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ORIGINAL INVESTIGATION

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Involvement of mu-, delta- and kappa-opioid receptor subtypes in the discriminative-stimulus effects of delta-9-tetrahydrocannabinol (THC) in rats

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Abstract *Rationale:* Many behavioral effects of delta-9-tetrahydrocannabinol (THC), including its discriminative-stimulus effects, are modulated by endogenous opioid systems. *Objective:* To investigate opioid receptor subtypes involved in the discriminative effects of THC. *Methods:* Rats trained to discriminate 3 mg/kg i.p. of THC from vehicle using a two-lever operant drug-discrimination procedure, were tested with compounds that bind preferentially or selectively to either mu-, delta- or kappa-opioid receptors. *Results:* The preferential mu-opioid receptor agonist heroin (0.3–1.0 mg/kg, i.p.), the selective delta-opioid receptor agonist SNC-80 (1–10 mg/kg, i.p.) and the selective kappa-opioid receptor agonist U50488 (1–10 mg/kg, i.p.) did not produce generalization to the discriminative effects of THC when given alone. However, heroin, but not SNC-80 or U50488, significantly shifted the dose–response curve for THC discrimination to the left. Also, the preferential mu-opioid receptor antagonist naltrexone (0.1–1 mg/kg, i.p.), the selective delta-opioid receptor antagonist, naltrindole (1–10 mg/kg, i.p.) and the kappa-opioid receptor antagonist nor-binaltorphimine (n-BNI, 5 mg/kg, s.c.), did not significantly reduce the discriminative effects of the training dose of THC. However, naltrexone, but not naltrindole or n-BNI, significantly shifted the dose–response curve for THC discrimination to the right. Finally, naltrexone, but not naltrindole or n-BNI, blocked the leftward shift in the dose–response curve for THC discrimination produced by heroin. *Conclusions:* mu- but not delta- or kappa-opioid receptors are involved in the discriminative effects of THC. Given the role that mu-opioid receptors play in THC's rewarding effects, the present findings suggest that discriminative-stimulus effects and rewarding effects of THC involve similar neural mechanisms.

Keywords THC · Cannabinoid · Drug discrimination · Heroin · Opioid receptor subtypes · Rat

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Introduction

In rats, cannabinoids and opioids produce many similar behavioral and physiological effects including analgesia, hypothermia, inhibition of intestinal mobility, hypolocomotion and, especially, reinforcement of drug self-administration behavior or induction of conditioned place preferences (Ameri 1999; Maldonado 2002; Maldonado and Rodriguez de Fonseca 2002; Manzanares et al. 1999; Tanda and Goldberg 2003). There is also strong experimental evidence that opioid and cannabinoid systems are anatomically and functionally coupled and, in fact, it has been repeatedly demonstrated in experimental animals that the behavioral effects of the active ingredient of cannabis, delta-9-tetrahydrocannabinol (THC), and other cannabinoid CB1 agonists depend to a large extent on the activation of endogenous opioid systems (Maldonado 2002; Maldonado and Rodriguez de Fonseca 2002; Manzanares et al. 1999; Tanda and Goldberg 2003; Justinova et al. 2004; Solinas et al. 2004).

Drug-discrimination procedures provide an animal model for the subjective effects of drugs in humans and are a useful tool for studying the pharmacology of abused drugs (Holtzman 1985; Kamien et al. 1993; Schuster and Johanson 1988; Stolerman 1992; Colpaert 1999, 2003). Drug discrimination studies with THC have long been used to study the mechanisms underlying the behavioral

effects of cannabis (Browne and Weissman 1981; Jarbe and Henriksson 1974; Prescott et al. 1992; Wiley 1999). The discriminative effects of THC show a high degree of pharmacological specificity. In fact, only drugs that activate CB1 receptors have been found to fully generalize to the discriminative effects of THC and, until recently, only drugs that block CB1 receptors have been found to antagonize the discriminative effects of THC (Barrett et al. 1995; Browne and Weissman 1981; Solinas et al. 2003, 2004; Wiley et al. 1995). We have recently demonstrated that the discriminative effects of THC are potentiated by opioid agonists and decreased by opioid antagonists and that a possible mechanism underlying the repeated demonstrations of opioid-cannabinoid interactions is a THC-induced release of the endogenous opioid beta-endorphin in brain areas such as the ventral tegmental area (Solinas et al. 2004).

Different opioid receptor subtypes differentially modulate the rewarding effects of opioid agonists such as heroin and morphine, as well the rewarding effects of other drugs such as ethanol and cocaine (Mansour et al. 1995; van Ree et al. 1999; Herz 1997; Mello and Negus 1996). In fact, mu-opioid and, to a lesser extent, delta-opioid receptors are involved in the rewarding effects of opioid agonists (Kieffer and Gaveriaux-Ruff 2002; Shippenberg and Herz 1987). In addition, mu-opioid, and, to a lesser extent, delta-opioid agonists produce rewarding effects on their own (Devine and Wise 1994), whereas kappa-opioid receptors are involved in negative motivation and kappa-opioid agonists produce dysphoric effects (Kieffer and Gaveriaux-Ruff 2002; Shippenberg and Herz 1987). Thus, investigating the role played by different opioid receptor subtypes in the discriminative-stimulus effects of THC would not only help to reveal the general neurobiological mechanisms involved in the behavioral effects of THC but also to elucidate similarities or differences between opioid system modulation of the discriminative and rewarding effects of THC.

