Cannabinoid Agonist, CP 55,940, Prevents Capsaicin-Induced Sensitization of Spinal Cord Dorsal Horn Neurons

Lisa M. Johanek and Donald A. Simone

Graduate Program in Neuroscience and Departments of Oral Sciences, Neuroscience, and Psychiatry, University of Minnesota, Minneapolis, Minnesota

Submitted 2 July 2004; accepted in final form 27 August 2004

Johanek, Lisa M. and Donald A. Simone. Cannabinoid agonist, CP 55,940, prevents capsaicin-induced sensitization of spinal cord dorsal horn neurons. J Neurophysiol 93: 989–997, 2005. First published September 22, 2004; doi:10.1152/jn.00673.2004. Low doses of cannabinoids applied intrathecally attenuate capsaicin-evoked heat and mechanical hyperalgesia via CB1 receptors. Although cannabinoids produce antinoceception, in part, by attenuating responses of nociceptive neurons in the spinal cord, few studies have examined the effect of cannabinoids on sensitization of spinal neurons. We therefore investigated whether a cannabinoid receptor agonist, CP 55,940, attenuated excitation and sensitization of spinal nociceptive neurons produced by intraplantar injection of 0.1% capsaicin (10 μl). In rats, wide-dynamic-range (WDR) and high-threshold (HT) neurons were classified according to responses evoked by mechanical stimuli of varying intensity. CP 55,940 (10 μg in 50 μl) or vehicle was applied directly to the spinal cord and responses to mechanical (von Frey monofilament) and heat stimuli were recorded 10 min after drug treatment. CP 55,940 alone did not alter responses to mechanical stimuli; however the enhanced responses to mechanical stimuli after injection of capsaicin into the receptive field were dose dependently attenuated in both HT and WDR neurons. Vehicle-treated neurons increased their response to 300.6 ± 52.1% of baseline after capsaicin, whereas CP 55,940-treated neurons responded at 153.0 ± 27.1% of baseline. The effects of CP 55,940 on sensitization of heat were less pronounced; however, CP 55,940 attenuated the capsaicin-evoked decrease in heat threshold in HT neurons. The attenuation by CP 55,940 of sensitization to mechanical stimuli was blocked by pretreatment of the spinal cord with the CB1 receptor antagonist, SR141716A. These studies demonstrate that cannabinoid application to the spinal cord prevents central sensitization.

INTRODUCTION

Several lines of evidence suggest that spinal cannabinoid receptors play a role in cannabinoid-mediated antinoceception and antihyperalgesia. First, cannabinoid receptors have been identified in the dorsal horn of the spinal cord. CB1 receptors are thought to be located on dorsal root ganglion cell bodies and on the central terminals of primary afferent fibers (Ahluwalia et al. 2000; Hohmann and Herkenham 1998; Khasabova et al. 2002; Ross et al. 2001; Salio et al. 2002b). Interneurons within the spinal cord also exhibit CB1 receptor immunoreactivity on their dendrites and cells bodies (Farquhar-Smith et al. 2000; Salio et al. 2002b). Second, intrathecal injection of cannabinoid receptor agonists produces antinoceception and antihyperalgesia via a CB1 receptor mechanism (Fox et al. 2001; Johanek et al. 2001; Lichtman and Martin 1991; Mao et al. 2000; Martin et al. 1999; Richardson et al. 1998). Finally, cannabinoids attenuate responses of dorsal horn neurons evoked by noxious mechanical and heat stimuli (Hohmann et al. 1995, 1999), inhibit activity-dependent facilitation (windup) of spinal neurons (Strangman and Walker 1999), and reduce C- and Aδ-fiber-evoked responses of dorsal horn neurons (Drew et al. 2000; Kelly and Chapman 2001).

Hyperalgesia can be localized to both the site of injury (primary hyperalgesia) and within surrounding, uninjured tissue (secondary hyperalgesia) (Hardy et al. 1950; Lewis 1936). Primary hyperalgesia is characterized by enhanced sensitivity to both heat and mechanical stimuli; only mechanical hyperalgesia is usually detected in the secondary zone (for review: Treede et al. 1992). Differences in the location and corresponding modality of hyperalgesia may arise from the differential activation and sensitization of neurons located in the peripheral and CNS. Whereas sensitization of nociceptors contributes to primary hyperalgesia, central sensitization contributes to primary and secondary hyperalgesia.

Capsaicin, the pungent ingredient in hot peppers, has been used to produce hyperalgesia and central sensitization. In rats, intraplantar (ipl) injection of capsaicin elicits nocifensive behavior characterized by licking, lifting, and shaking the injected paw (Gilchrist et al. 1996). Capsaicin also produces a decrease in withdrawal latency to radiant heat (heat hyperalgesia) as well as an increase in withdrawal frequency to mechanical stimuli applied to the injected paw (mechanical hyperalgesia). Intracutaneous injection of capsaicin into the receptive field (RF) of nociceptive dorsal spinal horn neurons produces an initial burst of excitation followed by sensitization to heat and mechanical stimuli (Khasabov et al. 2002; Simone et al. 1991).

