Cannabinoid Modulation of Cutaneous Aδ Nociceptors During Inflammation

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modulation of cutaneous Aδ nociceptors during inflammation. *J
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2008; doi:10.1152/jn.90809.2008. Previous studies have demon-
strated that locally administered cannabinoids attenuate allodynia and
hyperalgesia through activation of peripheral cannabinoid receptors
(CB_1 and CB_2). However, it is currently unknown if cannabinoids
alter the response properties of nociceptors. In the present study,
correlative behavioral and in vivo electrophysiological studies were
conducted to determine if peripheral administration of the cannabi-
noid receptor agonists arachidonyl-2′-chloroethylamide (ACEA) or
(R)-(+)-methanandamide (methAEA) could attenuate mechanical al-
lodynia and hyperalgesia, and decrease mechanically evoked re-
sponses of Aδ nociceptors. Twenty-four hours after intraplantar
injection of complete Freund's adjuvant (CFA), rats exhibited allodynia
(decrease in paw withdrawal threshold) and hyperalgesia (increase in
paw withdrawal frequency), which were attenuated by both ACEA
and methAEA. The antinociceptive effects of these cannabinoids were
blocked by co-administration with the CB_1 receptor antagonist *N*-(pi-
peridin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophen yl)-4-methyl-1H-
pyrazole-3-carboxamide (AM251) but not with the CB_2 receptor
antagonist 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-y l](4-
methoxyphenyl)methanone (AM630). ACEA and methAEA did not
produce antinociception under control, non-inflamed conditions 24 h
after intraplantar injection of saline. In parallel studies, recordings
were made from cutaneous Aδ nociceptors from inflamed or control,
non-inflamed skin. Both ACEA and methAEA decreased responses
evoked by mechanical stimulation of Aδ nociceptors from inflamed
skin but not from non-inflamed skin, and this decrease was blocked by
administration of the CB_1 receptor antagonist AM251. These results
suggest that attenuation of mechanically evoked responses of Aδ
nociceptors contributes to the behavioral antinociception produced by
activation of peripheral CB_1 receptors during inflammation.

INTRODUCTION

Several studies have demonstrated that locally administered
cannabinoids produce antinociception in animal models of both
acute and persistent pain through peripheral mechanisms (for
reviews, see Hohmann 2002; Mbvundula et al. 2004; Walker
and Huang 2002; Walker et al. 1999). Two receptors for
cannabinoids have been isolated and cloned to date, cannabi-
noid 1 (CB_1) and cannabinoid 2 (CB_2), and both are G-protein-
coupled receptors (Matsuda et al. 1990; Munro et al. 1993) that
have been localized to various tissues. CB_1 receptors are most
commonly expressed on neurons, and their activation can
decrease neuronal excitability by decreasing calcium channel
conductance and increasing potassium channel conductance
(for review, see Howlett et al. 2004). CB_2 receptors are

predominately expressed on leukocytes, and their activation
can produce a variety of different immunological effects (for
review, see Klein et al. 2003; Massi et al. 2006).

Locally administered cannabinoids have been shown to
produce antinociception through activation of CB_1 and CB_2
receptors. CB_1 receptor-mediated antinociception has been
attributed to activation of CB_1 receptors expressed by primary
afferent nociceptive dorsal root ganglion neurons (Ahluwalia
et al. 2000; Hohmann and Herkenham 1999) and their periph-
eral nerve terminals (Amaya et al. 2006; Ständer et al. 2005).
Conditional knockdown of CB_1 receptors in Nav1.8-express-
ing nociceptive sensory neurons prevents locally administered
cannabinoids from producing antinociception in models of
neuropathic and inflammatory pain (Agarwal et al. 2007).
Consistent with these observations, activation of CB_1 receptors
decreased high-voltage-activated calcium currents (Khasabova
et al. 2002, 2004; Ross et al. 2001) and reduced capsaicin-
evoked calcium transients (Millns et al. 2001; Sagar et al.
2005) in nociceptive dorsal root ganglion neurons in vitro. A
recent study also showed that mechanically evoked responses
of primary afferent neurons are decreased by CB_1 receptor
activation in vivo; however, the types of afferent fibers affected
are not known (Kelly and Donaldson 2008). The precise
mechanisms underlying CB_2 receptor-mediated antinocicep-
tion remain unclear but likely involve both indirect and direct
actions on neuronal tissue (for review, see Guidon and Hohm-
ann 2008).

Although behavioral studies have indicated that activation of
peripheral CB_1 and CB_2 receptors produces antinociceptive
effects, it is currently unknown if cannabinoids alter the re-
sponse properties of nociceptors and which subtypes of func-
tionally identified nociceptors are cannabinoid-sensitive. In the
present study, parallel behavioral and electrophysiological ex-
periments were conducted to determine how peripherally me-
diated antiallodynia and antihyperalgesia produced by canna-
binoids relate to changes in Aδ nociceptor activity and through
which cannabinoid receptor subtype these changes occur. In
behavioral studies, we examined the effects of intraplantar
administration of the cannabinoid receptor agonists arachido-
nyl-2′-chloroethylamide (ACEA) and (R)-(+)-methanandam-
ide (methAEA) on mechanical allodynia and hyperalgesia in
rats with complete Freund's adjuvant-induced inflammation of
the hindpaw. In parallel electrophysiological studies, we inves-
tigated the effects of intraplantar administration of ACEA and
methAEA on mechanically evoked responses of cutaneous Aδ
nociceptors innervating inflamed skin. Similar behavioral and
electrophysiological studies were also conducted to compare the

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effects of locally administered ACEA and methAEA in inflamed skin to any changes produced in control, non-inflamed skin.

METHODS

Subjects

A total of 542 adult, male, Sprague–Dawley rats weighing 280–350 g were used in this study. Animals were obtained from Harlan (Indianapolis, IN), housed on a 12-h light/dark schedule, and allowed ad libitum access to food and water. Experiments were performed during the light cycle. All procedures were approved by the Animal Care Committee at the University of Minnesota, and experiments were conducted according to the guidelines established by the International Association for the Study of Pain.

