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Cannabinoid-induced antinociception is mediated by a spinal α_2-noradrenergic mechanism

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The present study examined whether descending noradrenergic and serotonergic systems mediate the antinociceptive effect of the prototypical cannabinoid, delta-9-tetrahydrocannabinol (Δ^9-THC). Rats were administered vehicle or Δ^9-THC (10 mg/kg, i.v.) and subsequently given an intrathecal (i.t.) injection of either the α_2-noradrenergic antagonist, yohimbine, or the non-specific serotonin (5-HT) antagonist, methysergide, through chronically implanted spinal catheters. Whereas yohimbine significantly reversed the cannabinoid-induced elevation of tail-flick latencies, methysergide had no effect. To examine whether yohimbine was indeed blocking the antinociceptive effects of Δ^9-THC through a spinal mechanism, it was administered i.t. at either the lumbar or the upper thoracic level of the spinal cord. Antinociception was significantly reduced when yohimbine was administered in the lumbar region; however, administration in the upper thoracic region failed to have an effect. In addition, the effect of i.t. administered yohimbine and methysergide was assessed on two other indices sensitive to cannabinoids, hypothermia and ring immobility. As previously reported, i.v. administration of Δ^9-THC led to hypothermia as well as immobility in the ring test which were not blocked by i.t. administration of either monoamine antagonist. To the contrary, methysergide potentiated the hypothermic effect of Δ^9-THC. These findings indicate that cannabinoids activate descending noradrenergic neurons resulting in antinociception via the stimulation of spinal α_2-adrenoceptors.

INTRODUCTION

It has been widely reported that Δ^9-THC and cannabinoid analogs produce antinociception in mice and rats as assessed by the tail-flick response to radiant heat[7,25,28]. Although there has been much investigation on the structure activity relationship[25,35] as well as the receptor mechanisms of the cannabinoids[6,20,29], relatively little is known about the neurochemistry mediating its antinociceptive effects. Opiate receptors do not appear to be involved because opiate antagonists fail to reverse cannabinoid-induced antinociception[10,21,27,38]. On the other hand, several studies examining the pharmacology underlying cannabinoid-induced antinociception have implicated monoaminergic systems. Specifically, intracerebroventricular (i.c.v.) injections of either the 5-HT neurotoxin, 5,7-dihydroxytryptamine[21] or the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA)[10] attenuated cannabinoid-induced antinociception. Alternatively, noradrenergic depletion may also account for the blockade because neither study pretreated the animals with a noradrenergic uptake inhibitor to prevent a concomitant destruction of noradrenergic cell bodies. Further complicating the issue is that 5-HT depletion with *p*-chlorophenylalanine (PCPA) failed to prevent cannabinoid-induced antinociception[21].

Recently, spinal transection was found to significantly attenuate, but not completely block, the antinociceptive effects of i.v. administered Δ^9-THC as well CP-55,940, a more potent cannabinoid analog, suggesting both supraspinal and spinal components[24]. The circuitry for the tail-flick reflex is located in the lower lumbar and sacral segments of the spinal cord[15], suggesting that Δ^9-THC's supraspinal action is mediated through the activation of descending neurochemical systems. Although numerous descending mechanisms have been implicated in antinociception, spinal noradrenergic and serotonergic systems seem to play the most pervasive role. Specifically, i.t. injections of 5-HT and noradrenergic antagonists have been shown to block the antinociceptive effect produced by either microinjection of noncannabinoid drugs[4,22,37] or electric stimulation[1,12,17] at supraspinal sites. In the present study, 5-HT and noradrenergic antagonists were administered directly to the spinal cord of rats, in order to determine whether descending monoaminergic systems also mediate cannabinoid-induced antinocicep-

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tion. In addition to antinociception, the cannabinoids have been shown to have a variety of other pharmacological effects, including hypothermia and catalepsy[11,13,14,25,31,32]. Because monoaminergic systems have been implicated in the pharmacology underlying both of these cannabinoid effects[11,32] subjects were also assessed for catalepsy and hypothermia.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats (Dominion Labs, Dublin VA), with a mean body weight of approximately 350 g served as subjects. Each rat was individually housed, and Prolab 3000 Animal Chow and tap water were continuously available. All experiments were conducted during the light portion of a 14:10 h light/dark cycle.

