groups in the late phase of the formalin test. Thus, acute administration of Δ^9-THC reduced the paw-licking time during the two phases of the mouse formalin test in both groups of selected animals. In addition, these results suggest that Δ^9-THC-induced antinociception did not depend on the mouse's basal level of anxiety.

The results showing the effect of the selective cannabinoid receptor antagonist SR 141716A on Δ^9-THC-induced antinociception are presented in Fig. 2. Again, there was no apparent difference in the effects of control groups of mice in both phases of the test. A similar three-way ANOVA revealed a significant effect for treatment in the early phase [$F(1,69) = 7.75$, $P = .007$], as well as a significant Treat-

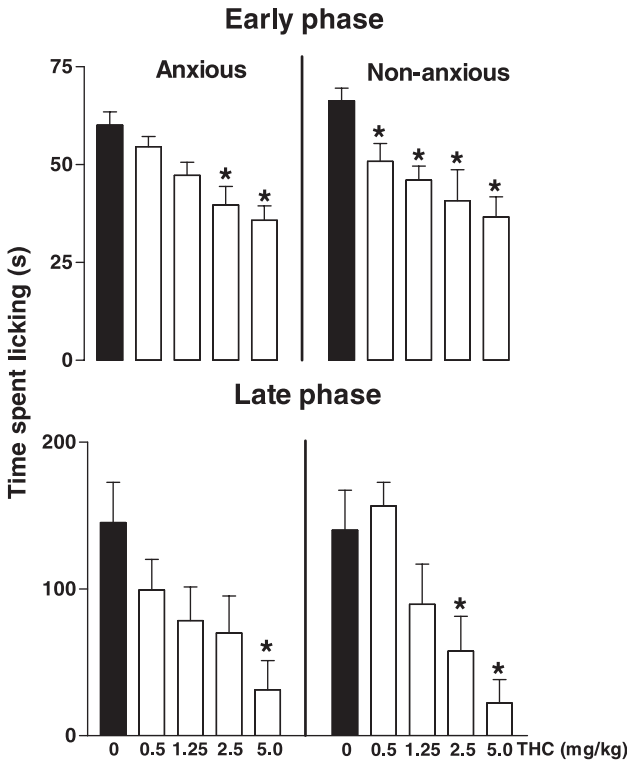

Fig. 1. Antinociceptive effects of acute treatment with Δ^9-THC (0.5–5.0 mg/kg ip) in "anxious" and "nonanxious" groups of mice. Nociceptive responses in the early phase (0–5 min after the formalin injection) and in the late phase (15–30 min after the formalin injection) were scored as the amount of time spent licking the hind-paw. Treatment was given 15 min before the injection of formalin. Data are expressed as mean ± S.E.M. of 7–11 animals. * $P < .05$ significantly different from the respective control group, Duncan's test.

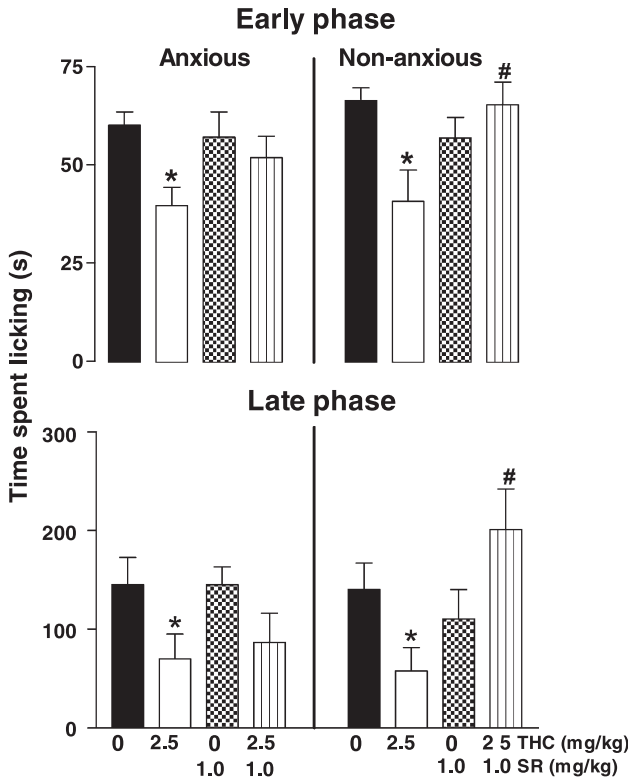

Fig. 2. Effect of the cannabinoid antagonist, SR 141716 (1 mg/kg ip), on the antinociceptive action of Δ^9-THC (2.5 mg/kg ip) in "anxious" and "nonanxious" groups. Nociceptive responses in the early phase (0–5 min after the formalin injection) and in the late phase (15–30 min after the injection) were scored as the amount of time spent licking the hind-paw. Pretreatment was given 15 min before the injection of Δ^9-THC. Data are expressed as mean ± S.E.M. of 7–11 animals. * $P < .05$ significantly different from the respective control group, Duncan's test. # $P < .05$ significantly different from the THC group, Duncan's test.

