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RAPID COMMUNICATION

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Local administration of Δ^9-tetrahydrocannabinol attenuates capsaicin-induced thermal nociception in rhesus monkeys: a peripheral cannabinoid action

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Abstract *Rationale:* Cannabinoids can reduce nociceptive responses by acting on peripheral cannabinoid receptors in rodents. *Objectives:* The study was conducted to evaluate the hypothesis that local administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) can attenuate capsaicin-induced nociception in rhesus monkeys. *Methods:* Capsaicin (100 µg) was applied locally in the tail of rhesus monkeys to evoke a nociceptive response, thermal allodynia, in normally innocuous 46°C water. Δ^9-THC (10–320 µg) was coadministered with capsaicin in the tail to assess local antinociceptive effects. In addition, a local antagonism study was performed to confirm the selectivity of Δ^9-THC action. *Results:* Δ^9-THC dose-dependently inhibited capsaicin-induced allodynia. This local antinociception was antagonized by small doses (10–100 µg) of the cannabinoid CB_1 antagonist, SR141716A, applied in the tail. However, 100 µg SR141716A injected subcutaneously in the back did not antagonize local Δ^9-THC. *Conclusions:* These results indicate that the site of action of locally applied Δ^9-THC is in the tail. It provides functional evidence that activation of peripheral cannabinoid CB_1 receptors can attenuate capsaicin-induced thermal nociception in non-human primates and suggests a new approach for cannabinoids in pain management.

Key words Capsaicin · Antinociception · Peripheral cannabinoid receptor · Inflammatory pain

Introduction

Since the cannabinoid receptor was cloned (Matsuda et al. 1990) and a selective cannabinoid antagonist was developed (Rinaldi-Carmona et al. 1994), there is a growing literature characterizing behavioral and biochemical effects of cannabinoids (for reviews, see Pertwee 1997; Felder and Glass 1998). Cannabinoid agonists may be effective treatments for nausea associated with chemotherapy, pain, migraine, and epilepsy. The major active constituent of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC), has been shown to possess antinociceptive function in rodents and monkeys (Sofia et al. 1973; Lichtman and Martin 1991; Compton et al. 1996; Vivian et al. 1998). It is well documented that cannabinoids produce antinociception at the spinal and supraspinal levels of the central nervous system (Lichtman and Martin 1991; Lichtman et al. 1996; Meng et al. 1998). However, complications of Δ^9-THC use in humans include decreased blood pressure, drowsiness, distortion of reality, and depersonalization (Voth and Schwartz 1997). Considering its therapeutic potential, it is valuable to explore the possibility of the peripheral action of cannabinoids in different experimental pain models.

Recently, two rodent studies have reported that cannabinoids reduce hyperalgesia and inflammation by acting on peripheral cannabinoid CB_1 receptors (Calignano et al. 1998; Richardson et al. 1998). In particular, cannabinoids inhibit carrageenan-induced hyperalgesia and neurosecretion from isolated hindpaw skin evoked by capsaicin (Richardson et al. 1998), which can be reversed by a selective CB_1 receptor antagonist, SR141716A (Rinaldi-Carmona et al. 1994; Pertwee 1997). Given the evidence that activation of the cannabinoid CB_1 receptors can inhibit adenylate cyclase and block certain calcium channels (Mackie and Hille 1992; Pertwee 1997), it is possible that cannabinoid inhibition of neurosecretion and decreased excitability from primary afferent fibers contribute to attenuation of nociceptive responses.

Previously, we have characterized capsaicin-induced nociception in non-human primates (Ko et al. 1998). Exposure of nociceptor terminals such as C-fibers to capsaicin initially leads to excitation of the neuron and the subsequent painful perception and local release of inflammatory pain mediators such as substance P and calcitonin gene-related peptide (Holzer 1991; Winter et al.

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1995; Caterina et al. 1997). Capsaicin-sensitive nerve fibers play an important role in many types of nociceptive conditions such as arthritis (Winter et al. 1995). It has been reported that topical or intradermal administration of capsaicin to human skin produces burning pain and allodynia/hyperalgesia responses (Simone et al. 1989). After capsaicin was subcutaneously administered into the tail of rhesus monkeys, it dose-dependently produced thermal allodynia, which was manifested as reduced tail-withdrawal latencies in normally innocuous warm water. More interestingly, when small, systemically inactive doses of opioid agonists were coadministered with capsaicin in the tail, they locally inhibited nociceptive responses (Ko et al. 1998, 1999). Thus, this experimental pain model could be used to investigate the function of peripheral cannabinoid receptors.

The aim of this study was to evaluate the hypothesis that local administration of a prototypical cannabinoid ligand, Δ^9-THC, can attenuate capsaicin-induced nociception in rhesus monkeys. In addition, a local antagonism study was performed to investigate the possible role of peripheral cannabinoid receptors in this procedure.

