Analgesic, Respiratory and Heart Rate Effects of Cannabinoid and Opioid Agonists in Rhesus Monkeys: Antagonist Effects of SR 141716A

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ABSTRACT

This study characterized the antinociceptive, respiratory and heart rate effects of the cannabinoid receptor agonists Δ9-tetrahydrocannabinol (Δ9-THC) and WIN 55212 (R)-(+) -2,3-dihydro-5-methyl-3-[4-morpholino]methyl]pyrrol-1,2,3-de]-1,4-benzoazin-6-yl] (1-naphthalenyl)methanone monomethanesulfonate), N-arachidonil ethanolamide (anandamide) and the mu and kappa opioid receptor agonists heroin and U69593, alone and in conjunction with a cannabinoid receptor antagonist, SR 141716A [N-(piperidin-1–1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1-

Acute administration of cannabinoid receptor agonists such as Δ9-THC, WIN 55212 (R)-(+) -2,3-dihydro-5-methyl-3-[4-morpholino]methyl]pyrrol-1,2,3-de]-1,4-benzoazin-6-yl] (1-naphthalenyl)methanone monomethanesulfonate) and CP 55940 ((-) cis-3-[2-hydroxy-4(1,1-dimethyl-heptyl)phenyl] trans-4-(3-hydroxypropyl)cyclohexanol) reliably produces antinociception (Herzberg et al., 1997; Pugh et al., 1997) and discriminative effects (Wiley et al., 1995a, 1995b), impairs tasks involving memory (Terranova et al., 1996; Lichtman and Martin, 1996) and reduces locomotor activity (Compton et al., 1996; Stark and Dews, 1980) and body temperature (Fan et al., 1994) in rodents. Chronic administration of Δ9-THC reveals the development of tolerance to the acute effects, and a withdrawal syndrome has been demonstrated (Aceto et al., 1996; Tsou et al., 1995).

Recently, a cannabinoid receptor antagonist has been synthesized and proved to be effective in reversing the effects of Δ9-THC. In vivo, pretreatment with SR 141716A blocks the antinociceptive (Compton et al., 1996), discriminative stimulus (Wiley et al., 1995c, 1995d), memory impairing (Lichtman and Martin, 1996) and hypolocomotor effects produced by Δ9-THC (Compton et al., 1996). SR 141716A also precipitates a withdrawal syndrome in rats treated chronically with Δ9-THC (Aceto et al., 1996). In vitro, SR 141716A binds selectively to central cannabinoid receptors (CB1) with high affinity (Kd = 2 nM), and blocks the inhibitory effects of cannabinoid receptor agonists in the mouse vas deferens, dopamine-stimulated adenyl cyclase (Rinaldi-Carmona et al., 1994) and WIN 55212-stimulated GTPγS binding (Selley et al., 1996).

Many of the previous in vivo investigations involving compounds targeting cannabinoid receptors have been performed...
in nonprimate species. The objectives of the current experiments were (1) to more fully characterize the antinociceptive, respiratory and heart rate effects of the cannabinoid receptor agonists Δ⁹-THC and WIN 55212 and (2) to evaluate the ability and selectivity of SR 141716A to block the antinociceptive, respiratory and heart rate effects of the cannabinoid agonists Δ⁹-THC and WIN 55212 and the opioid receptor agonists heroin and U69593 in rhesus monkeys.

Methods

Subjects

Twelve adult rhesus monkeys (Macaca mulatta) with complex experimental and drug histories were individually housed with free access to water in a vivarium maintained at 21 ± 1°C, 30% to 50% humidity and a 12:12-hr light/dark cycle. Monkeys were fed ~30 biscuits (Purina Monkey Chow) daily and fresh fruit twice weekly.

Apparatus and Procedure

Warm water tail withdrawal assay. Monkeys (n = 6) were seated in primate chairs, and the lower 10 cm of the shaved tail was immersed in a flask containing water maintained at either 40°, 50° or 55°C (Dykstra and Woods, 1986). Tail withdrawal latencies were timed manually, and a maximum latency of 20 sec was allowed to prevent tissue damage. Noninjection base-line withdrawal latencies were determined at each water temperature in a random order among the monkeys. Subsequent to base-line determinations, drugs were administered using a cumulative dosing procedure, with ascending doses of a test compound being administered at 30- (opioids) or 60- (cannabinoids) min intervals. Tail withdrawal latencies were determined at each of the three water temperatures in a random fashion —25 (opioids) or —55 (cannabinoids) min after drug administration. In antagonist studies, quazadocine or SR 141716A were administered 30 or 60 min before agonist administration, respectively.