We have shown that intrathecal application of a cannabinoid agonist attenuates capsaicin-evoked hyperalgesia via spinal CB1 receptors (Johanek et al. 2001). In the present study, we examined whether spinal administration of a cannabinoid receptor agonist, CP 55,940, produces a corresponding attenuation of capsaicin-evoked sensitization of spinal dorsal horn neurons. We investigated the effect of cannabinoids on evoked responses of both wide-dynamic-range (WDR) and high-threshold (HT) neurons and determined whether CP 55,940 attenuated capsaicin-induced excitation and sensitization of nociceptive neurons. A preliminary report has appeared (Johanek and Simone 2002).
METHODS

Subjects

Adult male Sprague Dawley rats (n = 73, Harlan Industries, Indianapolis, IN) weighing 290–500 g were housed on a 12-h light-dark cycle and given food and water ad libitum. Experiments were approved by the Animal Care Committee at the University of Minnesota and were conducted according to the guidelines set forth by the International Association for the Study of Pain.

Electrophysiological recordings

Rats were anesthetized by intramuscular injection of ketamine (100 mg/kg) and acepromazine (45 mg/kg). A catheter was inserted into the external jugular vein for supplemental anesthesia with pentobarbital sodium (10 mg·kg⁻¹·h⁻¹). Anesthesia was assessed by monitoring the corneal reflex at frequent intervals throughout the experiment. The trachea was cannulated to provide an unrestricted airway. The animal was placed in a stereotaxic frame, and the lumbar enlargement was exposed by a laminectomy. The vertebral column was then secured in a spinal frame. The spinal cord was continually bathed in a pool of warm (37°C) buffered saline (in mM) 25 HEPES, 135 NaCl, 2.5 CaCl₂, 2H₂O, 3.5 KCl, 1 MgCl₂, 6H₂O, and 3.3 glucose, pH 7.4. Core body temperature was maintained at 37°C by a feedback-controlled heating pad.

Stainless steel microelectrodes (Frederick Haer, Brunswick, ME; 10 MΩ) were used to obtain extracellular recordings of single dorsal horn neurons with RFs located on the plantar surface of the hindpaw. Recording electrodes were lowered into the spinal cord in 5-μm steps at the L4 and L5 segments using an electronic microdrive (Burleigh Instruments, Fishers, NY). Recordings were made only from single neurons the amplitude of which could be easily discriminated. Electrophysiological activity was amplified, audio monitored, and displayed on a storage oscilloscope before being sent to a computer for data collection using a customized version of Lab View (National Instruments, Austin, TX) software that enabled storage of raw data, discriminated impulses, stimulus temperature, and time of application of mechanical stimuli. In most experiments, recordings were obtained from two neurons, one on each side of the spinal cord. When two cells were recorded from a single animal, the first cell was treated with vehicle and the second cell was treated with CP 55,940. In some experiments performed to generate the dose-response curve, the first cell was treated with a lower dose of CP 55,940 and the second cell received a higher dose.

Functional classification of spinal neurons

Search stimuli applied to the rat hindpaw included stroking the skin and mild pinching with the experimenter’s fingers. The RFs of isolated neurons were mapped with a suprathreshold von Frey monofilament. Each spinal neuron was characterized according to its response evoked by graded intensities of mechanical stimulation applied to the RF. Innocuous stimuli consisted of stroking the skin with a soft bristled camel hairbrush. Noxious stimulation included mild pinching with an arterial clamp and with serrated forceps; the latter stimulus was applied sparingly to avoid neuronal sensitization. Neurons were classed functionally according to responses evoked by mechanical stimuli as: low threshold if they were excited maximally by innocuous brush stimulation, WDR if they responded in a graded manner to increasing intensity of stimulation, and HT if responses were evoked by noxious stimulation only. Only WDR and HT neurons were studied.

Evoked response measures and experimental design

To obtain responses evoked by mechanical stimuli, four test sites within the RF were marked on the skin with a felt-tip pen and stimulated with a von Frey monofilament (178 mN bending force applied for 2 s). Each test site was stimulated sequentially three times with a 10-s interval between stimuli. The mean number of impulses evoked at each site was determined. In 15 experiments, lower threshold mechanical stimuli (von Frey monofilament of 37 mN and hand-held brush) were used in addition to the 178-mN von Frey stimulus. The 37-mN von Frey filament was used to stimulate each test site three times as described in the preceding text, and the brush was lightly swept across the foot three times for 3 s, each with a 5-s interval between stimuli. To determine response evoked by heat, stimuli of 35–51°C were applied in ascending order of 2°C increments from a base temperature of 32°C using a Peltier thermode (contact area of 1 cm²). Stimuli were of 5-s duration and were delivered at a ramp rate of 18°C/s with an interstimulus interval of 60 s.