Surgery

Subjects were anesthetized with pentobarbital (50 mg/kg, i.p.) and supplemented with halothane as needed. After induction of anesthesia, each rat was implanted with an i.t. catheter (PE-10, approximately 0.75 mm diameter) through an incision in the atlanto-occipital membrane[40]. The tips of the cathethers were placed either just rostral to the lumbar enlargement (extending 8.5 cm in length) or at the upper thoracic region (extending 3.5 cm in length). Immediately prior to surgery, each catheter was flushed with sterile saline, and the external end was heat sealed. Approximately 10% of the subjects exhibited signs of motor dysfunction and were eliminated from the study. All subjects were given one week to recover prior to evaluation.

Drug preparation and administration

Δ^9-THC was provided by the National Institute on Drug Abuse. Yohimbine was obtained from RBI (Natick, MA) and methysergide was obtained from Sandoz (East Hanover, NJ). For i.v. administration, Δ^9-THC was dissolved in a 1:1 mixture of ethanol and emulphor (Rhone-Poulenc Surfactants and Specialties, Princeton, NJ) and then added to isotonic saline to yield a final vehicle of 1:1:8 (ethanol/emulphor/saline). Each subject was placed in a restraining chamber, and the tail was emerged in 37 °C water to dilate the veins for the i.v. injection. Injections were administered via the tail vein in a volume of 0.5 ml/kg. For i.t. injections, yohimbine (10, 30, or 100 μg) was dissolved in 10 μl of vehicle consisting of 50% DMSO and 50% isotonic saline followed by a 10 μl saline flush to clear the catheter. In the experiment comparing the effect of i.t. yohimbine (30 μg) administered to either the upper thoracic or the lumbar region of the spinal cord, the drug was administered in 1 μl of 100% DMSO followed by a 4 or 6 μl saline flush, respectively. Methysergide (5, 20, or 50 μg) was dissolved in 10 μl of saline and administered i.t. followed by a 10 μl saline flush. All i.t. injections were administered over a one-min period and a cap approximately 2 cm long made from PE 50 tubing was then fitted over the exposed catheter to prevent leakage.

Antinociceptive test

The tail-flick response to radiant heat[5,8] was used to assess antinociception. An automatic 10 s cut-off was used to prevent tissue damage.

Body temperature

Core temperature to the nearest 0.1 °C was recorded by inserting a rectal probe, connected to telethermometer (Yellow Spring Industries Inc., Yellow Springs, Ohio) to a depth of 4.5 cm.

Ring immobility

A ring test procedure that has been used for mice[31] was modified to assess catalepsy of rats[34]. Each subject was placed on a ring (13 cm diameter) which was elevated 38 cm from a table top. A CCD camera (Panasonic, BLV-200) was focused on the rat for a 5-min recording session. A black background provided a sharp contrast to the albino rat. The videotape was transmitted to a Macintosh II microcomputer via a Scion Image-Capture 2 board at the speed of 30 frames/s in 256 shades of gray. The captured image was divided into 56,000 individual picture elements (pixels) which were then assigned values of either 0 (white) or 256 (black). An image was assessed approximately one frame per s. Each pixel from any given image was subtracted from the corresponding pixel from the previous frame and recorded by the computer to determine whether the animal was immobile. This objective measure of ring immobility has been demonstrated to have a 0.95 reliability coefficient with trained human observers[34].

Procedure

One week after catheter implantation, each rat was weighed and baseline body tempratures and tail-flick latencies were assessed. Subjects were then injected i.v. with either Δ^9-THC or its vehicle. Ten min after the i.v. injection, subjects received an i.t. injection of either yohimbine, methysergide, or their respective vehicles. The rectal probe was inserted to assess body temperature at 15, 30 and 60 min for the yohimbine-treated animals and at 15 and 60 min for the methysergide-treated animals. Tail-flick latencies were assessed at 15 min and immobility in the ring test was then assessed for a 5-min period. Initially, animals receiving yohimbine or DMSO treatment were not evaluated for ring immobility; therefore, an additional study was conducted to assess whether yohimbine (30 μg, i.t.) would block Δ^9-THC-induced ring immobility. Each group was composed of 6–8 rats.

Statistical analysis

Tail-flick response latencies were transformed to the percent maximum effect (%MPE) by the following equation[18]:

$$\%\text{MPE} = 100\times \frac{\text{test latency}-\text{control latency}}{\text{cut-off-time}-\text{control latency}}$$

Rectal temperature was expressed as the difference between post- and pre-injection values calculated from each animal. Statistical analysis of the data was performed using either the Student *t*-test or an ANOVA with the Newman–Keuls test for multiple comparisons, when appropriate. Differences were considered significant at the $P < 0.05$ level.