Respiration and heart rate. Monkeys (n = 6) were seated in primate chairs and placed in ventilated, sound-attenuating primate chambers with custom-made polycarbonate respiratory helmets placed over their heads (Howell et al., 1988). The helmet was sealed around the neck of the monkeys with two polycarbonate shields. Gas chambers with custom-made polycarbonate respiratory helmets primate chairs and placed in ventilated, sound-attenuating primate chambers were individually housed with free access to water in a vivarium maintained at 21 ± 1°C, 30% to 50% humidity and a 12:12-hr light/dark cycle. Monkeys were fed ~30 biscuits (Purina Monkey Chow) daily and fresh fruit twice weekly.

Drugs

Δ⁹-THC (National Institute for Drug Abuse, Rockville, MD), WIN 55212 (Sterling Winthrop, Rensselaer, NY), N-arachidonyl-ethanolamine (anandamide; Organix, Woburn, MA) and SR 141716A (Sanofi Recherche, Montpellier, France) were dissolved in a vehicle containing emulphor, ethanol and distilled water (1:1:9). Heroin (National Institute for Drug Abuse), U69593 (Upjohn, Kalamazoo, MI), and quazazocine methanesulfate (Sterling Winthrop) were dissolved in distilled water. Pilot work revealed that tolerance developed to the antinociceptive effects of the cannabinoids rapidly; for this reason, cannabinoid test compounds were not administered more than once every 3 weeks. All drugs were administered intramuscularly at a volume of 0.1 ml/kg b.w.

Data Analysis

Tail-withdrawal data were converted to percent maximum possible effect (% MPE) with the calculation: % MPE = 100 × [(test latency — control latency)/(cutoff latency — control latency)]. Respiratory data were from the last 3-min exposure to air and 5% CO₂ and heart rate data were from the last 3-min exposure to air, during each cycle was converted to percent of vehicle control, and all data were analyzed with a one-factor (dose) repeated-measures ANOVA. When significant effects were demonstrated, post-hoc Dunnett’s t tests were performed. The α value was .05 (two-tailed).

Results

Antinociception. The cannabinoid receptor agonists Δ⁹-THC and WIN 55212 and the mu and kappa opioid receptor agonists heroin and U69593 dose-dependently increased the latency to remove the tail from a 50° and 55°C water bath (figs. 1 and 2). In 50°C water, Δ⁹-THC and WIN 55212 produced a 71% and 79% MPE [Δ⁹-THC: F(4,8) = 5.49, P < .05; WIN 55212: F(3,6) = 11.2, P < .05], whereas both heroin and U69593 produced a 100% MPE [heroin: F(5,10) = 11.4, P < .05; U69593: F(6,12) = 11449.0, P < .05], respectively. Base-line tail-withdrawal latencies are presented in table 1.

In 55°C water, the cannabinoids were less effective in producing antinociception than they were at 50°C and compared with the effects of the opioids at 55°C. At the highest doses tested, Δ⁹-THC (3.2 mg/kg) and WIN 55212 (1 mg/kg) produced a 15% and 9% MPE [Δ⁹-THC: F(4,8) = 6.02, P < .05; WIN 55212: F(3,6) = 7.65, P < .05], respectively. In contrast, the opioids were effective in abolishing the tail-withdrawal reflex [heroin: F(5,10) = 600.0, P < .05; U69593: F(6,12) = 26.9, P < .05].

In the presence of their respective antagonists (cannabinoids: SR 141716A, opioids: quazadocine), the antinociceptive effects of WIN 55212 were shifted approximately one-half log unit to the right at 50°C [F(4,8) = 10.1, P < .05] and 55°C [F(4,8) = 7.83, P < .05]. In the presence of SR 141716A (5.6 mg/kg), WIN 55212 did not produce antinociception. In contrast, SR 141716A (1.8 mg/kg) pretreatment, Δ⁹-THC did not reliably produce antinociception, and the antinociceptive effects of WIN 55212 were shifted approximately one-half log unit to the right at 50°C [F(4,8) = 10.1, P < .05; F(4,8) = 30.0, P < .05, respectively] and 55°C [F(5,10) = 1340.0, P < .05; F(4,8) = 259.5, P < .05].