After obtaining baseline responses to mechanical and heat stimuli, a small pool was made around the recording electrode using either petroleum jelly or a silicon-based adhesive (World Precision Instruments, Sarasota, FL). A 50-μl solution of vehicle (n = 29) or the cannabinoid receptor agonist CP 55,940 at a concentration of 0.53 mM (10 μg in 50 μl; n = 25) was applied to this area of the cord. In most experiments, vehicle or drug was left on the cord for the duration of the experiment. In some cases (n = 8 vehicle; n = 8 CP 55,940 cells), the cord was rinsed with HEPES-buffered saline 10 min after drug application. Data obtained from these two groups were pooled. Responses to mechanical and heat stimuli were always obtained 10 min after drug application. Next, capsaicin (10 μg in 10 μl) was injected into the RF and activity was recorded for 5 min after the injection. Responses evoked by mechanical and heat stimuli were again determined as described in the preceding text. Additional studies were performed as described above using 0.1, 1.0, and 5.0 μg doses of CP 55,940.

To determine whether effects of CP 55,940 occurred through the CB1 receptor, a separate group of cells was pretreated with the CB1 antagonist, SR141716A (5 μg) followed 5 min later by CP 55,940 (5 μg). Ten minutes after CP 55,940 administration, responses evoked by mechanical and heat stimuli were obtained. As in the experiments described in the preceding text, capsaicin was injected into the RF, capsaicin-evoked activity was recorded for a period of 3 min, and responses to mechanical and heat stimuli were again determined. In another group of cells (n = 9), the effect of SR141716A (5 μg) alone on responses to mechanical and heat stimuli was determined.

Drug preparation

Stock solutions of CP 55,940 (10 mM) and SR141716A (10 mM) were obtained from the National Institute on Drug Abuse and stored in ethanol at −20°C until use. All solutions were diluted to their final concentration in HEPES-buffered saline. The vehicle consisted of 5% ethanol in HEPES-buffered saline.

Histological localization of recording sites

At the end of each experiment, passing current (10 μA for 20 s) through the recording electrode marked the recording site. Animals were perfused with 0.9% saline followed by 10% formalin containing 1% potassium ferrocyanide. Serial transverse sections (50 μm) were cut using a vibratome. Recording sites were identified by Prussian Blue marks or small lesions.

Statistical analyses

The numbers of impulses evoked by capsaicin, the normalized response evoked by mechanical stimuli, and heat thresholds were compared among groups using repeated-measures ANOVA and Bon-
ferroni post hoc comparisons. Response thresholds for heat stimuli and responses evoked by mechanical stimuli were determined by subtracting the spontaneous discharge from the response that occurred during the stimulus and were calculated as percent of the baseline response. Based on preliminary and supplementary analyses, the percentage of baseline response data followed a long-tailed distribution inconsistent with the assumptions underlying ANOVA. Therefore when applicable, we analyzed data using a nonparametric method (Kruskal-Wallace 1-way ANOVA on ranks with Dunn’s post hoc method). For all statistical tests, a $P$ value of $<0.05$ was considered significant.

RESULTS

Location and classification of dorsal horn neurons

A total of 98 cells were studied. Of these, 54 cells were treated with either vehicle or 10 $\mu$g CP 55,940. Twenty-nine cells were pretreated with vehicle, and their recording sites were located at an average depth in the spinal cord of 601.1 ± 44.3 $\mu$m. Recording sites for cells pretreated with 10 $\mu$g CP 55,940 ($n = 25$) had an average depth of 720.4 ± 37.9 $\mu$m. Histological reconstruction of recording sites for 15 vehicle-treated cells and 15 CP 55,940-treated cells shows that the locations of recording sites were similar in both groups of animals (Fig. 1). Recording sites estimated by microelectrode depth had mean depths similar to the populations identified by lesions with recording sites of vehicle-treated neurons located at a mean depth of 595.9 ± 50.2 $\mu$m and recording sites for CP 55,940-treated neurons located at a mean depth of 731.1 ± 71.0 $\mu$m. Of the neurons pretreated with vehicle, 16 were HT and 13 were WDR neurons. Of the neurons pretreated with 10 $\mu$g CP 55,950, 13 were HT and 12 were WDR neurons.