Materials and methods

Subjects

One male and three female adult rhesus monkeys (*Macaca mulatta*) with body weights ranging between 7.7 and 12.2 kg (their mean weight during this study was 10.5 kg) were used. They were housed individually with free access to water and were fed approximately 25–30 biscuits (Purina Monkey Chow) and fresh fruit daily. All monkeys had experience in the tail-withdrawal procedure and had previously received opioids. These subjects did not have exposure to capsaicin and other analgesics for 1 month before the present study.

Animals used in this study were maintained in accordance with the University Committee on the Use and Care of Animals in the University of Michigan, and the Guide for the Care and Use of Laboratory Animals (7th edn) by the Institute of Laboratory Animal Resources (National Academic Press, Washington D.C., revised 1996).

Procedure

Thermal antinociception was measured by a warm water tail-withdrawal procedure which has been previously described (Ko et al. 1998). Briefly, the subjects were seated in restraint chairs and the lower part of the shaved tail (approximately 15 cm) was immersed into warm water maintained at temperatures of 42, 46, and 50°C. Tail-withdrawal latencies were timed manually by an experimenter. A maximum cutoff latency (20 s) was recorded if the subjects failed to remove their tails by this time. Each experimental session began with control determinations at each temperature. Subsequent tail-withdrawal latencies were determined at 5, 15, 30, 45, and 60 min following injection. The subjects were tested 1–2 times at three temperatures in a varying order, with approximately a 1- to 2-min interval between tests. Experimental sessions were conducted once per week. A single dosing procedure was used in all test sessions.

Experimental designs

Capsaicin was injected subcutaneously (SC) in the terminal 1–4 cm of the tail, in a constant 0.1 ml volume. In this procedure, the small amount of capsaicin dose-dependently produced transient allodynia (5–30 min) (Ko et al. 1998). Based on this former study, 100 µg capsaicin was chosen as a standard noxious stimulus in 46°C water for the present study.

Δ^9-THC (10–320 µg) was coadministered with capsaicin in the tail to assess local antinociceptive effects in 46°C water. A maximum effective dose of Δ^9-THC (320 µg) was also administered in the back against 46°C water in the presence of capsaicin, or was administered in the tail against 50°C water in the absence of capsaicin. Given that onset and distribution factors may be minimized with local administration, SR141716A (3.2–100 µg) was coadministered with capsaicin and Δ^9-THC in the tail, in order to investigate local antagonist effects. The highest locally effective dose of SR141716A was injected SC in the back, to verify whether the antagonist effect was localized in the tail. In addition, an opioid antagonist, quadazocine (100 µg), was coadministered with capsaicin and Δ^9-THC in the tail, in order to confirm the selectivity of local Δ^9-THC action. In this preparation, the results of the locally effective dose of Δ^9-THC (320 µg) and SR141716A (100 µg) were verified by two, different experimenters in the same subjects.

Data analysis

The 15-min time point was used for analysis because this was the time of peak effects of capsaicin and locally applied analgesics (Ko et al. 1998, 1999). Individual tail withdrawal latencies were converted to percent of maximum possible effect (%MPE) by the following formula: %MPE=[(test latency–control latency)/(cutoff latency, 20 s–control latency)]×100. Individual control latencies were averaged from two determinations following application of 100 µg capsaicin in the tail in 46°C water. The mean ED_{50} value of Δ^9-THC was obtained after log transformation of individual ED_{50} values, which were calculated by least-squares regression using the portion of the dose-effect curves spanning the 50% MPE; and the 95% confidence limit (95% CL) was also determined. The mean ID_{50} value of SR141716A was determined in the same manner by defining the dose which inhibited the 50% MPE induced by local Δ^9-THC. In addition, the dose-dependent effects were analyzed with one-way ANOVA followed by the Newman-Keuls test ($P<0.01$).

Drugs

Δ^9-THC (National Institute on Drug Abuse, Rockville, Md., USA) and SR141716A (Sanofi Recherche, Montpellier, France) were dissolved in a vehicle of emulphor/95%ethanol/sterile water in a ratio of 1:1:8. Quadazocine methanesulfonate (Sanofi, Malvern, Pa., USA) was dissolved in sterile water. Capsaicin (Sigma, St Louis, Mo., USA) was dissolved in a vehicle of Tween 80/95% ethanol/saline in a ratio of 1:1:8. For local coadministration, all compounds were mixed in a bottle and injected in 0.1 ml volume in the tail.