In the present experiments, rats were trained to discriminate injections of a 3-mg/kg dose of THC from injections of vehicle in a two-lever choice drug-discrimination procedure with food reinforcement. We then tested the ability of the preferential mu-opioid receptor agonist heroin, the selective delta-opioid receptor agonist SNC-80 and the selective kappa-opioid receptor agonist U50488 to produce or potentiate the discriminative effects of THC. We also tested the ability of the preferential mu-opioid receptor antagonist naltrexone, the selective delta-opioid receptor antagonist naltrindole and the selective kappa-opioid receptor antagonist nor-binaltorphimine (n-BNI) to block or decrease the discriminative effects of THC.

Materials and methods

Subjects

Ten Male Sprague–Dawley rats initially weighing 350–380 g (Charles River, Wilmington, MA, USA) were housed individually. Rats' weights were gradually reduced to ~85% of free feeding by limiting daily access to food before the start of drug-discrimination training sessions. Once drug-discrimination sessions were started, weight was maintained at about 85% of free feeding by giving about 15 g of food pellets shortly after the end of each daily session. Water was available ad libitum. Rats were housed in a temperature- and humidity-controlled room and were maintained on a 12-h light/dark cycles. The lights were on from 6:45 A.M. to 6:45 P.M and experiments were conducted during the light phase. Animals were maintained in facilities fully accredited by the American Association for the Assessment and Accreditation of Laboratory Animal Care; all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, National Institute on Drug Abuse (NIDA), National Institutes of Health and the Guide for Care and Use of Laboratory Animals (National Research Council 1996).

Drug-discrimination apparatus and procedure

Standard operant-conditioning chambers (Coulbourn Instruments, Lehigh Valley, PA, USA) were used. Each chamber contained two levers, separated by a recessed tray into which a pellet dispenser could deliver 45-mg food pellets (F0021; Bioserv, Frenchtown, NJ, USA). Each press of a lever with a force of 0.4 N through 1 mm was recorded as a response and was accompanied by an audible click. The operant-conditioning chambers were controlled by computers using the MED Associates MED-PC software package (Med Associates, East Fairfield, VT, USA). Rats were trained under a discrete-trial schedule of food-pellet delivery, as described previously (Solinas et al. 2003, 2004), to respond on one lever after an injection of a training dose of 3 mg/kg THC and on the other lever after an injection of 1 ml/kg of THC vehicle. Injections of THC or vehicle were given intraperitoneally (i.p.) 30 min before the start of the session. At the start of the session, a white house light was turned on and in its presence the rats were required to make ten consecutive responses (fixed-ratio 10 schedule of food delivery, FR10) on the lever appropriate to the pre-session treatment. The completion of ten consecutive responses on the injection-appropriate lever produced delivery of a 45-mg food pellet and initiated a 45-s time-out during which lever-press responses had no programmed consequences and the chamber was dark. Responses on the injection-inappropriate lever reset the FR requirement on the injection-appropriate lever. After each time-out, the white house light was again turned on and the next trial began. Each session ended after completion of 20 fixed-ratio trials or after 30 min has elapsed, whichever occurred first.

Discrimination-training sessions were conducted 5 days/week under a double alternation schedule (i.e., DDVV DDVV, etc.; D=drug, THC; V=Vehicle). Training continued until there were eight consecutive sessions during which rats completed at least 90% of their responses during

the session on the correct lever and no more than four responses occurred on the incorrect lever during the first trial. Test sessions were then initiated. Test sessions were identical to training sessions with the exception that ten consecutive responses on either one of the two levers were reinforced. Switching responding from one lever to the other lever reset the ratio requirement. In a test phase, a single alternation schedule was introduced and test sessions were usually conducted on Tuesdays and Fridays. Thus, a 2-week sequence starting on Monday was: DTVDTV TDVT (T=test). In this way, test sessions occurred with equal probability after saline and drug sessions. Test sessions were conducted only if the criterion of 90% accuracy and not more than four incorrect responses during the first trial was maintained in the two preceding training sessions. If rats failed to meet this criterion, training sessions were run according to the normal single alternation schedule until criterion was met for at least two consecutive sessions. The first test sessions consisted of different doses of the training drug in order to establish a THC dose–response curve. Afterwards, tests with other compounds given alone or in combination with THC began. At the end of the experiments shown in Fig. 4 and just before the experiments shown in Fig. 5, a THC dose–response curve was reestablished (shown in Fig. 5) and was not different from the dose–response curve obtained at the start of the experiment (shown in Fig. 1).