Induction of inflammation

Rats were anesthetized with a mixture of isoflurane gas in air (2% for induction and maintenance; Phoenix Pharmaceuticals, St. Joseph, MO) and received a single intraplantar injection of complete Freund's adjuvant (CFA; (Sigma Chemical, St. Louis, MO) or sterile isotonic saline as a control (Baxter, Deerfield, IL). CFA (1 mg/ml) and saline were given in a volume of 50 μl using a 28-gauge needle. Electrophysiological and behavioral experiments were performed 24 h after injection of CFA or saline.

Drug preparation and administration

The cannabinoid receptor agonists were N-(2-chloroethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide (ACEA) and (R)-N-(2-hydroxy-1-methylethyl)-5Z,8Z,11Z,14Z-eicosatet raenamide (methAEA). The CB_1 receptor antagonist was N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) which exhibits ≈300-fold selectivity over CB2 receptors (Gatley et al. 1996). The CB_2 receptor antagonist was 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-y l](4-methoxyphenyl)methanone (AM630) which exhibits ≈160-fold selectivity over CB_1 receptors (Hosohata et al. 1997; Pertwee et al. 1995). All drugs were obtained from Tocris Bioscience (Ellisville, MO). ACEA was supplied predissolved in ethanol (5 mg/ml). methAEA was supplied predissolved in a water soluble emulsion (Tocrisolve; 5 mg/ml). AM251 was dissolved in anhydrous ethanol (25 μg/μl). AM630 was dissolved in a vehicle containing 5% Tween80 and 5% DMSO in isotonic saline (20 μg/μl). All drugs were administered via subcutaneous intraplantar injection in a volume of 50 μl for behavioral studies or 20 μl for electrophysiological studies.

Behavioral studies

PAW WITHDRAWAL THRESHOLDS. Paw withdrawal thresholds to mechanical stimuli were used as a measure of mechanical allodynia. Withdrawal thresholds were determined using a series of eight calibrated Semmes-Weinstein von Frey monofilaments of logarithmic incremental stiffness (0.40–15 g; Stoelting, Wood Dale, IL). Animals were placed on an elevated wire-mesh platform under individual plastic cages and allowed to acclimate to the testing environment for 30 min prior to testing. The monofilaments were applied to the mid-plantar surface of the hindpaw for 1–2 s with an interstimulus interval of 5–6 s. The paw withdrawal threshold (g) was calculated according to the methods described by Chaplan et al. (1994). Baseline measures were determined for each animal for three consecutive days prior to injection of saline or CFA. Mechanical allodynia was defined as a decrease in the paw withdrawal threshold.

PAW WITHDRAWAL FREQUENCIES. The frequency of paw withdrawal from a standard mechanical stimulus was used to measure mechanical hyperalgesia. The frequency of paw withdrawal evoked by mechanical stimulation was determined using a 26 g Semmes-Weinstein von Frey monofilament (Stoelting). Animals were placed on an elevated wire-mesh platform under individual plastic cages and allowed to acclimate to the testing environment for 30 min prior to testing. The filament was applied to the mid-plantar surface of the hindpaw 10 times for 1–2 s each with an interstimulus interval of 5–6 s, and the paw withdrawal frequency (%) was determined. Baseline measures were determined for each animal for three consecutive days prior to injection of saline or CFA. Mechanical hyperalgesia was defined as an increase in the paw withdrawal frequency.

Experimental design for behavioral studies

Separate groups of animals were used for studies of paw withdrawal thresholds or paw withdrawal frequencies. After three consecutive days of baseline testing, animals received an intraplantar injection of either CFA or saline into the left hindpaw. Twenty-four hours after injection, animals were randomly divided into groups of 8–10 rats each. Paw withdrawal thresholds or paw withdrawal frequencies were determined for both hindpaws before and 30, 60, and 120 min after cannabinoid or vehicle administration into the left hindpaw. In a separate group of rats, ACEA or methAEA was injected into the contralateral hindpaw and paw withdrawal thresholds or frequencies were determined in the inflamed (ipsilateral) hindpaw. The doses of either ACEA or methAEA given were 0.1, 1, or 10 μg. AM251 or AM630 was each given in doses of 30 μg and was co-injected with either ACEA (10 μg) or methAEA (10 μg). Each animal was used in only one experiment, and the experimenter was blinded to the identity of the drug administered.

Electrophysiological studies

SURGICAL PREPARATION. Rats were initially anesthetized with ketamine (100 mg/kg im) and xylazine (45 mg/kg im). The trachea was cannulated, and a catheter was placed in the external jugular vein to provide supplemental anesthesia with sodium pentobarbital (10 mg·kg^{-1}·h^{-1}). Core body temperature was maintained at 37°C using a feedback-controlled heating pad (Harvard Apparatus, Holliston, MA).

ELECTROPHYSIOLOGICAL RECORDING. Recordings were made from cutaneous afferent fibers of the left tibial nerve using a teased-fiber approach. The tibial nerve was dissected from the surrounding tissue, and the overlaying skin was sewn to a metal ring to form a pool that was filled with warm mineral oil. The tibial nerve was placed onto a mirror platform for fine dissection with sharpened Dumont No. 5 forceps (Fine Science Tools, Foster City, CA). Teased fibers were placed onto a tungsten wire electrode, and action potentials were recorded extracellularly. Action potentials were amplified, audio monitored, displayed on an oscilloscope, and stored on a PC computer for data analysis. Only fibers with clearly discriminated single unitary action potentials (units) were studied. Responses of individual units were analyzed off-line using a customized data analysis program (LabVIEW, version 5.1; National Instruments, Austin, TX).

IDENTIFICATION OF UNITS. Afferent units were found by mechanically stimulating the plantar surface of the hindpaw with the experimenter's finger or by stimulation with calibrated von Frey monofilaments. Once a single unit was identified, its mechanical receptive field (RF) was marked on the skin using a felt-tipped pen.