The opioid antagonist quazadocine (1 mg/kg) did not alter the antinociceptive effects of Δ⁹-THC. In the presence of the quazadocine (0.1 mg/kg), heroin dose-dependently increased tail-withdrawal latencies [50°C: F(5,10) = 10.7, P < .05; 55°C: F(5,10) = 4.7, P < .05], albeit at a reduced potency. In the presence of quazadocine (1 mg/kg), U69593 dose-dependently increased tail-withdrawal latencies [50°C: F(6,12) = ...
55.7, $P < .05$; 55°C: $F(6,12) = 786.4, P < .05$, also at a reduced potency. Neither SR 141716A nor quadazocine altered base-line tail-withdrawal latencies when administered alone at these doses (data not shown).

In a separate time course study, the initial exposure to the putative endogenous cannabinoid agonist anandamide (10 mg/kg i.m.) produced a 100% MPE 30 to 60 min after administration in 50°C $[F(12,24) = 7.44, P < .05]$. In subsequent weekly anandamide challenges (1, 3.2 and 5.6 mg/kg), modest but not dose-related antinociceptive effects ($<35\%$ MPE) were observed. Anandamide (10 mg/kg) administered 4 weeks after the initial 10 mg/kg administration failed to produce an antinociceptive effect. Anandamide did not alter tail-withdrawal latencies in 55°C at any time point tested.

**Respiration.** Increased respiratory $f$, $V_t$ and $V_e$ during 5% $CO_2$ breathing were observed and are depicted in table 1. Drug effects on respiratory measures during air/5% $CO_2$ breathing were similar; only the respiratory data during 5% $CO_2$ challenges are presented. Δ-9-THC, WIN 55212, heroin and U69593 dose-dependently decreased $V_t$ and $V_e$ and did not produce reliable decreases in $f$ (figs. 3 and 4). At the highest doses tested, Δ-9-THC produced a 64% suppression of $V_t$ [1 mg/kg; $F(4,8) = 6.16, P < .05$, WIN 55212 produced a 40% [1 mg/kg; $F(3,6) = 25.8, P < .05$, heroin produced a 40% suppression of $V_t$ [0.32 mg/kg; $F(4,8) = 19.0, P < .05$] and U69593 produced a 42% suppression of $V_t$ [0.32 mg/kg; $F(5,10) = 18.4, P < .05$]. Similarly, Δ-9-THC produced a 60% suppression of $V_e$ [4.8, $F(4,8) = 9.47, P < .05$, WIN 55212 produced a 40% suppression of $V_e$ [4.8, $F(3,6) = 16.0, P < .05$, heroin produced a 40% suppression of $V_e$ [4.8, $F(4,8) = 40.2, P < .05$] and U69593 produced a 43% suppression of $V_e$ [4.8, $F(5,10) = 10.6, P < .05$]. Respiratory (and heart rate) effects of anandamide were not evaluated.

In the presence of their respective antagonists, the respi-
ratory suppressant effects of Δ-9-THC, WIN 55212, heroin and U69593 were reduced. After SR 141716A (1.8 mg/kg) pretreatment, Δ-9-THC dose-dependently decreased Vt and Ve, now requiring a dose of 10 mg/kg to produce a 27% and 39% suppression of these measures, respectively [Vt: F(4,8) = 6.16, P < .05; Ve: F(4,8) = 9.47, P > .05]. Two doses of SR 141716A were evaluated in conjunction with WIN 55212. SR 141716A (0.56 and 1.8 mg/kg) produced a dose-related inhibition of WIN 55212 respiratory depressant effects. After pretreatment with the lower dose of SR 141716A, WIN 55212 (1 mg/kg) produced a 20% suppression of Vt [F(3,6) = 5.94, P < .05] and a 29% suppression of Ve [F(3,6) = 15.9, P < .05], and pretreatment with the higher dose of SR 141716A abolished the ability of WIN 55212 to suppress these respiratory measures. In contrast, SR 141716A (1.8 mg/kg) did not alter the respiratory suppressant effects of heroin or U69593; heroin and U69593 dose-dependently decreased Vt [F(4,8) = 6.08, P < .05; F(5,10) = 5.17, P < .05, respectively] and Ve [F(4,8) = 9.98, P < .05; F(5,10) = 4.48, P < .05, respectively].