Two measures were analyzed: (1) percentage of total lever-presses made on the THC lever, which gives a quantitative indication of how much the drug or the combination of drugs tested produce discriminative effects similar to those of the 3 mg/kg training dose of THC; (2) overall rate of lever-press responding, which gives an indication of any disruption of motor responses produced by the drug or the combination of drugs tested. When rates of responding were significantly reduced compared to basal levels, administrations of higher doses of that specific drug or combination of drugs were normally avoided. The use of some combinations of THC and opioid compounds was also avoided when long-lasting disruptions of lever-press responding were found in the sessions after the administration of such combinations in pilot experiments.

Drugs

Delta-9-tetrahydrocannabinol (National Institute on Drug Abuse, Baltimore, MD, USA) 50 mg/ml in ethanol was dissolved in a solution 40% w/v of β-hydroxy-cyclodextrine (RBI-Sigma; St. Louis, MO, USA). Doses of heroin HCl (NIDA), U50488 HCl, naltrexone HCl, naltrindole HCl; nor-binaltorphimine 2 HCl (n-BNI) (all purchased from RBI-Sigma and dissolved in sterile saline) were calculated as salts. n-BNI was administered subcutaneously (s.c.) 24 h before the session. Naltrexone and naltrindole were administered i.p. 45 min before the session (15 min before THC), whereas all opioid agonists were administered i.p. 15 min before the session (15 min after THC).

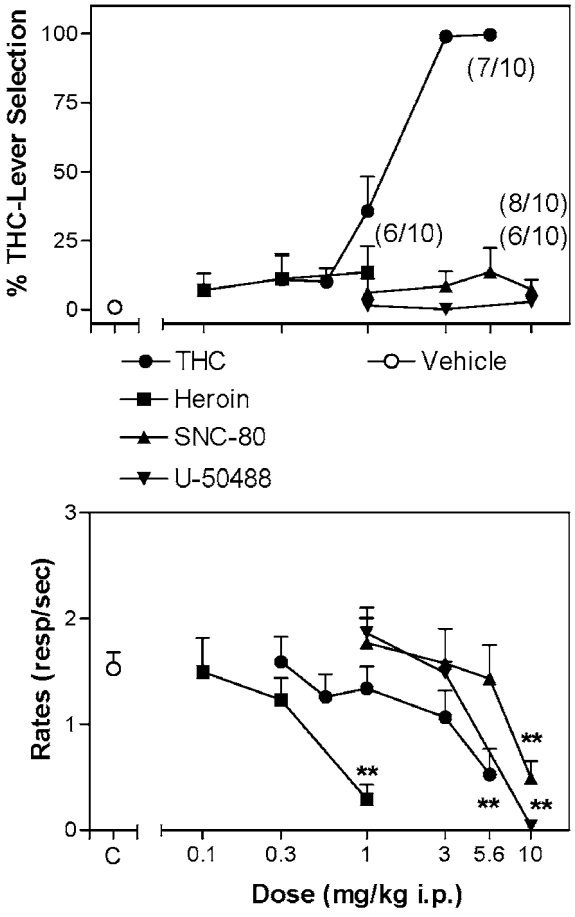

Fig. 1 Effects of heroin, SNC-80 and U50488 in rats trained to discriminate 3 mg/kg of THC from vehicle. *Ordinates*: overall percentage of responses on the lever associated with THC administration (*upper panels*) and overall rate of lever pressing expressed as responses per seconds (*lower panels*) averaged over the entire session. *Abscissae*: dose in mg/kg (log scale). Results represent means±SEM from ten rats. *C* Control value for vehicle alone. Repeated-measures ANOVA followed by post-hoc Dunnet's test: $^{**}p<0.01$ compared to saline. *Numbers in parentheses* at higher doses indicate the number of rats that completed at least one fixed ratio during the session over the total number of rats in which the dose was tested. Dose–response curve data for THC are the same as shown in Figs. 2 and 4

Data analysis

Discriminative-stimulus data were expressed as the percentage of the total responses (on both levers) that were made on the THC-appropriate lever during the entire test session. Response-rate data were expressed as responses per second averaged over the session, with responding during time-out periods not included in calculations. The data from sessions during which rats did not complete at least one fixed-ratio were excluded from analysis of drug-lever selection. All results are presented as group means (±SEM).

Statistical analysis of the ability of opioid compounds to produce generalization to the discriminative effects of the training dose of THC and the ability of opioid antagonists to reduce the discriminative effects of the training dose of

THC were carried out by using one-way ANOVA for repeated measures in comparison with vehicle treatments, followed, when appropriate, by the Dunnet's post-hoc test. A probability value of $p<0.05$ was considered significant.