CONDUCTION VELOCITY. The conduction velocity was determined by electrically stimulating the skin outside the unit's RF with pin electrodes to electrically activate the unit (200 μs pulse width at 0.5 Hz). The unit was stimulated 1.5× its electrical threshold and the conduction latency was measured from the time of the electrical stimulus artifact to the evoked unitary action potential. Conduction distance was determined by measuring the distance from the unit's RF

to the recording electrode. Conduction velocity (m/s) was calculated by dividing conduction distance by conduction latency.

FUNCTIONAL CLASSIFICATION OF NOCICEPTORS. Units were classified functionally according to their responsiveness to mechanical and heat stimulation. Mechanical stimuli used to classify units included light brushing with the tip of a cotton swab, mildly pinching with a pair of forceps, and application of von Frey filaments. Mechanical response thresholds were determined using a series of calibrated von Frey monofilaments and defined as the weight (g) required to evoke at least one impulse when applied to a unit's RF for 1 s. Heat stimuli were delivered using a feedback-controlled Peltier device (Yale Electronics, New Haven, CT) with a contact area of 1 cm^2. A unit was considered heat-responsive if it responded with at least one impulse to a stimulus temperature of 51°C for 5 s. Units were classified as Aδ nociceptors if they had conduction velocities between 2.4 and 25.0 m/s and exhibited a slowly adapting response to noxious pinch but not to light touch (Leem et al. 1993).

Experimental design for electrophysiological studies

Once an Aδ nociceptor was characterized, baseline responses evoked by a 26 g von Frey monofilament were determined. This stimulus was above the mechanical response threshold for all Aδ nociceptors sampled in the present study and was the same filament used to determine mechanical hyperalgesia in our behavioral studies. The monofilament was secured in a manipulator and lowered onto the mechanical RF for 5 s. The monofilament was applied twice to the same location with an interstimulus interval of 180 s. The number of evoked impulses and the discharge rate (from the first to the last evoked impulse) were averaged over the two trials. For nociceptors that exhibited ongoing activity, the number of impulses that occurred 5 s prior to the stimulus was subtracted from the number of impulses evoked during stimulation. To assess the variability between stimulus trials, the number of impulses elicited during the second stimulus trial was expressed as a percent of the number of impulses elicited during the first stimulus trial (Wenk et al. 2006).

After baseline responses were determined, cannabinoid or vehicle was injected into the unit's RF. The needle was inserted outside the RF, and the injectate was observed as a bleb of fluid centered in the unit's RF. Ongoing activity was recorded before, during, and for 300 s after injection of drug or vehicle. Responses were separated into injection responses, the response during injection, and post-injection responses, the response after injection of drug and withdrawal of the needle from the skin. Injection and post-injection response magnitudes are indicated as both the number of impulses elicited and discharge rate (Hz). Mechanical response thresholds, mechanically evoked responses, and the variability between stimulus trials were determined 30, 60, 90, and 120 min after injection of drug or vehicle as described above. The dose of either ACEA or methAEA given was 10 μg. AM251 or AM630 was each given in doses of 30 μg and pre-injected prior to injection of ACEA (10 μg) or methAEA (10 μg). Only one nociceptor was studied per animal.

Data analysis

BEHAVIORAL STUDIES. To determine the effect of cannabinoids or their vehicles on paw withdrawal thresholds and paw withdrawal frequencies compared with baseline measures, comparisons were made using one-way repeated-measures ANOVA followed by paired *t*-test with the Bonferroni correction for multiple comparisons. A one-way ANOVA followed by unpaired *t*-test with the Bonferroni correction for multiple comparisons was used to determine the effect of cannabinoids or their vehicles on paw withdrawal thresholds and paw withdrawal frequencies between groups. Within group comparisons of the main effect of CFA and saline injection on paw withdrawal thresholds and paw withdrawal frequencies were made using paired

t-tests. For all statistical analyses, a probability value <0.05 was considered significant. All data are presented as means ± SE.

ELECTROPHYSIOLOGICAL STUDIES. To determine the effect of cannabinoids or their vehicles on mechanically evoked responses and variability between stimulus trials compared with baseline measures, comparisons were made using a one-way repeated-measures ANOVA followed by paired *t*-test with the Bonferroni correction for multiple comparisons. A two-way ANOVA followed by unpaired *t*-test with the Bonferroni correction for multiple comparisons was used to determine the effect of cannabinoids or their vehicles on mechanically evoked responses between groups. Between-group comparisons of conduction velocity and mechanically evoked responses on nociceptors isolated from CFA- and saline-injected hindpaws were made using unpaired *t*-tests. To determine the effect of cannabinoids or their vehicles on mechanical response thresholds compared with baseline measures, comparisons were made using the Kruskal-Wallis ANOVA followed by Mann-Whitney rank sum tests. To compare the effect of drug or vehicle on mechanical response thresholds between groups, Mann-Whitney rank sum tests were used. Injection and post-injection response magnitudes were compared between groups using one-way ANOVA followed by unpaired *t*-tests with the Bonferroni correction for multiple comparisons. The proportions of units exhibiting injection and post-injection responses between groups were made using χ^2 test followed by pair-wise comparisons using the Fisher exact test. For all statistical analyses, a probability value <0.05 was considered significant. All data are presented as means ± SE. All statistical analysis was performed using Sigma Stat software (Systat Software, San Jose, CA).

RESULTS

Behavioral studies

MECHANICAL HYPERALGESIA AND MECHANICAL ALLODYNIA AFTER INDUCTION OF INFLAMMATION. Twenty-four hours after intraplantar injection of CFA, rats exhibited mechanical allodynia and mechanical hyperalgesia in the injected hindpaw. Paw withdrawal thresholds decreased from 13.6 ± 0.2 to 5.0 ± 0.2 g (n = 140, P < 0.001), and paw withdrawal frequencies increased from 26.9 ± 0.7 to 94.8 ± 0.6% (n = 188, P < 0.0001). Paw withdrawal thresholds (n = 37) and frequencies (n = 32) did not change 24 h after intraplantar injection of saline (from 13.1 ± 0.3 to 12.9 ± 0.4 g and from 24.6 ± 1.7 to 25.7 ± 1.3%, respectively). No changes in withdrawal thresholds or withdrawal frequencies were observed in the contralateral hindpaw in either CFA- or saline-treated rats.