Quadazocine (1 mg/kg) did not alter the respiratory suppressant effects of Δ-9-THC or WIN 55212. In the presence of quadazocine, Δ-9-THC and WIN 55212 dose-dependently decreased Vt [F(4,8) = 8.92, P < .05; F(3,6) = 9.34, P < .05, respectively] and Ve [F(4,8) = 34.3, P < .05; F(3,6) = 21.1, P < .05, respectively]. After pretreatment with quadazocine (0.1 mg/kg), the potency of heroin to reduce Vt and Ve was decreased [F(4,8) = 4.95, P < .05; F(4,8) = 7.33, P < .05, respectively]. Similarly, pretreatment with quadazocine (1 mg/kg) reduced the potency of U69593 to decrease Vt [F(5,10) = 9.12, P < .05] and Ve (P = N.S.). Neither SR 141716A nor quadazocine altered base-line respiratory measures (data not shown).

Heart rate. Δ-9-THC dose-dependently decreased heart rate (fig. 5). At the highest doses tested, Δ-9-THC and WIN 55212 reduced heart rate to 70% of control [Δ-9-THC: F(4,8) = 11.6, P < .05; WIN 55212: P = N.S.], and heroin and U69593 reduced heart rate to 80% of control [heroin: F(4,8) = 4.03, P < .05; U69593: P = N.S.].

SR 141716A (1.8 mg/kg), but not quadazocine (1 mg/kg), reduced the potency of Δ-9-THC to reduce heart rate [F(5,10) = 3.52, P < .05; F(4,8) = 30.3, P < .05, respectively]. In contrast, quadazocine (0.1 mg/kg), but not SR 141716A
(1.8 mg/kg), reversed the suppressive effects of heroin on heart rate \[F(5,10) = \text{n.s.}; F(4,8) = 3.61, P < .05, \text{ respectively}\]. Neither SR 141716A nor quadazocine altered the baseline heart rate (data not shown).

**Discussion**

The current experiments reveal that cannabinoid receptor agonists such as Δ-9-THC, WIN 55212 and anandamide produce antinociception and suppress respiratory function and heart rate. These effects were reversed with the cannabinoid receptor antagonist SR 141716A but not the opioid receptor antagonist quadazocine, thus revealing a cannabinoid receptor mechanism of action. Despite data that support the proposal that cannabinoid and opioid systems may modulate common behavioral events (e.g., antinociception; Reche et al., 1996a; Welch, 1994), the current data were ineffective in revealing such an interaction.

The antinociceptive effects of acutely administered cannabinoid receptor agonists are quite reliable. Previously, Δ-9-
THC, WIN 55212, CP 55940 and anandamide were effective in increasing tail-flick latencies and reducing stretch reflexes induced by p-phenylquinone in rats and mice (Herzberg et al., 1997; Pugh et al., 1997; Compton et al., 1996; Smith et al., 1994). Similarly, these agonists produced antinociception in rhesus monkeys, yet their efficacy as antinociceptive agents was limited. As observed in the current experiment, all three of the tested cannabinoids failed to produce an antinociceptive effect against more intense thermal stimuli, specifically 55°C water. This is in contrast to heroin and U69593, which produced 100% MPE values at the highest doses tested. Pretreatment with SR 141716A reduced the antinociceptive potency of Δ-9-THC and WIN 55212, but not heroin and U69593, providing further in vivo evidence that SR 141716A is an effective and selective cannabinoid receptor antagonist.

The interaction of antinociceptive effects produced by cannabinoids and opioids in mice is well documented. The mu opioid receptor agonists morphine and DAMGO produced parallel and leftward shifts in the antinociceptive effects produced by Δ-9-THC (Reche et al., 1996a), and the administration of Δ-9-THC decreased the antinociceptive ED$_{50}$ values produced by morphine (Pugh et al., 1997; Welch and Stevens, 1992). Furthermore, dynorphin antisera, a kappa opioid antisense oligonucleotide, and the kappa opioid receptor antagonist norbinaltorphimine blocked Δ-9-THC antinociception in rodents (Pugh et al., 1995, 1997; Reche et al., 1996b). SR 141716A has been found to inhibit morphine antinociception (Compton et al., 1996). In the current experiments, there was no evidence for a cannabinoid/opioid interaction because SR 141716A failed to alter the antinociception produced by heroin or U69593 and quazadoxine, at a dose that targeted both mu and kappa receptor types, failed to alter the antinociceptive effects of Δ-9-THC. Previously, failure of nonselective opioid antagonists to alter cannabinoid-induced antinociception has been demonstrated, and interactions between opioids and cannabinoids have been most evident through the use of selective kappa antagonists and intrathecal administration of cannabinoid agonists (Welch et al., 1995; Welch, 1994).