The ability of opioid compounds to modulate the discriminative effects of different doses of THC, i.e., shift the dose–response curve for THC discrimination, was established by nonlinear regression analysis using a sigmoidal dose–response (variable slope) equation. ED_{50} values were obtained for THC alone and for THC in combination with opioid compounds. Shifts in the dose–response curves were considered significant when 95% confidence intervals of ED_{50} values did not overlap.

Statistical analysis of the effect of any treatment on rates of responding was done by using one-way ANOVA for repeated measures in comparison with vehicle treatment, followed, when appropriate, by the Dunnet's post-hoc test. A probability value of $P<0.05$ was considered significant.

Results

Opioid agonists do not produce generalization to the discriminative effects of THC

Rats consistently discriminated the 3-mg/kg training dose of THC (about 100% THC-lever selection, Fig. 1, upper panel) from injections of vehicle (about 0% THC-lever selection, Fig. 1, upper panel, bottom left corner) and the discriminative effects of THC were clearly dose-dependent (Fig. 1, upper panel), with an ED_{50} value of 1.222 (95% confidence intervals=0.8703–1.573). The highest dose of THC tested (5.6 mg/kg, i.p.) significantly decreased rates of responding (Fig. 2, lower panel) [$F(5,45)=5.293$, $p<0.001$] as compared to vehicle-control levels (Fig. 1, lower panel, left side).

The preferential mu-opioid receptor agonist heroin (0.1–1 mg/kg, i.p.), the selective delta-opioid receptor agonist SNC-80 (1–10 mg/kg, i.p.) and the selective kappa-opioid receptor agonist U50488 (1–10 mg/kg, i.p.) did not produce significant generalization to the discriminative effects of THC (<20% THC-lever selection, Fig. 1, upper panel), even at doses of the opioid agonists that markedly and significantly depressed rates of responding [$F(3,27)=8.552$, $p<0.001$ for heroin, $F(4,36)=6.278$, $p<0.001$ for naltrindole and $F(3,27)=40.215$, $p<0.0001$ for U50488] (Fig. 1, lower panel).

mu-Opioid receptor agonists, but not delta- or kappa-opioid receptor agonists, potentiate discriminative effects of low doses of THC

Heroin, at a dose of 0.3 mg/kg, significantly shifted to the left (i.e., potentiated) the dose–response curve for THC discrimination (Fig. 2, upper panel) ($ED_{50}=0.4706$, 95% confidence intervals=0.2599–0.6812). Combinations of 0.3 mg/kg of heroin and different doses of THC produced small and non-significant decreases in rates of responding (Fig. 2, lower panel). In contrast, combinations of 3 mg/kg of SNC-80 or 1 mg/kg of U50488 with different doses of THC did not significantly modify the dose–response curve for THC discrimination (Fig. 2, upper panel) and did not produce changes in rates of responding (Fig. 2, lower panel). The higher doses of 5.6 mg/kg of SNC-80 and 3 mg/kg of U50488 in combination with low doses of THC were tested in a few rats, but they produced long-lasting (five to ten sessions) disruptions in lever-press responding and, thus, were not further studied. However, there was no indication of a potentiation or of an attenuation of the discriminative effects of THC in these pilot experiments.

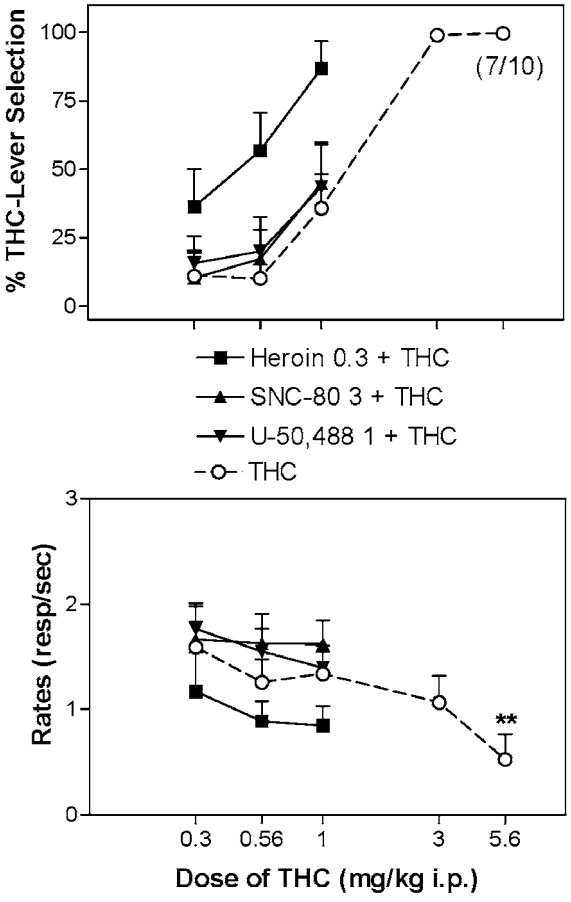

Fig. 2 Effects of heroin (0.3 mg/kg), SNC-80 (3 mg/kg) and U50488 (1 mg/kg) on the discriminative effects of THC. *Ordinates*: overall percentage of responses on the lever associated with THC administration (*upper panels*) and overall rate of lever pressing expressed as responses per seconds (*lower panels*) averaged over the entire session. *Abscissae*: dose of THC in mg/kg (log scale). Results represent means±SEM from ten rats. Repeated-measures ANOVA followed by post-hoc Dunnet's test: $^{**}p<0.01$ compared to saline. *Numbers in parentheses* at the high dose of THC indicate the number of rats that completed at least one fixed ratio during the session over the total number of rats in which the dose was tested. Dose–response curve data for THC are the same as shown in Figs. 1 and 4