EFFECTS OF ACEA AND METHAEA ON MECHANICAL ALLODYNIA. Intraplantar injection of ACEA or methAEA, but not vehicle, dose-dependently attenuated mechanical allodynia produced by CFA. Increases in withdrawal threshold occurred after the 1 and 10 μg doses of both cannabinoids (Fig. 1, *A* and *B*). The antiallodynic effects of both cannabinoids peaked 30 min after administration and withdrawal thresholds returned to baseline values by 60 min after administration (Fig. 1, *C* and *D*).

To determine if the antiallodynia produced by ACEA and methAEA was mediated by peripheral cannabinoid receptors, rather than through a systemic mechanism, ACEA (10 μg) or methAEA (10 μg) was injected into the contralateral hindpaw and paw withdrawal thresholds were determined in the inflamed (ipsilateral) hindpaw. Injection of either ACEA or methAEA into the contralateral hindpaw did not alter withdrawal thresholds in the inflamed (ipsilateral) hindpaw (data not shown). These results indicate that the antiallodynia after
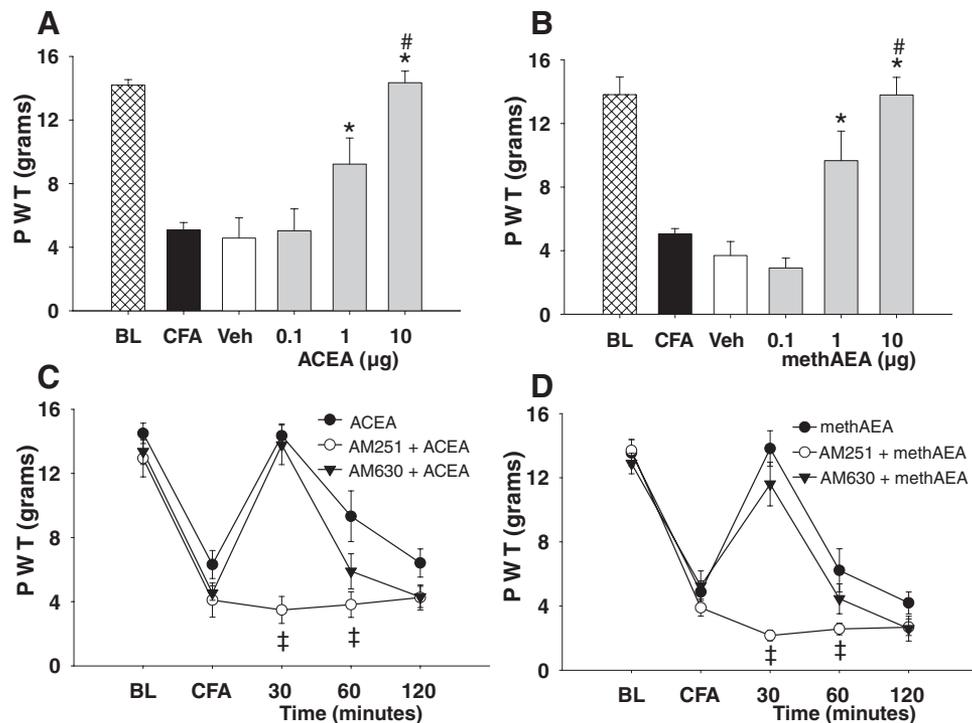
FIG. 1. arachidonyl-2′-chloroethylamide (ACEA) and (R)-(+)-methanandamide (methAEA) attenuate mechanical allodynia via activation of peripheral cannabinoid 1 (CB$_1$) receptors. Peripheral administration of either ACEA (*A*) or methAEA (*B*) dose-dependently increased paw withdrawal thresholds at doses of 1 and 10 μg (values shown indicate 30 min after administration). Co-administration with the CB$_1$ receptor antagonist *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophen yl)-4-methyl-1H-pyrazole-3-carboxamide (AM251, 30 μg), but not the cannabinoid 2 (CB$_2$) receptor antagonist 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-y l](4-methoxyphenyl)methanone (AM630, 30 μg), blocked the antiallodynic effects of 10 μg ACEA (*C*) and 10 μg methAEA (*D*). BL, mean baseline paw withdrawal thresholds 24 h prior to injection of complete Freund's adjuvant (CFA); CFA, mean paw withdrawal thresholds 24 h after intraplantar injection of CFA and also serves as the predrug baseline; PWT, paw withdrawal threshold; Time, time after intraplantar injection of drugs; *, significant difference from vehicle ($P < 0.05$); #, significant difference from 1 μg of methAEA/ACEA ($P < 0.05$); ‡, significant difference from 10 μg of methAEA/ACEA ($P < 0.05$). $n = 8$–10 animals per dose.

administration of ACEA and methAEA was mediated by peripheral cannabinoid receptors.

ACEA and methAEA were co-administered with either the CB$_1$ receptor antagonist, AM251, or the CB$_2$ receptor antagonist, AM630, to determine which cannabinoid receptor subtype mediated the antiallodynic effects produced by both cannabinoids. Co-administration of either ACEA (10 μg) or methAEA (10 μg) with AM251 (30 μg), but not AM630 (30 μg), blocked the increase in withdrawal thresholds produced by ACEA and methAEA (Fig. 1, *C* and *D*). These results suggest that the antiallodynia after administration of ACEA and methAEA are mediated by peripheral CB$_1$ receptors. Administration of AM251 or AM630 alone, or their vehicles, did not alter withdrawal thresholds (data not shown).