ment × Pretreatment interaction [$F(1,69) = 10.35$, $P = .002$]. The post hoc tests on these data indicated that SR 141716A (1.0 mg/kg) significantly reversed the antinociceptive activity of Δ^9-THC in mice selected as "nonanxious." The same ANOVA carried out on the results of the late phase of the formalin test showed a significant effect only for the Pretreatment × Treatment [$F(1,69) = 5.77$, $P = .0189$] and Anxiety × Pretreatment × Treatment interactions [$F(1,69) = 3.90$, $P < .05$]. Post hoc comparisons confirmed that the antagonism of SR 141716A on Δ^9-THC-induced antinociceptive action is evident only in "nonanxious" mice. It is noteworthy that during the late phase, the coadministration of SR 141716A + Δ^9-THC in this group of "nonanxious" mice significantly increased the paw-licking behavior causing an apparent hyperalgesic effect, however, when compared to the vehicle-treated group this response did not reach statistical significance, in addition SR 141716A injected alone did not induce hyperalgesia in the formalin test.

The evaluation of the naloxone pretreatment in the antinociceptive effects of Δ^9-THC is depicted in Fig. 3.

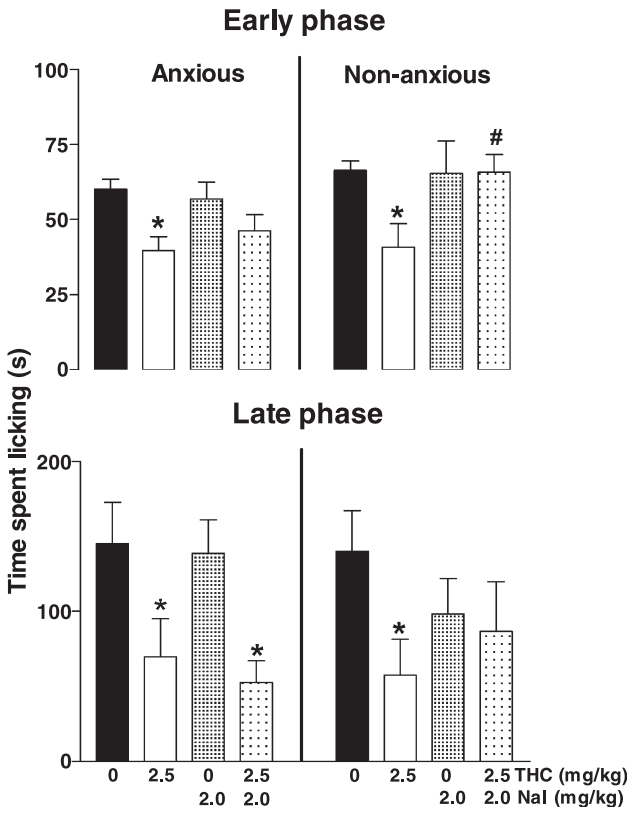

Fig. 3. Effect of the opioid antagonist, naloxone (2 mg/kg ip), on the antinociceptive action of Δ^9-THC (2.5 mg/kg ip) in "anxious" and "nonanxious" groups. Nociceptive responses in the early phase (0–5 min after the formalin injection) and in the late phase (15–30 min after the formalin injection) were scored as the amount of time spent licking the hind-paw. Pretreatment was given 15 min before the injection of Δ^9-THC. Data are expressed as mean ± S.E.M. of 7–11 animals. $^*P < .05$ significantly different from the respective control group, Duncan's test. $^{\#}P < .05$ significantly different from the Δ^9-THC group, Duncan's test.

A separate three-way ANOVA on the data collected during the early phase of the formalin test indicated a significant effect for treatment factor [$F(1,67) = 11.21$, $P = .0013$] and for the interaction factor between Treatment × Pretreatment [$F(1,67) = 4.55$, $P = .0366$]. Post hoc tests revealed that naloxone significantly blocked the antinociceptive activity of Δ^9-THC in mice selected as "nonanxious." Concerning the results of the late phase of the formalin test, similar analysis by ANOVA revealed a significant effect only for the treatment factor [$F(1,67) = 12.54$, $P = .0007$]. Thus, no effect was found for the pretreatment experiments with the opioid antagonist in both groups of mice in the late phase of the test.