Opioid antagonists do not block the discriminative effects of the 3-mg/kg training dose of THC

The preferential mu-opioid antagonist naltrexone (0.1–1 mg/kg), the selective delta-opioid antagonist naltrindole (1–10 mg/kg) and the selective kappa-opioid antagonist n-BNI (5 mg/kg) did not produce generalization to the discriminative effects of THC (Fig. 3, upper panel). In addition, none of these opioid antagonists significantly reduced the discriminative effects of the 3-mg/kg training dose of THC (<80% of THC-lever selection) (Fig. 3, upper panel). Rates of responding were significantly decreased after administration of the highest dose (10 mg/kg, i.p.) of naltrindole tested alone or in combination with training dose of THC [$F(3,27)=3.533$, $p<0.05$ and $F(3,27)=3.189$, $p<0.05$, respectively] (Fig. 3, lower panel). Since n-BNI has been shown to produce long-lasting blockade of kappa-receptors (Spanagel 1996; Spanagel et al. 1994), the establishment of a full-dose response curve for this compound was avoided. However, the 5-mg/kg dose of n-BNI used in this study has been shown to be effective in blocking the effects of kappa-opioid agonists (Spanagel et al. 1994).

mu-Opioid receptor antagonists, but not delta- or kappa-opioid receptor antagonists reduce discriminative effects of low doses of THC

A dose of 0.3 mg/kg of naltrexone significantly shifted to the right (i.e., reduced) the dose–response curve for THC discrimination (Fig. 4, upper panel) (ED_{50}=2.477, 95% confidence intervals=1.966–2.989). However, naltrexone

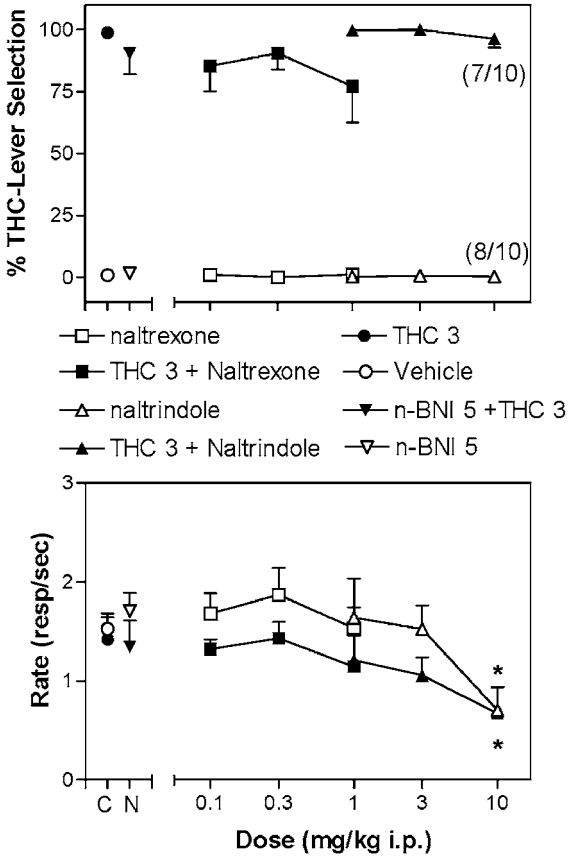

Fig. 3 Effects of naltrexone, naltrindole and nor-binaltorphimine (n-BNI) on the discriminative effects of the 3-mg/kg training dose of THC. *Ordinates*: overall percentage of responses on the lever associated with THC administration (*upper panels*) and overall rate of lever pressing expressed as responses per seconds (*lower panels*) averaged over the entire session. *Abscissae*: dose in mg/kg (log scale). *C* Control values for 3 mg/kg THC alone (*filled circle*) and THC's vehicle alone (*open circle*); *N*=values for combination of n-BNI 5 mg/kg+THC 3 mg/kg (*filled triangle*) and n-BNI 5 mg/kg alone (*open triangle*). Results represent means±SEM from ten rats. Repeated-measures ANOVA followed by post-hoc Dunnet's test: *$p<0.05$ compared to saline. *Numbers in parentheses* at higher doses indicate the number of rats that completed at least one fixed ratio during the session over the total number of rats in which the dose was tested