EFFECTS OF ACEA AND METHAEA ON MECHANICAL HYPERALGESIA. Intraplantar injection of ACEA or methAEA, but not vehicle, dose-dependently attenuated mechanical hyperalgesia. Decreases in withdrawal frequencies occurred after the 1 and 10 μg doses for both cannabinoids (Fig. 2, *A* and *B*). The antihyperalgesic effects of both cannabinoids peaked 30 min after administration and paw withdrawal frequencies returned to baseline values by 60 min after administration (Fig. 2, *C* and *D*).

Similar to the results for mechanical allodynia, intraplantar injection of either ACEA (10 μg) or methAEA (10 μg) into the contralateral hindpaw did not alter paw withdrawal frequencies in the inflamed (ipsilateral) hindpaw (data not shown). Again, these results indicate that the antihyperalgesic effects of locally

administered ACEA and methAEA are mediated by peripheral cannabinoid receptors.

The decrease in withdrawal frequencies produced by either ACEA (10 μg) or methAEA (10 μg) was blocked by the CB$_1$ receptor antagonist AM251 (30 μg) but not by CB$_2$ receptor antagonist AM630 (30 μg; Fig. 2, *C* and *D*). These results suggest that the antihyperalgesia produced by ACEA and methAEA is mediated by peripheral CB$_1$ receptors. Administration of AM251 or AM630 alone, or their vehicles, did not alter paw withdrawal frequencies (data not shown).

EFFECTS OF ACEA AND METHAEA IN CONTROL, NON-INFLAMED RATS. Twenty-four hours after intraplantar injection of saline, rats received an intraplantar injection of ACEA (10 μg), methAEA (10 μg), or vehicle. In contrast to the antinociceptive effects observed in CFA-injected rats, ACEA and methAEA, as well as their vehicles, produced a small decrease in paw withdrawal thresholds and a trend for an increase paw withdrawal frequencies (Fig. 3, *A–D*). The decrease in withdrawal thresholds produced by ACEA and methAEA did not differ from their vehicles at any time point tested. These results suggest that peripheral administration of ACEA or methAEA does not produce antinociception to mechanical stimuli during control, non-inflamed conditions.

Electrophysiological studies

GENERAL PROPERTIES OF Aδ NOCICEPTORS. A total of 145 Aδ nociceptors were studied: 40 from control, non-inflamed (saline-
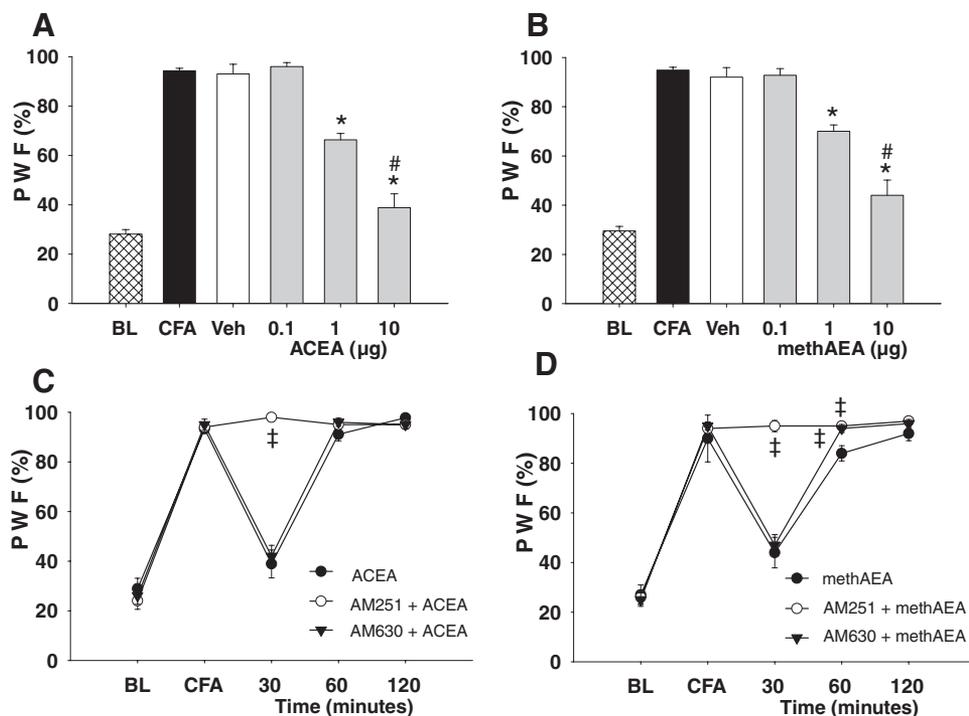
FIG. 2. ACEA and methAEA attenuate mechanical hyperalgesia by activation of peripheral CB_1 receptors. Peripheral administration of either ACEA (*A*) or methAEA (*B*) dose-dependently decreased paw withdrawal frequencies at doses of 1 and 10 µg (values shown indicate 30 min after administration). Co-administration with the CB_1 receptor antagonist AM251 (30 µg), but not the CB_2 receptor antagonist AM630 (30 µg), blocked the antihyperalgesic effects of 10 µg ACEA (*C*) and 10 µg methAEA (*D*). BL, mean baseline paw withdrawal frequency 24 h prior to injection of CFA; CFA, mean paw withdrawal frequency 24 h after intraplantar injection of CFA and also serves as the predrug baseline; PWF, paw withdrawal frequency; Time, time after intraplantar injection of drugs; *, significant difference from vehicle ($P < 0.05$); #, significant difference from 1 µg of methAEA/ACEA ($P < 0.05$); ‡, significant difference from 10 µg of methAEA/ACEA ($P < 0.05$). $n = 8–10$ animals per dose.