RESULTS

The %MPE of subjects given an i.v. injection of either Δ^9-THC or vehicle prior to i.t. administration of yohimbine or DMSO is depicted in Fig. 1. ANOVA revealed a significant effect of drug treatment, $F_{5,29} = 7.$ $P < 0.05$. Specifically, i.v. administration of Δ^9-THC led to significantly longer tail-flick latencies than the two groups injected with i.v. emulphor/ethanol/saline vehicle, Newman–Keuls, $P < 0.05$. Moreover, i.t. administration of yohimbine (30 or 100 μg) reversed this antinociception, Newman–Keuls, $P < 0.05$. the Δ^9-TH group administered 10 μg of i.t. yohimbine only differed from the emulphor/ethanol/saline vehicle group which received 100 μg of yohimbine i.t., Newman–Keuls, P 0.05. No other differences reached statistical significance.

Shown in Fig. 2 is the effect of i.t. yohimbine (30 μ or DMSO administered to either the lumbar or the u

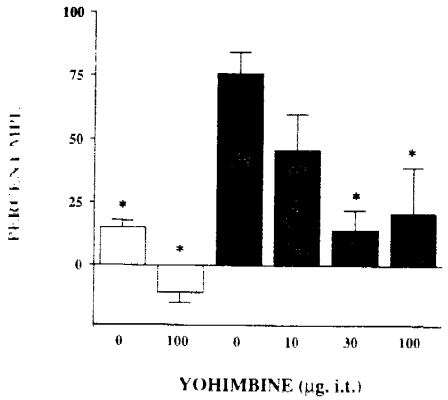

Fig. 1. Effect of i.t. administered yohimbine on cannabinoid-induced antinociception. Subjects were administered i.v. vehicle (□) or 10 mg/kg of Δ^9-THC (■) 5 min prior to the i.t. injection of DMSO or yohimbine (10, 30, or 100 μg). The animals were tested at 15 min after the i.v. injection. The results are presented as means ± S.E.M. of %MPE (n = 6 per group). *Significantly different from group receiving i.v. Δ^9-THC and i.t. vehicle.

per thoracic levels of the spinal cord on Δ^9-THC-induced antinociception. Rats administered yohimbine via the lumbar catheters exhibited significantly less antinociception than the animals administered with DMSO, t_{13} = 2.4, $P < 0.05$. In contrast, yohimbine administered in the upper thoracic region failed to decrease the %MPE relative to the control animals, $t_{13} < 1$.

The antinociceptive effect of i.v. administered Δ^9-THC or vehicle prior to i.t. administration of methysergide or saline is shown in Fig. 3. There was a significant effect of drug treatment $F_{5,30}$ = 7.1, $P < 0.05$; each group administered Δ^9-THC had significantly longer latencies than the two groups given i.v. emulphor/ethanol/saline vehicle, Newman–Keuls, $P < 0.05$. Although it appears that methysergide produced some antagonism, it failed to have any significant effects at any of the doses tested.

The effect of i.t. yohimbine on Δ^9-THC-induced hypothermia and immobility in the ring test is presented in the top panel of Table I. ANOVA revealed a significant interaction between drug treatment and time on body temperature, $F_{10,58}$ = 4.8, $P < 0.05$. Each Δ^9-THC-treated group had significantly lower rectal temperatures than either of the two emulphor/ethanol/saline vehicle groups at each time point, Newman–Keuls, $P < 0.05$. In addition, the body temperatures of Δ^9-THC-treated animals continued to decline over time. Yohimbine administered i.t. had no impact on this hypothermia at all doses tested. Administration of Δ^9-THC also led to a dramatic increase in immobility, $F_{1,2}$ = 164, $P < 0.05$, which was not influenced by i.t. yohimbine administration.