Responses to capsaicin

Injection of 10 $\mu$g capsaicin into the RF resulted in a large initial burst of activity in 24 of 29 vehicle and 21 of 25 CP 55,940-pretreated cells. A response of an individual neuron to capsaicin was defined as an evoked discharge that was at least two times above the mean ongoing spontaneous discharge rate. Capsaicin-evoked activity usually persisted over a 3-min period (Fig. 2, B and C). A two-way ANOVA revealed no difference in capsaicin-evoked discharge over the 3-min time period between cells pretreated with vehicle or CP 55,940 for either neuron type. Discharge rates where typically high in the first minute after capsaicin injection. In the first minute after capsaicin injection, 1,158.0 ± 228.2 spikes occurred in vehicle-treated neurons, whereas 701.2 ± 183.7 spikes occurred in CP 55,940-treated neurons (Fig. 2A; HT and WDR neurons combined).
Responses to mechanical stimuli

A suprathreshold von Frey monofilament with a bending force of 178 mN was used to evoke responses in HT and WDR neurons before any treatment (baseline), after treatment with either vehicle or CP 55,940 (before capsaicin injection), and after injection of capsaicin (Fig. 3). Neither pretreatment with vehicle or with 10 μg CP 55,940 altered mechanically evoked responses as compared with baseline responses. After application of vehicle, the response to the von Frey stimulus was 116 ± 8.5% of the baseline response (Fig. 4A; HT and WDR combined). However, after capsaicin injection, responses of all neurons (HT and WDR) pretreated with vehicle increased to 300.6 ± 52.1% of their baseline response ($P < 0.001$). After pretreatment with CP 55,940 alone (before injection of capsaicin), responses to mechanical stimuli remained unchanged and were 132.5 ± 13.5% of their baseline response. After capsaicin injection, sensitization to mechanical stimuli was blocked by application of CP 55,940. Responses were 153.0 ± 27.1% of the baseline response.

Sensitization to the von Frey monofilament injection was blocked by CP 55,940 in both HT and WDR neurons (Fig. 4, B and C). After capsaicin injection responses of HT neurons pretreated with vehicle increased to 395.9 ± 87.7% of their baseline response ($P < 0.001$), whereas responses of HT neurons pretreated with CP 55,940 were 172.3 ± 49.2% of baseline after capsaicin injection. Likewise, capsaicin injection increased mechanically evoked responses of WDR neurons.

FIG. 3. Examples of responses evoked by a von Frey monofilament (178 mN) of single HT (A) and single WDR (B) neurons treated with either VEH or CP 55,940. Numbers refer to the stimulation sites in the receptive field. Shown are responses at baseline (before drug application), after drug application to the spinal cord (drug treatment), and after injection of capsaicin into the receptive field.

FIG. 4. CP 55,940 attenuated sensitization to von Frey mechanical stimuli (178 mN) after capsaicin injection. All graphs show percent of baseline response after VEH or CP 55,940 application (drug treatment) and after capsaicin. In VEH-treated neurons, capsaicin increased the response to mechanical stimuli; however, capsaicin did not alter responses in CP 55,940-treated cells (A). The response to the von Frey monofilament was attenuated after capsaicin in both HT (B) and WDR (C) neurons pretreated with CP 55,940. *, $P < 0.001$ compared with “drug treatment” VEH group; #, $P < 0.05$ compared with “after capsaicin” VEH group.
pretreated with vehicle to 183.4 ± 15.6% of baseline (P < 0.001), whereas responses of WDR neurons pretreated with CP 55,940 were 132.0 ± 20.3% of baseline and did not differ from baseline values.

Responses evoked by a brush stimulus and a von Frey monofilament that delivered a lower force (37 mN) were also obtained in eight neurons pretreated with vehicle (3 WDR and 5 HT) and in seven neurons pretreated with CP 55,940 (4 WDR and 3 HT). Responses evoked by these stimuli did not increase after capsaicin injection in vehicle or CP 55,940-treated cells (data not shown). Although there was no apparent sensitization to these stimuli, it is interesting to note that neither vehicle nor CP 55,940 affected brush-evoked responses (91.5 ± 13.6 and 90.0 ± 9.5% of baseline, respectively) or responses evoked by the weak von Frey stimulus (111.1 ± 15.7 and 105.1 ± 15.3% of baseline, respectively). These results demonstrate that there was no effect of CP 55,940 on responses of HT and WDR neurons to innocuous mechanical stimulation.

Response to heat stimuli

Responses of WDR and HT neurons were initially combined to determine the effects of CP 55,940 on heat thresholds. Neither vehicle nor CP 55,940 alone altered response thresholds to heat stimuli. However, response thresholds after intraplantar injection of capsaicin were lowered after pretreatment with either vehicle or CP 55,940 (Fig. 5A). Mean response threshold after pretreatment with vehicle decreased from 43.3 ± 0.6°C before injection of capsaicin to 38.3 ± 0.7°C after injection of capsaicin (P < 0.05). Similarly, heat thresholds in cells pretreated with CP 55,940 decreased from 42.8 ± 0.4°C before injection of capsaicin to 38.7 ± 0.8°C after injection of capsaicin (P < 0.05). Cells that had no response to heat after capsaicin injection (3 vehicle-treated cells and 1 CP 55,940-treated cell) or that had a very low (37°C) heat threshold before capsaicin injection (1 vehicle-treated cell) were excluded from the analyses.