injected) skin and 105 from inflamed (CFA-injected) skin. The mean conduction velocity of Aδ nociceptors isolated from non-inflamed skin was 15.7 ± 0.6 m/s with a range of 4.2–20.8 m/s and was similar to the mean conduction velocity of Aδ nociceptors from inflamed skin (15.1 ± 0.4 m/s with a range of 3.1–21.8 m/s). Examples of conduction latency traces are displayed in Fig. 4*A*. The median mechanical response threshold of Aδ nociceptors from inflamed skin was 2.5 g (interquartile range = 3.4 g), which was lower than the median mechanical response threshold of Aδ nociceptors from non-inflamed skin (4.7 g; interquartile range = 4.3 g; $P < 0.001$). None of the Aδ nociceptors from

non-inflamed skin exhibited ongoing activity, whereas 25% (26/105) of Aδ nociceptors from inflamed skin exhibited ongoing activity with an average discharge rate of 0.16 ± 0.03 Hz (range = 0.02–0.61 Hz). None of the Aδ nociceptors from non-inflamed skin were excited by noxious heat, whereas 4% (4/105) of Aδ nociceptors from inflamed skin were excited by heat. Examples of heat responses of a single Aδ nociceptor from inflamed skin are shown in Fig. 4*C*.

MECHANICALLY EVOKED RESPONSES OF Aδ NOCICEPTORS. Preliminary studies were initially conducted to determine the
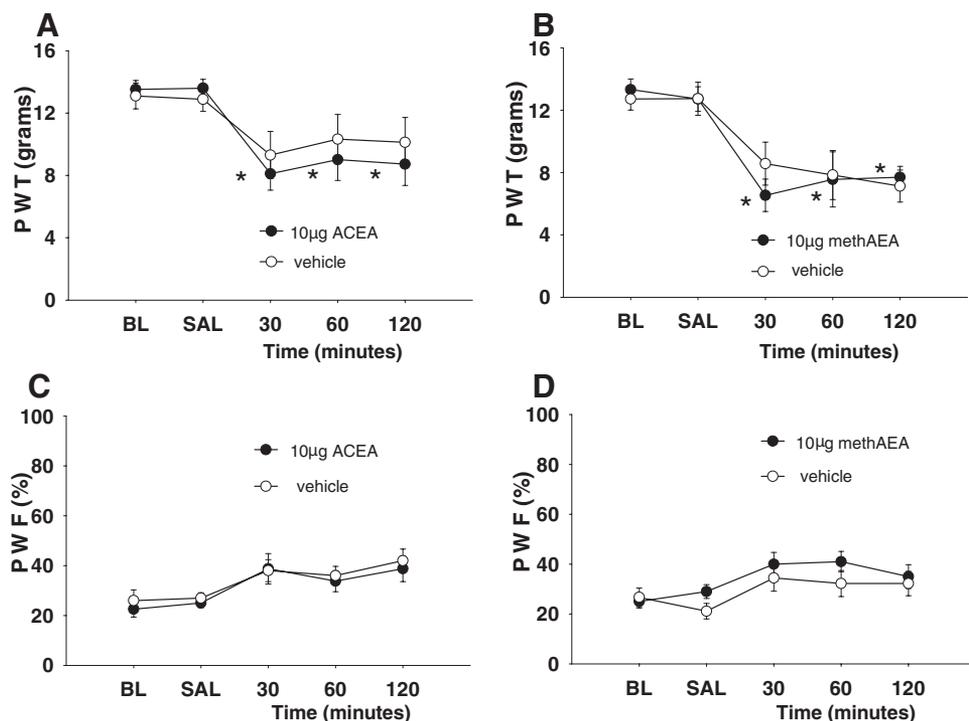

FIG. 3. Effects of ACEA and methAEA on withdrawal responses during non-inflamed conditions. Effects of intraplantar injection of ACEA (10 µg) or vehicle on mean paw withdrawal thresholds (*A*) and mean paw withdrawal frequencies (*C*) 24 h after intraplantar injection of saline. Effects of intraplantar injection of methAEA (10 µg) or vehicle on mean paw withdrawal thresholds (*B*) and mean paw withdrawal frequencies (*D*) 24 h after intraplantar injection of saline. BL, mean baseline paw withdrawal threshold and withdrawal frequency 24 h before intraplantar injection of saline; SAL, mean paw withdrawal threshold and mean withdrawal frequency 24 h after intraplantar injection of saline and also serves as the predrug baseline; *, significant difference from SAL ($P < 0.05$). $n = 8–10$ animals per dose.
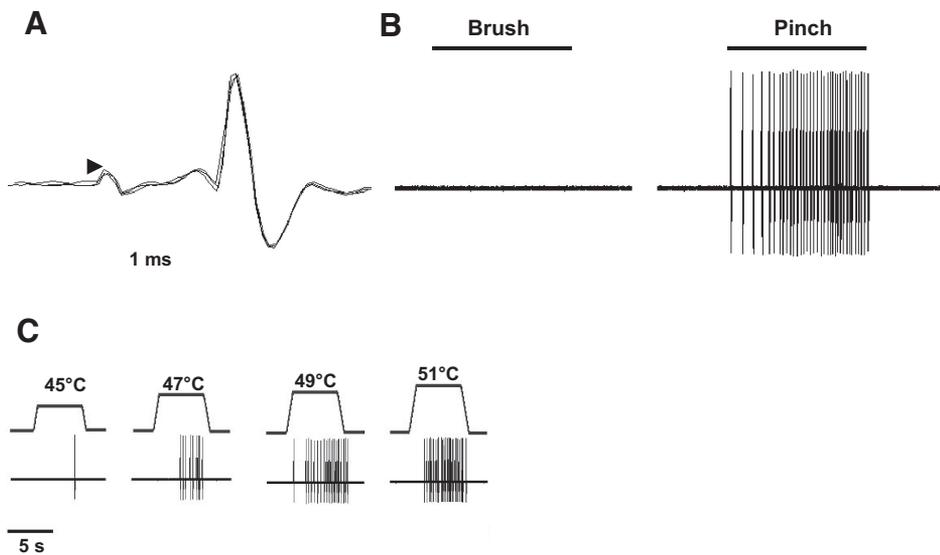
FIG. 4. Examples of Aδ nociceptor activity. *A*: 3 overlaying conduction latency traces of a single Aδ nociceptor from non-inflamed skin. Arrowhead indicates electrical stimulus artifact. *B*: the response of this nociceptor to noxious pinch but not brushing in its receptive field (RF). The line above each trace in *B* represents 2 s. *C*: responses of a single Aδ nociceptor from inflamed skin to increasing heat stimuli applied to the unit's RF.