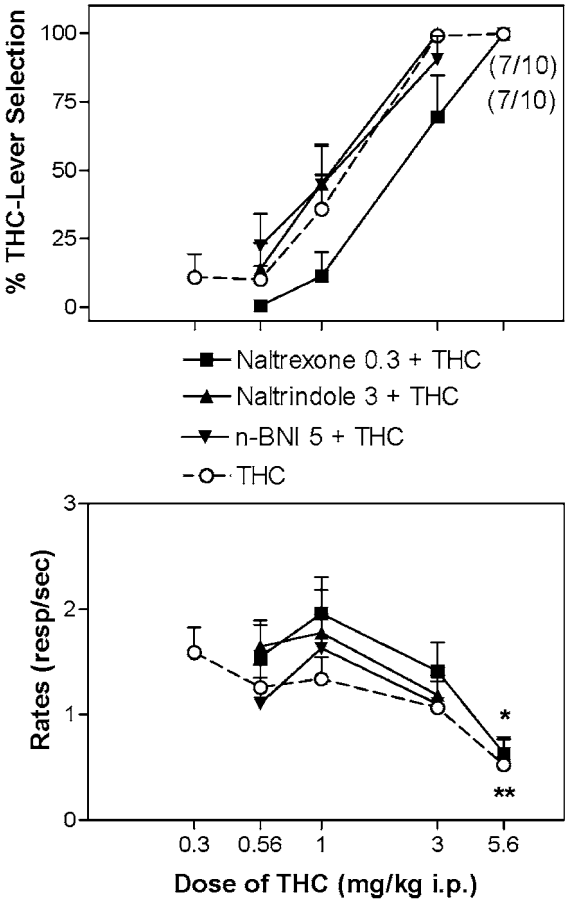

Fig. 4 Effects of naltrexone (0.3 mg/kg), naltrindole (3 mg/kg) and nor-binaltorphimine (n-BNI, 5 mg/kg) on the discriminative effects of THC. *Ordinates*: overall percentage of responses on the lever associated with THC administration (*upper panels*) and overall rate of lever pressing expressed as responses per seconds (*lower panels*) averaged over the entire session. *Abscissae*: dose in mg/kg (log scale). Results represent means±SEM from ten rats. Repeated-measures ANOVA followed by post-hoc Dunnet's test: *$p<0.05$ and **$p<0.01$ compared to saline. *Numbers in parentheses* at higher doses indicate the number of rats that completed at least one fixed ratio during the session over the total number of rats in which the dose was tested. Dose–response curve data for THC are the same as shown in Figs. 1 and 2

did not block the decreases in rates of responding produced by the high 5.6-mg/kg dose of THC [$F(4,36)=5.673$, $p<0.01$] (Fig. 4, lower panel). Neither naltrindole, at a dose of 3 mg/kg, nor n-BNI, at a dose of 5 mg/kg, significantly modified the dose–response curve for THC (Fig. 4, upper panel). Combinations of 3 mg/kg of naltrindole or 5 mg/kg of n-BNI with different doses of THC did not produce any significant change in rates of responding (Fig. 4, lower panel).

Heroin-induced potentiation depends on mu-opioid receptor activation

A replication of the THC dose–response curve (Fig. 5, upper panel), obtained after the results shown in Fig. 4, demonstrated that the ability of rats to discriminate THC did not change over time, as demonstrated by no significant difference from the initial dose–response curve (ED_{50}= 1.117, 95% confidence intervals=0.8902–1.344).

An i.p. dose of 0.3 mg/kg of naltrexone completely blocked the leftward shift in THC discrimination produced by heroin (0.3 mg/kg, i.p.) (ED_{50}=1.004, 95% confidence intervals=0.8207–1.188). In contrast, neither naltrindole (3 mg/kg, i.p.) nor n-BNI (5 mg/kg, i.p.) was able to antagonize the heroin-induced potentiation of THC discrimination (ED_{50}=0.5881, 95% confidence intervals=0.3585–0.8176 for naltrindole+THC+heroin and ED_{50}=0.5373, 95% confidence intervals=0.3197–0.7549 for n-BNI+THC+heroin). Combinations of naltrexone, naltrindole or n-BNI with THC and heroin did not significantly alter the rates of responding compared to vehicle control levels. Not only naltrexone, but also naltrindole and n-BNI, appeared to antagonize the non-significant decreases in rates of responding produced by 0.3 mg/kg of heroin in combination with different doses of THC.