Further analyses demonstrated that the effect of CP 55,940 on heat threshold differed for WDR and HT neurons. Whereas WDR neurons pretreated with CP 55,940 became sensitized to heat after injection of capsaicin (Fig. 5C), sensitization to heat was prevented in HT neurons. Expressing the data as a percent of baseline response threshold, HT neurons treated with CP 55,940 exhibited no decrease in response threshold to heat stimuli. Thresholds were 97.9 ± 2.0% of baseline before and 94.6 ± 2.6% of baseline after capsaicin injection (Fig. 5B). HT neurons pretreated with vehicle exhibited a decrease in threshold to heat stimuli after capsaicin injection (P < 0.01), with thresholds changing from 96.9 ± 1.3% of baseline before to 87.3 ± 1.3% of baseline after injection of capsaicin. After capsaicin injection, HT neurons treated with CP 55,940 had a higher threshold to heat stimuli than HT neurons treated with vehicle (P < 0.01).

Effect of CP 55,940 on sensitization is dose-dependent

CP 55,940 produced a dose-dependent attenuation of capsaicin-evoked sensitization to the 178 mN suprathreshold von Frey monofilament (P < 0.05; Fig. 6A), and responses were converted to percent inhibition to determine the ED$_{50}$ (Fig. 6B). To determine percent inhibition, the mean normalized responses to mechanical stimuli after capsaicin injection were calculated for vehicle-pretreated neurons. Responses to mechanical stimuli after injection of capsaicin in neurons pretreated with CP 55,940 were then calculated as a percent of the average response of vehicle pretreated cells. The ED$_{50}$ generated from the percent inhibition was 3.7 µg (95% CL 1.3–10.5).

FIG. 5. Neurons sensitized to heat stimuli after capsaicin injection. A: mean heat thresholds for all neurons were lowered in both vehicle- and CP 55,940-treated cells after capsaicin injection. *, $P < 0.05$ compared with “baseline”; #, $P < 0.05$ compared with “drug treatment.” B: sensitization to heat stimuli was attenuated in HT neurons, but not WDR neurons (C). *, $P < 0.05$ compared with “vehicle-treated group after capsaicin.”

J Neurophysiol • VOL 93 • FEBRUARY 2005 • www.jn.org
Attenuation of central sensitization occurs through the CB1 receptor.

To determine whether the effect of CP 55,940 was mediated through CB1 receptors, spinal cord neurons (n = 6 HT; n = 7 WDR) were pretreated with the CB1 receptor antagonist SR141716A (5 μg). Topical application of SR141716A to the spinal cord blocked the ability of CP 55,940 (5 μg) to attenuate sensitization to the 178 mN mechanical stimulus after capsaicin injection (P < 0.05; Fig. 7A). In neurons pretreated with SR141716A and CP 55,940, responses were 508.2 ± 199.6% of baseline response after injection of capsaicin, whereas responses of neurons pretreated with 5 μg CP 55,940 were 130.4 ± 18.3% of baseline after injection of capsaicin.

To determine whether the antagonist altered neuronal excitability, SR141716A was applied alone to the spinal cord. SR141716A (5 μg) did not change evoked responses of dorsal horn neurons to the 178 mN mechanical stimulus in 9 HT neurons tested (Fig. 7A). At 10 min after application of SR 141716A, mechanically evoked responses were not significantly different from baseline (151.9 ± 35.5% of baseline values). After injection of capsaicin, responses to mechanical stimuli in neurons pretreated with SR141716A increased to 763.9 ± 249.0% of baseline values (P < 0.05). Although mechanically evoked responses in these neurons after capsaicin were large, they did not differ from responses of neurons pretreated with vehicle (300.6 ± 52.1% of their baseline response after capsaicin injection).

**Fig. 6.** CP 55,940 dose-dependently attenuated capsaicin-induced mechanical (178 mN von Frey) sensitization. In A, responses to mechanical stimuli after capsaicin injection are depicted as a percent of baseline in all neurons (HT and WDR). To determine the ED₅₀, responses were converted to percent inhibition (B). *, P < 0.05 compared with vehicle group.

**Attenuation of central sensitization occurs through the CB1 receptor.**

To determine whether the effect of CP 55,940 was mediated through CB1 receptors, spinal cord neurons (n = 7 WDR; n = 6 HT) were pretreated with the CB1 receptor antagonist SR141716A (5 μg). Topical application of SR141716A to the spinal cord blocked the ability of CP 55,940 (5 μg) to attenuate sensitization to the 178 mN mechanical stimulus after capsaicin injection (P < 0.05; Fig. 7A). In neurons pretreated with SR141716A and CP 55,940, responses were 508.2 ± 199.6% of baseline response after injection of capsaicin, whereas responses of neurons pretreated with 5 μg CP 55,940 were 130.4 ± 18.3% of baseline after injection of capsaicin.