responses of Aδ nociceptors to graded mechanical stimuli. Examples of graded responses to mechanical stimulation are displayed in Fig. 5*A*. As expected, Aδ nociceptors from non-inflamed skin responded montonically to graded von Frey filaments of 10, 26, and 60 g ($n = 6$ per group; Fig. 5*B*). Similarly, Aδ nociceptors from inflamed skin also responded monotonically, although the magnitude of response to each stimulus was greater than those of Aδ nociceptors from non-inflamed skin ($n = 6$ per group; $P < 0.001$; Fig. 5*B*).

We determined the effects of cannabinoids or vehicle on responses evoked by the 26 g von Frey filament, the same stimulus used in behavioral studies. A concern was the potential variability of responses to repeated application of the stimulus because responses at each time point represented are the average of two stimulus trials (see METHODS). We accounted for the variability of responses by expressing the number of impulses evoked during the second stimulus trial as a percent of the number of impulses evoked during the first stimulus trial. Overall the variability between stimulus trials for baseline responses of Aδ nociceptors isolated from non-inflamed and inflamed hindpaws did not differ and was $110.8 \pm 4.9\%$ ($n = 40$) and $105.6 \pm 3.0\%$ ($n = 105$), respectively. Units were classified as cannabinoid-sensitive if the evoked responses after cannabinoid administration were 2 SD below the baseline response (Wenk et al. 2006). Thus units that had a decrease in response of $\leq 44\%$ after cannabinoid administration were considered cannabinoid-sensitive.

Across all 40 Aδ nociceptors from non-inflamed skin, the mean baseline response to 26 g von Frey filament was 33.4 ± 1.9 impulses (6.6 ± 0.3 Hz). For Aδ nociceptors from inflamed skin ($n = 105$), the mean baseline response was 78.4 ± 3.9 impulses (16.0 ± 0.8 Hz) and was significantly greater than the responses of nociceptors from non-inflamed skin ($P < 0.001$).

EFFECTS OF METHAEA ON Aδ NOCICEPTORS FROM INFLAMED SKIN. Administration of the cannabinoid receptor agonist methAEA (10 μg) attenuated mechanically evoked responses from 81.1 ± 10.1 to 42.3 ± 6.6 impulses (a decrease of $\approx 48\%$) that peaked 30 min after administration and returned to baseline values 60 min after administration (Fig. 6). Unlike the decreases in mechanically-evoked responses observed after injection of methAEA (10 μg), injection of vehicle increased

mechanically evoked responses at all time points tested ($P < 0.01$) (Fig. 6). Seven of the 20 (35%) Aδ nociceptors treated with methAEA were cannabinoid-sensitive. Neither vehicle nor methAEA altered mechanical response thresholds or the variability in responses (number of evoked impulses) between stimulus trials at any time point tested (data not shown).

To determine if the attenuation of mechanically evoked responses by methAEA was mediated by CB_1 receptors, the CB_1 receptor antagonist AM251 (30 μg) was administered 5 min prior to injection of methAEA (10 μg). Pre-treatment with AM251 blocked the attenuation of mechanically evoked responses produced by methAEA (Fig. 6). No changes in mechanical response thresholds or the variability in responses between stimulus trials occurred at any time point tested after administration of AM251 followed by methAEA (data not shown).

EFFECTS OF ACEA ON Aδ NOCICEPTORS FROM INFLAMED SKIN. Administration of the cannabinoid receptor agonist ACEA (10 μg) also attenuated mechanically evoked responses from 80.0 ± 9.1 to 51.3 ± 6.1 impulses (a decrease of $\approx 36\%$) that peaked at 30 min after administration and returned to baseline values 60 min after administration (Fig. 7). Unlike the decreases in mechanically-evoked responses observed after injection of ACEA, the injection of vehicle did not alter mechanically evoked responses. Six of the 17 (35%) Aδ nociceptors treated with ACEA were cannabinoid-sensitive. Neither vehicle nor ACEA altered mechanical response thresholds or the variability in responses between stimulus trials at any time point tested (data not shown).

As with methAEA, AM251 (30 μg) also blocked the attenuation of mechanically evoked responses produced by ACEA (Fig. 7). No changes in mechanical response thresholds or the variability in responses between stimulus trials occurred at any time point tested after administration of AM251 followed by ACEA (data not shown).