In the bottom panel of Table I, the effect of i.t. methysergide on Δ^9-THC-induced hypothermia and immobility is shown. Once again, there was a significant interaction between drug treatment and time on body temperature, $F_{5,30}$ = 8.6, $P < 0.05$. Animals treated with Δ^9-THC exhibited lower body temperatures than the two emulphor/ethanol/saline vehicle groups at each time point, Newman–Keuls, $P < 0.05$, and this hypothermia increased over time. Methysergide not only failed to block the hypothermic effect of Δ^9-THC but further reduced body temperature at 60 min. Specifically, the Δ^9-THC-treated animals administered 20 or 50 μg of methysergide were more hypothermic than the Δ^9-THC-treated animals that received i.t. saline, Newman–Keuls, $P < 0.05$. The body temperatures of subjects administered 5 μg of methysergide did not differ from any of the other Δ^9-THC-treated groups. Finally, there was a

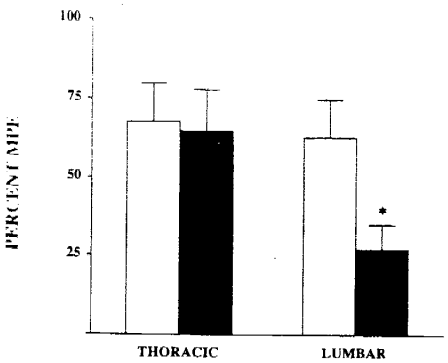

Fig. 2. Effect of yohimbine administered i.t. at either the upper thoracic or lumbar segments of the spinal cord on cannabinoid-induced antinociception. All subjects received an i.t. injection of either DMSO (□) or 30 μg of yohimbine (■) 5 min after Δ^9-THC (10 mg/kg, i.v.) administration. They were then evaluated for the tail-flick response 15 min after Δ^9-THC administration. The results are presented as means ± S.E.M. of %MPE (n = 7 per group). *Significantly different from the respective i.t. DMSO group.

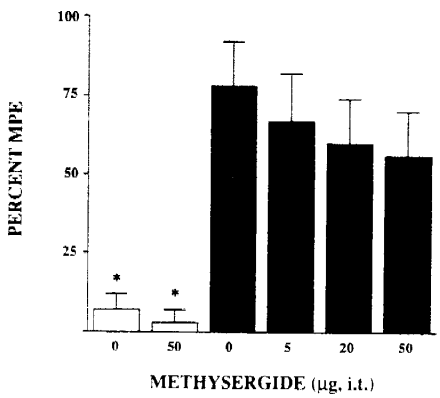

Fig. 3. Effect of i.t. administered methysergide on cannabinoid-induced antinociception. Subjects were administered i.v. vehicle (□) or 10 mg/kg of Δ^9-THC (■) 5 min prior to the i.t. injection of saline or methysergide (5, 20, or 50 μg). The animals were tested at 15 min after the i.v. injection. The results are presented as means ± S.E.M. of %MPE (n = 6 per group). *Significantly different from the group receiving i.v. Δ^9-THC and i.t. vehicle.

TABLE I

The effect of I.T. administered yohimbine and methysergide on Δ^9-THC-induced hypothermia and catalepsy

I.V. drug	*I.T. drug*	*Temprature change (°C)*			*Percent of time immobile*
		15 min	*30 min*	*60 min*	
	Yohimbine (μg)				
Vehicle	0	0.5 ± 0.5	0.8 ± 0.4	0.8 ± 0.3	16 ± 4
"	30				2 ± 1
"	100	0.3 ± 0.1	0.3 ± 0.2*	0.9 ± 0.3*	
Δ^9-THC	0	−0.9 ± 0.2*	−1.3 ± 0.2*	−1.8 ± 0.2*	68 ± 6*
"	10	−0.7 ± 0.3*	−1.3 ± 0.4*	−1.5 ± 0.4*	
"	30	−0.6 ± 0.1*	−1.4 ± 0.1*	−1.6 ± 0.3*	70 ± 5*
"	100	−1.1 ± 0.2*	−1.9 ± 0.2*	−2.4 ± 0.3*	
	Methysergide (μg)				
Vehicle	0	1.0 ± 0.2		0.8 ± 0.3	18 ± 5
"	50	0.2 ± 0.4*		0.2 ± 0.2	11 ± 5
Δ^9-THC	0	−1.3 ± 0.3*		−2.1 ± 0.2*	79 ± 7*
"	5	−1.6 ± 0.1*		−2.7 ± 0.4*	76 ± 12*
"	20	−1.6 ± 0.2*		−3.4 ± 0.4*	65 ± 10*
"	50	−1.6 ± 0.1*		−3.5 ± 0.3*	75 ± 6*

* Significantly different than the respective vehicle–vehicle control group ($P < 0.05$).

significant effect of drug treatment on the ring test, $F_{5,30} = 20.5$, $P < 0.05$. Each Δ^9-THC-treated group was significantly more immobile than the animals administered i.v. vehicle, Newman–Keuls, $P < 0.05$. Methysergide administered i.t. failed to have any impact on this immobility.