To determine whether the antagonist altered neuronal excitability, SR141716A was applied alone to the spinal cord. SR141716A (5 μg) did not change evoked responses of dorsal horn neurons to the 178 mN mechanical stimulus in 9 HT neurons tested (Fig. 7A). At 10 min after application of SR 141716A, mechanically evoked responses were not significantly different from baseline (151.9 ± 35.5% of baseline values). After injection of capsaicin, responses to mechanical stimuli in neurons pretreated with SR141716A increased to 763.9 ± 249.0% of baseline values (P < 0.05). Although mechanically evoked responses in these neurons after capsaicin were large, they did not differ from responses of neurons pretreated with vehicle (300.6 ± 52.1% of their baseline response after capsaicin injection).

**Fig. 7.** The effect of CP 55,940 was prevented by treatment with SR141716A. SR141716A attenuated the effects of CP 55,940 on mechanical sensitization (A). After capsaicin, responses to mechanical stimuli of cells pretreated with CP 55,940 (CP; n = 8) were decreased compared with cells pretreated with vehicle (n = 29) and SR141716A (SR) and CP (n = 13). *, P < 0.05 compared with vehicle group; #, P < 0.05 compared with CP group. Responses to mechanical stimuli after SR141716A alone were not elevated as compared with the responses of the vehicle treated group. (B). Cells pretreated with CP did not exhibit a significant decrease in heat threshold after capsaicin; however, thresholds of cells pretreated with SR141716A followed by CP 55,940 decreased from baseline values. Heat thresholds for cells treated with SR141716A alone were decreased from baseline after capsaicin. *, P < 0.05 compared with “baseline.”
Heat response thresholds after capsaicin injection decreased for cells pretreated with SR141716A and CP 55,940 (5 HT and 5 WDR cells; *P < 0.05; Fig. 7B). The mean heat thresholds for vehicle-treated neurons decreased from 41.4 ± 1.3°C after drug treatment to 38.6 ± 1.0°C after injection of capsaicin. The mean heat threshold of neurons treated with CP 55,940 (3 HT cells, 3 WDR cells) did not decrease after capsaicin injection (40.7 ± 0.8°C before injection of capsaicin and 40.3 ± 1.5°C after injection of capsaicin). However, heat thresholds of neurons that received CP 55,940 did not differ from those that were given SR141716A and CP 55,940. No differences were apparent between the groups when data were analyzed as a percent of baseline.

Neurons treated with SR141716A alone displayed a significant decrease in heat threshold from 44.3 ± 1.1°C before injection of capsaicin to 40.3 ± 1.8°C after injection of capsaicin (*P < 0.05; Fig. 7B).

**DISCUSSION**

Previous studies have reported that cannabinoids depress acute responses of dorsal horn neurons evoked by noxious heat (Hohmann et al. 1998, 1999), mechanical (Hohmann et al. 1995; Kelly and Chapman 2003), and electrical stimuli (Drew et al. 2000; Kelly and Chapman 2001). The data presented here extend these studies by showing that spinal application of cannabinoids prevents sensitization that occurs after a peripheral injection of capsaicin. The doses of CP 55,940 that blocked sensitization did not alter responses of WDR or HT neurons to subthreshold mechanical stimuli; however, the sensitization to mechanical stimuli that normally occurs after injection of capsaicin was prevented in both classes of neurons in a dose-dependent manner. The prevention of sensitization to mechanical stimuli by CP 55,940 was mediated through CB1 receptors because the CB1 receptor specific antagonist, SR141716A, blocked the effects of CP 55,940. Inhibition by CP 55,940 of sensitization to heat stimuli was less pronounced, yet the decrease in heat threshold that normally occurs after capsaicin injection was prevented in HT neurons. Because the effect of CP 55,940 on heat response thresholds was not as robust as for responses to mechanical stimuli, it was difficult to demonstrate antagonism of its effects on sensitization to heat stimuli.