In humans, Δ-9-THC produced inconsistent effects on respiratory measures: decreased tidal volume (Johnstone et al., 1975), no change in tidal volume (Malit et al., 1975) and increased respiration rate (Mathew et al., 1992) have been observed. In rats, Δ-9-THC and Δ-8-THC dose-dependently decreased respiration rate (Estrada et al., 1987), yet cannabinoid receptor agonist effects on respiration in rhesus monkeys are undocumented. In the current experiments, both Δ-9-THC and WIN 55212 produced dose-related decreases in minute and tidal volume, while not affecting respiratory frequency. These respiratory suppressant effects were prevented by SR 141716A, but not quazadoxine, revealing a cannabinoid mechanism of action. Similar to the results from the antinociceptive experiments (described above), there were no indications of an interaction between the cannabinoid and opioid systems influencing respiratory function in that quazadoxine did not alter cannabinoid respiratory effects and SR 141716A failed to change the respiratory effects of both heroin and U69593.

Heart rate effects of Δ-9-THC are robust in rats and humans, although the direction of the effect diverges. In humans, smoked marijuana produced tachycardia, and this increased heart rate was further demonstrated after oral administration of Δ-9-THC (e.g., Chait and Burke, 1994; Zacny and Chait, 1992). In rats, bradycardia was observed after cannabinoid administration: Δ-9-THC, Δ-8-THC and RU-210 (−11-1OH-Δ-8-tetrahydrocannabinol-dimethylheptyl) decreased the heart rate in rats (Bidrio et al., 1996; Estrada et al., 1987; Hine et al., 1977). In monkeys, increased and decreased heart rates have been observed after the administration of Δ-9-THC. When administered intraperitoneally, Δ-9-THC (0.75–4 mg/kg) dose-dependently decreased heart rate, producing a maximal (40%) suppression at the highest dose tested (Matsuzaki et al., 1987). Conversely, when administered intravenously, Δ-9-THC (0.5 mg/kg) produced an increase in heart rate (Fredericks et al., 1981). In the current experiment, intramuscular Δ-9-THC and WIN 55212 dose-dependently decreased heart rate, an effect that was antagonized in the presence of SR 141716A, suggesting cannabinoid receptor mediation. Whether the route of administration is an important factor for the heart rate effects of Δ-9-THC remains unknown. Although quazadoxine failed to alter the heart rate effect of Δ-9-THC, there is evidence that cross-tolerance develops to the THC- and morphine-induced decrease in heart rate after a regimen of morphine (50 mg/kg/day × 23 days) or Δ-9-THC (10 mg/kg/day × 7 days; Hine, 1985). The use of selective opioid antagonists in conjunction with acutely or chronically administered cannabinoids might help to further illuminate cannabinoid-opioid interactions on heart rate.

The current experiments demonstrated the ability of SR 141716A to selectively block cannabinoid antinociceptive, respiratory and heart rate effects in rhesus monkeys. In general, each of the tested agonists (cannabinoids and opioids) produced dose-related effects that were reversed through the use of the appropriate antagonist. SR 141716A is an important tool in the understanding of cannabinoid pharmacology and neurobiology, and its apparent affinity for the central cannabinoid receptor (CB1; Rinaldi-Carmona et al., 1996, 1995) will help to elucidate central and peripheral actions of cannabinoid receptor systems. Although the neurobiology of cannabinoids is becoming better understood, it must be noted that the effectiveness of Δ-9-THC as an analgesic agent is limited. Most important, Δ-9-THC is not particularly effective as an analgesic against more intense pain, and untoward side effects, including respiratory suppression and the rapid development of tolerance, are manifest at doses that produce analgesic effects.

References


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