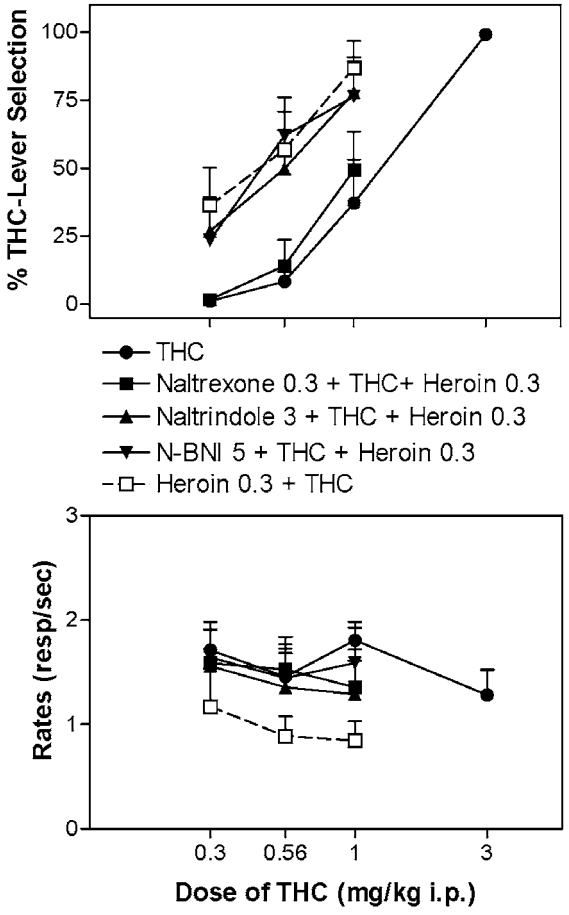

Fig. 5 Effects of naltrexone (0.3 mg/kg), naltrindole (3 mg/kg) and nor-binaltorphimine (n-BNI, 5 mg/kg) on heroin-induced potentiation of the discriminative effects of THC. *Ordinates*: overall percentage of responses on the lever associated with THC administration (*upper panels*) and overall rate of lever pressing expressed as responses per seconds (*lower panels*) averaged over the entire session. *Abscissae*: dose in mg/kg (log scale). Results represent means±SEM from ten rats. Dose–response curve data for the combinations of 0.3 mg/kg of heroin with different doses of THC are the same as shown in Fig. 2. Dose–response curve data for THC alone come from a re-evaluation of the THC dose–response curve after experiments shown in Fig. 4 and before the experiments with drug combinations shown in this figure

Discussion

We have recently demonstrated that the discriminative effects of THC can be potentiated by the opioid agonist morphine and reduced by the opioid antagonist naloxone, and that this modulation of THC's discriminative effects is related to its ability to increase the extracellular levels of beta-endorphin in the ventral tegmental area (Solinas et al. 2004). The present results confirm and extend those findings by showing that the preferential mu-opioid agonist heroin (like morphine) potentiates and the preferential mu-opioid antagonist naltrexone (like naloxone) reduces the discriminative effects of THC. We also show that selective agonists or antagonists at delta-opioid and kappa-opioid receptors do not alter the discriminative effects of THC and do not block the heroin-induced potentiation of THC discrimination. Thus, the discriminative effects of THC appear to be modulated by mu-opioid receptor activation.

Heroin is a semisynthetic analog of morphine in which two hydroxyl groups have been acetylated, rendering the molecule more lipophylic. Heroin is rapidly metabolized to morphine (and 6-monoacetylmorphine) (Kamendulis et al. 1996), which then activates opioid receptors. The present findings of potentiation of THC's discriminative effects by heroin are in complete agreement with our previous results showing that morphine potentiated THC discrimination (Solinas et al. 2004), with heroin being more potent than morphine. Both heroin and morphine are relatively selective for the mu-opioid receptor. In fact, the affinities of heroin and morphine for mu-opioid receptors are about 100 times higher than for delta- and kappa-opioid receptors (Goldstein and Naidu 1989). Consistently, the behavioral effects of heroin and morphine, especially the reinforcing effects, are believed to be mediated mainly by mu-opioid receptors (De Vries and Shippenberg 2002; van Ree et al. 1999).

Naltrexone is a long-lasting analog of naloxone with a similar opioid-receptor binding profile. The affinity of both naltrexone and naloxone for mu-opioid receptors is about ten times higher than for kappa-opioid receptors and 100 times higher than for delta-opioid receptors (Goldstein and Naidu 1989). The present findings with naltrexone are in agreement with our previous findings that naloxone reduces the discriminative effects of THC (Solinas et al. 2004). The doses of both heroin and naltrexone in the present study, as well as those of morphine and naloxone in our previous study (Solinas et al. 2004) were relatively low and this suggests a preferential, if not selective, activation or blockade of mu-opioid receptor.

It should be noted that, as previously reported for opioid agonists (Jarbe and Henriksson 1974; Wiley et al. 1995; Browne and Weissman 1981; Solinas et al. 2004), heroin did not produce THC-like effects when administered alone and, as previously reported for opioid antagonists (Jarbe and Ohlin 1977; Browne and Weissman 1981; Solinas et al. 2004), naltrexone did not completely block the effects of the training dose of THC. As we previously discussed (Solinas et al. 2004), these results suggest that the endogenous opioid system does not mediate, but instead facilitates, the discriminative effects of THC.