DISCUSSION

The primary goal of the present study was to ascertain whether spinal noradrenergic and 5-HT systems mediate cannabinoid-induced antinociception. Yohimbine reversed the antinociception when administered in the lumbar region of the spinal cord at similar doses to those which have been demonstrated to block antinociception produced by either miroinjection of noncannabinoid compounds[4,22,37] as well as electric stimulation[1,12,17] at specific brain sites or the direct administration of α_2-agonists to the spinal cord[39]. In contract, yohimbine administered to the upper thoracic level of the spinal cord failed to attenuate the antinociceptive effect of Δ^9-THC, suggesting that α_2-adrenoceptors in the lower spinal cord mediate cannabinoid-induced antinociception. These data taken together with the general concensus that noradrenergic projections to the spinal cord originate supraspinally[39] is consistent with a previous report that indicated an important supraspinal component of cannabinoid-induced antinociception[24]. It is unlikely that α_1-receptors play a significant role because the α_1-antagonist, phenoxybenzamine, failed to block cannabinoid-induced antinociception[21]. The lack of a methysergide effect suggests that spinal 5-HT receptors are not involved. It is important to note that the dose range employed has been previously shown to completely block 5-HT-induced analgesia at the spinal level[41]; thus, doses greater than 50 μg were not evaluated. When these results are considered along with the observation that 5-HT depletion with PCPA had no impact on cannabinoid-induced antinociception[21], it would appear unlikely that 5-HT plays an important role in this phenomenon.

The second goal of this investigation was to examine whether spinal 5-HT and noradrenergic systems are involved in other cannabinoid effects. Both methysergide and yohimbine failed to reverse cannabinoid-induced hypothermia. In fact, i.t. administered methysergide led to a further decline in body temperature 60 min after Δ^9-THC administration. Pretreatment with PCPA has also been reported to potentiate the hypothermic activity of cannabis resin in rats[36]. In light of the fact that i.t. 5-HT administration leads to a robust increase in body temperature[26], it is possible that cannabinoids induce hypothermia by inhibiting spinal 5-HT transmission. In addition, supraspinal catacholaminergic systems may play a role since 6-OHDA pretreatment has been shown to block the hypothermic action of Δ^9-THC[11,36]. Both methysergide and yohimbine failed to affect immobility in the ring test, suggesting that spinal α_2-noradrenergic and 5-HT receptors do not mediate the cataleptic effect of i.v. administered Δ^9-THC. However, these data do not preclude the possibility of supraspinal involvement of norepinephrine and 5-HT in cannabinoid-induced catalepsy.

The neural substrates subserving cannabinoid-induced antinociception are yet to be determined. The recent lo-

calization of putative cannabinoid receptors through autoradiography[19] provides a powerful tool to investigate brain structures that may mediate cannabinoid-induced behavior. Of consequence, the periaqueductal gray (PAG), a region strongly implicated in the centrifugal modulation of spinal nociceptive transmission[16,37], also contains cannabinoid binding sites[19]. Furthermore, antinociception produced by microinjection of morphine[16] or glutamate[22] to the PAG is also attenuated by i.t. administration of noradrenergic antagonists. Other brain regions that have been characterized as possessing high-affinity cannabinoid binding sites[19] and have also been implicated in endogenous pain inhibitory mechanisms are the dorsal hippocampus[33], substantia nigra[2,3,but9], and caudate–putamen areas[30]. Alternatively, Δ^9-THC may be acting at areas containing relatively few cannabinoid binding sites, but which play important roles in antinociception, such as the lateral reticular nucleus, one of the regions where descending noradrenergic fibers are believed to originate[12].

In conclusion, the present results indicate that cannabinoid-induced antinociception is mediated, in part, through the activation of a descending spinal noradrenergic mechanism. Although cannabinoids do not act at opiate sites, the effects of both drugs may be mediated through a common descending noradrenergic mechanism. This noradrenergic system has also been implicated in mediating antinociception produced by other pharmacological[4,22,37], electrophysiological[1,12,17], and environmental[23] manipulations. Finally, the fact that i.t. yohimbine administration had no impact on either the cataleptic or hypothermic effects of Δ^9-THC indicates that this noradrenergic system is relatively specific to antinociception.

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