4. Discussion

In the present study, we investigated the relationship between different levels of anxiety and the antinociceptive effects of Δ^9-THC in mice. Our results have shown that paw injections of formalin in "anxious" and "nonanxious" mice produced a similar biphasic nociceptive response in both control groups consistent with the results of our previous studies using "normal" mice (Bittencourt and Takahashi, 1997; Rodrigues-Filho and Takahashi, 1999). This result is at variance with the hypothesis that the degree of anxiety may contribute to the perception of and response to the noxious stimulus (Rhudy and Meagher, 2000). This apparent discrepancy was further confirmed when pain reactivity in these preselected groups was tested following Δ^9-THC administration. Mice displaying different basal levels of anxiety in the elevated plus-maze did not differ in terms of responsiveness to the antinociceptive effect of Δ^9-THC in both phases of the formalin test. To the best of our knowledge, this study constitutes the first attempt to correlate experimental anxiety and the antinociceptive action of cannabinoids.

Although anxiety defined operationally in a given animal model may differ from that generated by other models in respect to its nature, one likely explanation for the present results is that animals were selected from a "normal" heterogeneous group of mice exposed to a single plus-maze test, the results of which clearly do not reflect a predominant inborn trait. Moreover, it is worthy to remind that the elevated plus-maze has been suggested to be an "ethologically" valid animal model of human anxiety (Dawson and Tricklebank, 1995). A major difficulty, however, is to determine a specific form of clinical anxiety that can be associated with a particular animal model. As proposed by Lister (1990), behavioral responses evaluated in tests, such as the plus-maze, which include a temporary anxiety-provoking situation, are thought to reflect transient states of anxiety rather than a chronic anxiety-related trait. Regardless of anxiety definition, it is important to mention that some clinical studies examining the influence of nonpathological levels of anxiety on pain perception have also

yielded contradictory results (Arntz and De Jong, 1993; Arntz et al., 1991; Rhudy and Meagher, 2000).

Higher doses of Δ^9-THC significantly decreased the licking responses in the two phases in a quite similar manner in both groups of mice selected as "anxious" and "nonanxious." This may suggest a common way of reducing inflammatory and noninflammatory pain by cannabinoids. Thus, in the present study, the antinociceptive effects of Δ^9-THC did not differ between the groups of mice with contrasting levels of anxiety. These results support the earlier study of Moss and Johnson (1980) describing the tonic analgesic effects of Δ^9-THC measured with the formalin test in rats, as well as the recent evidence showing that synthetic cannabinoids, such as WIN-55212-2 and HU-210, block the two phases of pain behavior induced by formalin in mice (Calignano et al., 1998).

The antinociceptive activity of Δ^9-THC reported here appears to be predominantly mediated by CB1 receptors, since SR 141716A, a selective antagonist, prevented the antinociceptive effect of Δ^9-THC in both phases of the formalin test. In the light of this result, it is interesting to note that Calignano et al. (1998) reported that the antinociceptive effects of synthetic cannabinoids were prevented by systemic administration of the CB1 antagonist SR 141716A, but not of the CB2 antagonist SR 144528. Moreover, the existence of a high correlation between the antinociceptive effects and binding affinity of the cannabinoids strongly supports a receptor-mediated mechanism of action (Thomas et al., 1992). More importantly, despite the apparent hyperalgesic effect following the coadministration of SR 141716A + Δ^9-THC only in "nonanxious" mice, late phase of the formalin test, it is noteworthy that the injection of the cannabinoid antagonist alone did not exert any hyperalgesic action under the same experimental conditions. This result confirms the recent study of Beaulieu et al. (2000) showing that SR 141716A was unable to induce hyperalgesia in several models of pain, including the mouse formalin test. Curiously, the antagonism following pretreatment with naloxone, an opioid antagonist, occurred only during the early phase of the formalin test in "nonanxious" mice. One likely explanation for this result is that the endogenous opioid–cannabinoid systems may modulate the early and late phases differently. Nevertheless, these findings confirm and extend the recent literature reporting the participation of cannabinoid and opioid mechanisms in the antinociceptive action of cannabis derivatives (Fuentes et al., 1999; Welch and Eads, 1999) and in the anxiety-related effects induced by Δ^9-THC (Berrendero and Maldonado, 2002).

In conclusion, these findings demonstrate that the antinociceptive effects of Δ^9-THC are unrelated to the basal levels of anxiety in the animals and that the responses may involve mainly cannabinoid mechanisms. Further, these results are in line with some reports that were unable to find a correlation between emotional states and human pain reactivity.

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