Results

Normally, the monkeys kept their tails in 42 and 46°C water until the cutoff time (20 s), which indicated that both temperatures were innocuous. In contrast, they removed their tails from 50°C water rapidly, typically within 1–3 s. When 100 µg capsaicin was injected into the tail, it evoked a nociceptive response, thermal allodynia, which was shown as reduced tail-withdrawal latencies. In particular, from 5 min following injection, capsa-

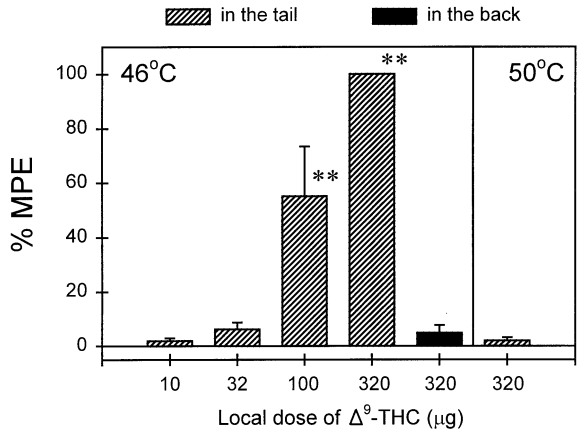

Fig. 1 Local antinociceptive effects of Δ^9-THC administered in the tail (*hashed bars*) or in the back (*filled bars*) against 46°C water in the presence of capsaicin (100 μg) or 50°C water in the absence of capsaicin. Each value represents the mean±SEM (*n*=4). *Asterisks* represent a significant difference (**P<0.01) from control. *Abscissa*: Δ^9-THC local doses in μg. *Ordinate*: percent of maximum possible effect (%MPE). Each data point was obtained 15 min after injection. See Materials and methods for other details

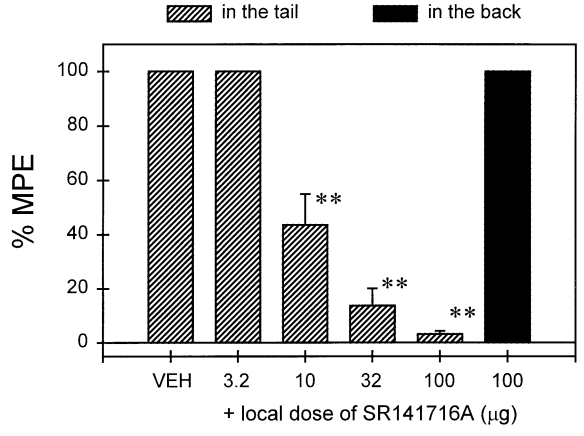

Fig. 2 Local antagonist effects of SR141716A administered in the tail (*hashed bars*) and in the back (*filled bars*) against local Δ^9-THC in 46°C water in the presence of capsaicin. *VEH* represents the vehicle effect in the condition of coadministration of 100 μg capsaicin and 320 μg Δ^9-THC in the tail. Other details as in Fig. 1

icin caused rapid tail-withdrawal latencies of approximately 2 s in 46°C water and this effect lasted for 30 min (Ko et al. 1998).

Figure 1 illustrates that coadministration of Δ^9-THC (10–320 μg) with capsaicin (100 μg) in the tail inhibited capsaicin-induced thermal allodynia in 46°C water in a dose-dependent manner (ED_{50}: 94 μg; 95% CL: 41.1–216.6 μg). However, the highest dose of Δ^9-THC (320 μg), when applied in the back, was not effective against capsaicin; and it was not locally effective against 50°C water in the absence of capsaicin. Although the 15-min time point was used to analyze the data, it was worth noting that this ineffectiveness was observed over 1 h in the test session. In addition, the locally effective dose of Δ^9-THC did not produce any behavioral changes such as sedation following its injection.

Figure 2 illustrates that local administration of SR141716A (3.2–100 μg) antagonized the local antinociceptive effects of Δ^9-THC (320 μg) against capsaicin in a dose-dependent manner (ID_{50}: 9.5 μg; 95% CL: 4.8–18.8 μg). When a locally effective dose of SR141716A (100 μg) was applied in the back, it did not antagonize local Δ^9-THC. After this dose of SR141716A was injected alone in the tail, there were no reduced or elevated tail-withdrawal latencies in 46 and 50°C water, respectively; and SR141716A also did not interfere with capsaicin-induced nociceptive responses after it was coadministered with capsaicin in the tail (data not shown). In addition, local administration of quadazocine (100 μg) did not antagonize local Δ^9-THC in this preparation (data not shown).