SNC-80 and U50488 are selective agonists at delta- and kappa-opioid receptors, respectively. SNC-80's affinity for delta-opioid receptors is about 500 times higher than for mu-opioid receptors and 300 times higher than for kappa-opioid receptors (Bilsky et al. 1995), while U50488's affinity for kappa-opioid receptors is about 50 times higher than for mu- and delta-opioid receptors (Clark and Pasternak 1988). Similarly, naltrindole and n-BNI are selective antagonists at delta- and kappa-opioid receptors, respectively. Naltrindole's affinity for delta-opioid receptors is about 1,000 times higher than its affinity for mu- and kappa-opioid receptors (Yamamura et al. 1992) and n-BNI's affinity for kappa-opioid receptors is 20 times higher than its affinity for mu- and delta-opioid receptors (Portoghese et al. 1987). The selectivity of these compounds and their lack of effects on THC discrimination strongly suggests that neither delta- or kappa-opioid receptors are involved in the discriminative effects of THC.

Some behavioral effects of THC might depend on the simultaneous activation of mu- and delta-opioid receptors. For example, it has been shown that withdrawal symptoms elicited by administration of the cannabinoid CB1 receptor antagonist SR-141716 in THC-dependent mice are attenuated in mu- and delta-opioid receptor double knockout mice, but not in either mu or delta-opioid receptor single knockout mice (Castane et al. 2003; Ghozland et al. 2002). Since heroin and naltrexone are preferential, but not highly selective, mu-opioid receptor agonists and antagonists, their potentiation and attenuation of the discriminative effects of THC could have been mediated by the simultaneous activation or blockade of mu- and delta-opioid receptors or mu- and kappa-opioid receptors. However, the fact that heroin-induced potentiation of THC's discriminative effects was not significantly reduced by naltrindole or n-BNI demonstrates that activation of mu-opioid receptors alone is sufficient to facilitate THC's effects and that only mu-opioid receptors are involved in THC's discriminative effects.

Although delta- and kappa-opioid compounds did not alter the discriminative effects of THC, both SNC-80 and U50488 at high doses produced long-lasting disruption of lever-press responding. Also, naltrindole and n-BNI did not antagonize the heroin-induced potentiation of THC discrimination but did appear to attenuate the depressant effects of combinations of heroin and THC on rates of responding. These results suggest that although they are not involved in the discriminative effects of THC, delta- and kappa-opioid receptors can interact with cannabinoid CB1 receptors and produce some behavior-depressant effects. Discrepancies between the effects of pharmacological manipulations on discriminative performance and rates of responding are not uncommon in drug-discrimination procedures. For example, in a previous study, we found that the cannabinoid CB1 antagonist SR-141716A at the dose of 3 mg/kg significantly reduced rate of responding but produced only partial blockade of THC's discriminative effects when given 45 min before the beginning of the test session and completely blocked THC's discriminative effects without producing any change on rates of responding when given 60 or 75 min before the beginning of the test session (Solinas et al. 2003). Given the nonspecific nature of rate of responding measures in drug-discrimination procedures, it is difficult to establish the cause and the meaning of the effects that lead to disruption of lever-press responding found in this study and future experiments using techniques other than drug discrimination will be needed to further investigate this issue.

The involvement of endogenous opioid systems, in particular the mu-opioid subtype of opioid receptors, in the abuse-related effects of THC has been repeatedly demonstrated (Maldonado and Rodriguez de Fonseca 2002; Manzanares et al. 1999; Tanda and Goldberg 2003). For example, systemic administration of opioid antagonists such as naltrexone or naloxone reduces intravenous self-administration of THC in monkeys (Justinova et al. 2004) or the synthetic cannabinoid CB1 agonist WIN55212-2 in mice (Navarro et al. 2001). Also, THC-induced changes in brain stimulation reward threshold in rats (Gardner and Lowinson 1991), and THC-induced dopamine elevations in the nucleus accumbens in rats (Chen et al. 1990; Tanda et al. 1997) are blocked by low doses of systemic naloxone or by local injection of the selective mu-1 opioid antagonist naloxonazine directly into the ventral tegmental area (Tanda et al. 1997). Finally, the involvement of different opioid receptor subtypes in the rewarding effects of THC measured by conditioned place preference procedures in opioid receptor deficient mice has been extensively studied (Maldonado 2002; Maldonado and Valverde 2003). In these studies, the development of THC-induced conditioned place preferences was dependent on mu-opioid activation and independent of delta-opioid receptor activity, while the aversive effects of THC (development of conditioned place aversions) that appeared at higher doses were dependent on the activation of kappa-opioid

receptors and the endogenous opioid dynorphin (Ghozland et al. 2002; Zimmer et al. 2001). The present findings demonstrate that activation of mu-opioid receptors, but not delta- or kappa-opioid receptors, contributes to the discriminative effects of THC. Given the role played by mu-opioid receptors in reward processes, including THC reward, our results indicate that similar neural processes are involved in the discriminative and in the positive, rewarding effects of THC.

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