**Attenuation of neuronal sensitization by cannabinoids**

A small number of studies have examined the effects of cannabinoids on sensitization of dorsal horn neurons. Cannabinoids administered intravenously were shown to inhibit windup of WDR and nociceptive specific neurons that occurs after repeated electrical stimulation (Strangman and Walker 1999). As in our study, cannabinoids were shown to suppress only the windup response and not acute responses. The site of action for cannabinoid attenuation of windup, however, is unclear because WIN 55,212-2 was administered intravenously. Subsequent electrophysiological studies have applied cannabinoids directly to the spinal cord and have demonstrated a direct inhibitory effect on responses of spinal cord neurons. Repetitive, high-intensity transcutaneous electrical stimulation generates C-fiber-dependent post-discharge (PDC) activity in central neurons that is thought to represent neuronal hyperexcitability. PDC activity was attenuated in spinal cord (Chapman 2001; Drew et al. 2000; Kelly and Chapman 2001) and brain stem neurons (Papanastassiou et al. 2004) after application of cannabinoids to these areas. Interestingly, the nonconditioned or normal responses of these neurons were not as strongly attenuated by cannabinoids (Drew et al. 2000; Papanastassiou et al. 2004). Again, our findings are similar to these because sensitization of spinal neurons after capsaicin injection was attenuated, but responses prior to capsaicin injection were not altered.

In our study, neither innocuous (brush and 37-mN von Frey monofilament) nor noxious (178-mN von Frey monofilament) mechanically evoked responses were altered 10 min after application CP 55,940. In contrast, a study by Kelly and Chapman (2003) reported inhibition of mechanically evoked responses in spinal neurons from uninjured animals by a cannabinoid agonist, arachidonyl-2-chloroethylamide (ACEA). This difference in results may be due to the different time points at which cannabinoid effects on uninjured neurons were tested. In our study, responses were examined 10 min after application of CP 55,940, a time point we chose to correspond with our behavioral study (Johanek et al. 2001). In their study (Kelly and Chapman 2003), cells were treated with ACEA for 1 h and tested every 10 min within that hour. It is possible that we would have observed inhibition had we tested >10 min after drug application. Differences in results may also be due to the use of different agonists and different procedures for delivering mechanical stimuli.

**Cannabinoids and capsaicin-evoked excitation of spinal neurons**

CP 55,940 did not attenuate the initial burst of dorsal horn activity that occurs after capsaicin injection. This finding is comparable to our behavioral studies, where another cannabinoid agonist, WIN 55,212-2, did not attenuate the nocifensive behavior that occurs in rats during the first 5 min after capsaicin injection (Johanek et al. 2001). Interestingly, although the initial excitation was not prevented, the ensuing sensitization was attenuated. This may be due to the ability of intrathecal cannabinoids acting through CB1 receptors to inhibit cellular cascades that promote central sensitization in the capsaicin model. Activation of CB1 receptors inhibits Ca2+ channels (Caulfield and Brown 1992; Khasabova et al. 2004; Mackie and Hille 1992; Pan et al. 1996; Ross et al. 2001; Twitchell et al. 1997) and inhibits adenylyl cyclase activity (Howlett 1984). Inhibiting these currents or second-messenger systems may decrease the cellular excitability and decrease transmitter release, which could prevent sensitization of central neurons.

A number of intracellular cascades have been shown to mediate the sensitization of nociceptive spinal cord neurons after capsaicin injection (for review, see Willis 2002). Among these transduction pathways are protein kinase C, protein kinase A, and the nitric oxide and protein kinase G pathways. In addition, ionotropic receptors become phosphorylated during sensitization. Spinally administered cannabinoids may be acting to block one or more of these pathways to prevent the ensuing sensitization. For example, calcium/calmodulin dependent kinase (CaMKII) is upregulated during spinal cord sensitization; cannabinoids may prevent the influx of calcium needed to activate this kinase and thereby attenuate sensitization.
Pre- and postsynaptic mechanisms may contribute to cannabinoid-evoked antihyperalgesia

Because of the design of our study, we were unable to differentiate pre- and postsynaptic effects of cannabinoids. The ability of spinally administered cannabinoids to attenuate sensitization likely occurs through CB1 receptors located on multiple cell types and potentially at both pre- and postsynaptic sites within the spinal cord. Because of the multiple locations of CB1 receptors, there are several potential mechanisms by which intrathecal cannabinoids can attenuate capsaicin-induced sensitization. Cannabinoids may act at the central terminals of primary afferent fibers to attenuate neurotransmitter release (Richardson et al. 1998). Because the vanilloid receptor, TRPV1, is co-localized with CB1 receptors in cultured dorsal root ganglion neurons (Ahluwalia et al. 2000) and at low levels in native dorsal root ganglion and trigeminal neurons (Price et al. 2003), cannabinoids could act on the same neurons that are excited by capsaicin.

A second potential site of action for spinal cannabinoids is at CB1 receptors located on interneurons (Farquhar-Smith et al. 2000; Salio et al. 2001, 2002b). Electrophysiological studies suggest that cannabinoids may have a large role in regulating presynaptic neurotransmitter release, possibly from interneurons. Presynaptic CB1 receptors on neurons in the substantia gelatinosa attenuate glutamate release (Morisset and Urban 2001) and inhibit the release of GABA and glycine from rat superficial medullary dorsal horn neurons (Jennings et al. 2001).