Discussion

Local administration of a cannabinoid agonist Δ^9-THC inhibited capsaicin-induced thermal nociception in rhesus monkeys. The locally effective dose of Δ^9-THC, when applied in the back, did not inhibit capsaicin-induced allodynia (Fig. 1). This indicates that the site of Δ^9-THC-induced antinociception against capsaicin may be located in the tail. A similar observation was also reported in a rodent study, supporting a local site of action (Richardson et al. 1998). The systemic dose of Δ^9-THC to produce thermal antinociception is 3.2 mg/kg by an intramuscular route. At this dose, monkeys display severe respiratory depression, reduced heart rate, and sedation (Vivian et al. 1998). In contrast, the peripherally effective dose of Δ^9-THC (100–320 μg) did not cause any behavioral changes in the present study. This observation strengthens the notion that peripheral antinociception can be achieved by local administration of compounds into the injured tissue without producing central side effects (Stein 1995; Ko et al. 1998, 1999). To our knowledge, it is the first report of Δ^9-THC exerting such an action in non-human primates.

When a noxious thermal stimulus, 50°C water, was assessed in the absence of capsaicin, local application of Δ^9-THC did not produce antinociception (Fig. 1). This was similar to opioid analgesic studies, in which the antinociceptive potency of opioid agonists is enhanced on the peripheral terminals of nociceptive primary afferents innervating inflamed tissue, but not in normal tissue (Stein 1995; Nagasaka et al. 1996; Ko et al. 1998). The activity of peripheral sensory fibers is dynamically regulated by the products of tissue injury and inflammation as well as by a number of exogenous irritant chemicals (Dray 1997). The mechanisms by which cannabinoids act under these circumstances remain unknown.

Capsaicin evokes pain sensations by activating C-fiber nociceptors and stimulating the release of neuropeptides such as substance P and calcitonin gene-related

peptide (CGRP) from primary nociceptive afferents (Holzer 1991; Winter et al. 1995; Caterina et al. 1997). Both substance P and CGRP play an important role in neurogenic inflammation and contribute to the transmission of nociceptive information. Activation of peripheral cannabinoid CB_1 receptors have been shown to inhibit the release of CGRP from capsaicin-sensitive primary afferent fibers (Richardson et al. 1998). In addition, in vitro studies also showed that cannabinoids can inhibit adenylate cyclase and block N-type and P/Q-type calcium channels in membranes of cultured cells expressing CB_1 receptors (Mackie and Hille 1992; Pertwee 1997). These mechanisms may account for the inhibitory effects of cannabinoids against nociception induced by capsaicin or other irritant agents (Calignano et al. 1998; Richardson et al. 1998; present study). Nevertheless, the extent to which cannabinoids can relieve pain in clinical situations remains to be determined.

Local administration of SR141716A, a cannabinoid CB_1 receptor antagonist, dose-dependently antagonized the local inhibition of Δ^9-THC against capsaicin-induced allodynia (Fig. 2). However, the locally effective dose of SR141716A (100 µg), when applied in the back, did not antagonize local Δ^9-THC. In particular, the peripherally effective dose of SR141716A (32–100 µg) was much less than the systemically effective dose (1.8 mg/kg) in rhesus monkeys with the mean body weight of 10 kg (Vivian et al. 1998). This observation confirms the local agonist study, indicating that the site of action of locally applied Δ^9-THC is in the tail. SR141716A displays the high selectivity for CB_1 receptors in vitro and it has been shown to reverse behavioral effects induced by cannabinoids including Δ^9-THC (Compton et al. 1996; Pertwee 1997; Vivian et al. 1998). It was reported that SR141716A produced and prolonged hyperalgesia measured by formalin-evoked nociception in mice, indicating the involvement of endogenous cannabinoids (Calignano et al. 1998). However, both systemic and local administration of SR141716A did not change baseline pain threshold and capsaicin-induced nociception in rhesus monkeys (Vivian et al. 1998; present study). In addition, local administration of an opioid antagonist, quadazocine (100 µg), did not antagonize local Δ^9-THC. The same dose of quadazocine has been shown to antagonize the local antinociceptive effects of *mu* and *kappa* opioid agonists in the same procedure (Ko et al. 1998, 1999). This lack of quadazocine antagonism against Δ^9-THC supports the previous study, showing the selective antagonism of cannabinoid behavioral effects by SR141716A in rhesus monkeys (Vivian et al. 1998). These antagonism studies indicate that local Δ^9-THC produces antinociception against capsaicin mainly via peripheral cannabinoid CB_1 receptors in this species.

In summary, the present study showed that local administration of Δ^9-THC significantly diminished capsaicin-induced thermal nociception in non-human primates. The antagonist study confirmed that this local antinociception was in the tail and could be mediated by cannabinoid CB_1 receptors. These results support the hypothesis that activation of peripheral cannabinoid receptors can relieve nociception induced by capsaicin, which is thought to be mediated by stimulating primary afferent C-fibers. This experimental pain model is useful for evaluating peripherally antinociceptive action and suggests a new approach utilizing cannabinoids in pain management.

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