Finally, in addition to neurons, CB1-like immunoreactivity has also been described on astrocytes within laminae I and II of the rat spinal cord (Salio et al. 2002a). It is less clear how cannabinoids might attenuate sensitization through astrocytes, although there is evidence that primary afferent input into the spinal cord (including capsaicin mediated excitation) alters the phosphorylation of proteins that maintain neural-glial interactions via gap junctions (Li and Nagy 2000). Pro-nociceptive molecules such as cytokines released from astrocytes may sensitize dorsal horn neurons after injury (for review, see Watkins et al. 2001). Thus cannabinoids may decrease the release of cytokines and other sensitizing agents from astrocytes.

Role of CB1 receptors in antihyperalgesia produced by CP 55,940

Attenuation of capsaicin-evoked sensitization to mechanical stimuli by CP 55,940 appears to be mediated by CB1 receptors because the effect was blocked by SR141716A. However, it is important to note that at this dose of SR141716A, other cannabinoid receptors may also be blocked. Although it is possible that other types of cannabinoid receptors exist in the spinal cord, data suggest that CB2 receptors are not found within the CNS under normal conditions (Griffin et al. 1999; Zhang et al. 2003). It has been reported that expression of CB2 receptor mRNA in the spinal cord occurred after nerve injury (Zhang et al. 2003). Thus CB2 receptors may be expressed, potentially on microglia after certain types of persistent pain conditions. However, in our capsaicin model sensitization occurs rapidly with insufficient time for the infiltration of microglia and expression of CB2 receptors to develop.

In the current study, although SR141716A blocked the effect of CP 55,940 on mechanical sensitization, there was a tendency for sensitization to mechanical stimuli to be increased after SR141716A and CP 55,940 (although this was not statistically significant). This may be due to a potential excitatory effect of SR141716A. However, after examining the responses obtained from individual cells in this portion of the study, the resulting increase was attributed to two HT neurons that exhibited large increases in mechanically evoked responses after capsaicin. When these two cells are removed from the analysis, neurons treated with CP 55,940 and SR141716A increase from 133.0 ± 16.0% of baseline response to 214.9 ± 22.1% of baseline response. Thus these two cells appeared to have an unusually large response after capsaicin.

Endogenous cannabinoid tone and neuronal excitability

To examine whether endogenous cannabinoid tone modulated neuronal excitability, we applied the CB1 receptor antagonist to the cord. SR141716A alone had no effect on mechanically evoked responses of dorsal horn neurons. In contrast, other studies found that in naïve rats, spinal administration of SR141716A tended to increase responses of dorsal horn neuron to mechanical stimuli (Kelly and Chapman 2003) and produced a significant increase in the nonpotentiated C-fiber response of electrically evoked dorsal horn neurons (Chapman 1999).

After capsaicin application in the present study, mechanically evoked responses tended to increase with SR141716A pretreatment; however, the increase was not significantly higher than responses after pretreatment with vehicle. SR141716A has been shown to increase capsaicin-evoked release of substance P from spinal cord slices (Lever and Malcangio 2002). However, capsaicin applied directly to the spinal cord could cause a much greater release of neurotransmitter than evoked in our study after intraplantar capsaicin injection, and SR141716A could act differentially in these two systems. In addition, SR141716A often demonstrates inverse agonist activity at the CB1 receptor (Bouaboula et al. 1997; Landsman et al. 1997) making it difficult to differentiate endogenous cannabinoid effects from inverse agonist effects. Thus the role of the endogenous cannabinoid system in mediating nociceptive responses in the spinal cord remains unclear.

Conclusions

This study provides evidence that cannabinoids act at spinal CB1 receptor sites to prevent capsaicin-evoked sensitization to mechanical stimuli. These data provide further rational for the use of spinal cannabinoids to treat painful syndromes associated with hyperalgesia. Anti-hyperalgesia after spinal administration of cannabinoids may be particularly attractive because unwanted side effects associated with cannabinergic mechanisms generated via activation of supraspinal CB1 receptors would be avoided.

Acknowledgments

The authors thank Dr. Jim Hodges, Department of Biostatistics at the University of Minnesota, for data analysis and statistical advice and Drs. Virginia Seybold and Glenn J. Giesler Jr. for reading an earlier version of this manuscript.

Grants

This work was supported by grants from the National Institutes of Health (DA-11471 and P30 DE-09737). L. Johanek was supported by NIH Training...
Cannabinoids prevent sensitization of spinal cord neurons

Grant T32 DA-07234 and a Louise Dosdall Fellowship from the Graduate School at the University of Minnesota.

REFERENCES


Landsman RS, Burke TJ, Consore P, Roeseke WR, and Yamamura HI. SR141716A is an inverse agonist at the human cannabinoid CB1 receptor. Eur J Pharmacol 334: R1–2, 1997.