EFFECTS OF ACEA AND METHAEA ON Aδ NOCICEPTORS FROM NON-INFLAMED SKIN. In contrast to the effects observed on Aδ nociceptors from inflamed skin, responses to the 26 g monofilament increased after methAEA (10 μg) or its vehicle (Fig. 8). The small increase in mechanically evoked responses
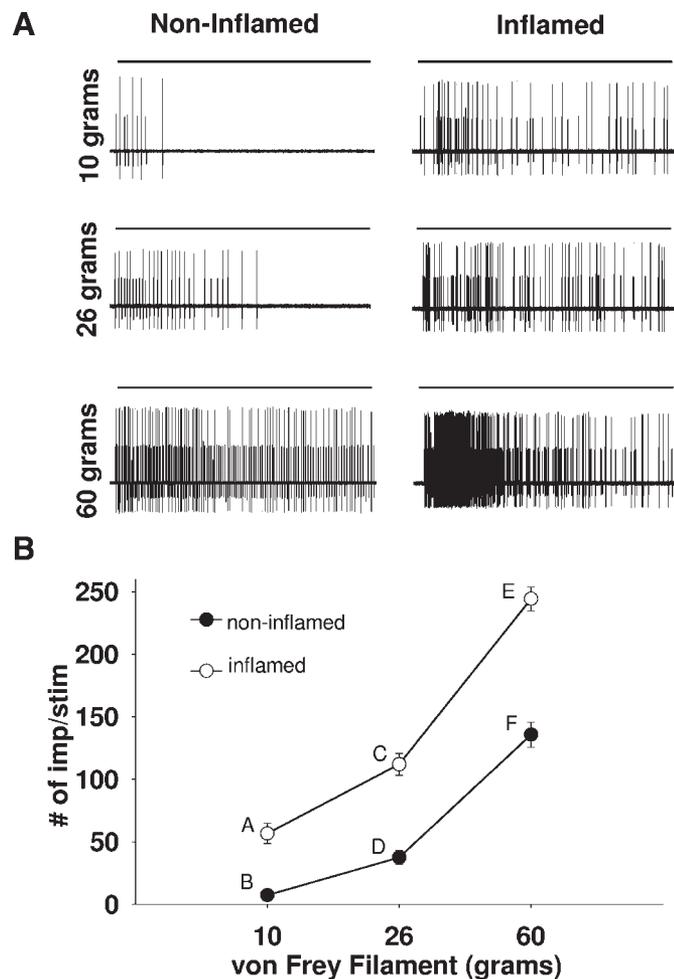
A

Non-Inflamed   **Inflamed**

B

FIG. 5. Responses of Aδ nociceptors to mechanical stimulation. *A*: examples of responses of Aδ nociceptors evoked by stimulation with 10, 26, and 60 g von Frey monofilaments from non-inflamed (*left*) and inflamed (*right*) skin (line above each traces represent stimulation for 5 s). *B*: the mean number impulses evoked by stimulation with 10, 26, and 60 g von Frey filaments of Aδ nociceptors are shown. # of imp/stim: number of impulses elicited by stimulation with a von Frey monofilament for 5 s. Groups that do not share letters are significantly different ($P < 0.05$). $n = 6$ units per group.

produced by methAEA and vehicle did not differ at any time point tested. Neither methAEA nor its vehicle altered mechanical response thresholds or variability in responses between stimulus trials at any time point tested. Similarly, administration of ACEA (10 μg) increased, and its vehicle did not alter, mechanically evoked responses compared with baseline measures (Fig. 9). As with methAEA, the changes in mechanically evoked responses produced by ACEA and vehicle did not differ at any time point tested. Neither ACEA nor vehicle altered mechanical response thresholds or the variability in responses between stimulus trials at any time point tested (data not shown).

RESPONSES EVOKED BY METHAEA, ACEA, AND THEIR VEHICLES. Previous studies have demonstrated that both ACEA (Price et al. 2004) and methAEA (Ralevic et al. 2001; Roberts et al. 2002) are able to activate TRPV1 receptors. To account for this potential excitatory effect, we recorded nociceptor activity during injection of drug (injection response) and for a 5-min period after injection (post-injection response). Injection responses of Aδ nociceptors from non-inflamed skin did not

differ in either proportion or magnitude regardless of the cannabinoid or vehicle injected. Similarly injection responses of Aδ nociceptors from inflamed skin did not differ in either proportion or magnitude regardless of the cannabinoid or vehicle injected. The magnitude of injection responses of Aδ nociceptors from inflamed skin, but not proportions, were greater than injection responses of Aδ nociceptors from non-inflamed skin ($P < 0.01$). Because only a small proportion of Aδ nociceptors from non-inflamed skin exhibited post-injection responses (5/40), differences between cannabinoids and vehicle could not be ascertained. Post-injection responses of Aδ nociceptors (53/105) from inflamed skin did not differ in either proportion or magnitude regardless of cannabinoid or vehicle injected. Overall the magnitude ($P < 0.01$) and proportion ($P < 0.0001$) of post-injection responses of Aδ nociceptors from inflamed skin were greater than those of Aδ nociceptors from non-inflamed skin. A complete listing of responses during and after injection is provided in Supplementary Table 1.[1] These data show that injection of cannabinoids or their vehicles into the plantar surface of the hindpaw evokes non-specific excitation of Aδ nociceptors, and inflammation increases the magnitude of this response.

DISCUSSION

In the present study, local administration of the cannabinoid receptor agonists, methAEA or ACEA, attenuated inflammatory mechanical allodynia and hyperalgesia and decreased mechanically evoked responses of Aδ nociceptors from inflamed skin. Both the antinociceptive effects and the decrease in mechanically evoked responses produced by methAEA and ACEA were blocked by the CB1 receptor antagonist AM251, strongly suggesting that activation of peripheral CB1 receptors underlies these effects. Administration of neither methAEA nor ACEA produced antinociception to mechanical stimuli in control, non-inflamed rats nor did it decrease mechanically evoked responses of Aδ nociceptors from non-inflamed skin. These data suggest that attenuation of evoked-responses of Aδ nociceptors contributes to the antiallodynia/antihyperalgesia produced by activation of peripheral CB1 receptors during inflammation.

Cannabinoid attenuation of allodynia and hyperalgesia

The results of our behavioral studies agree with prior studies demonstrating antiallodynia/antihyperalgesia after local administration of cannabinoids into inflamed tissue through activation of peripheral CB1 receptors (Amaya et al. 2006; Gutierrez et al. 2007; Richardson et al. 1998). Local administration of cannabinoids have also been shown to attenuate hyperalgesia produced by nerve injury (Fox et al. 2001; Guindon and Beaulieu 2006), cutaneous heat injury (Johanek and Simone 2004), and cancer (Guerrero et al. 2008; Potenzieri et al. 2008) through activation of peripheral CB1 receptors.

Previous studies have demonstrated that ACEA (Hillard et al. 1999; Meybohm et al. 2008) and methAEA (Abadji et al. 1994) given systemically produced typical cannabinemic effects such as hypothermia, hypolocomotion, catalepsy, and antinociception through activation of CB1 receptors in the CNS. The doses required to produce these cannabinemic ef-

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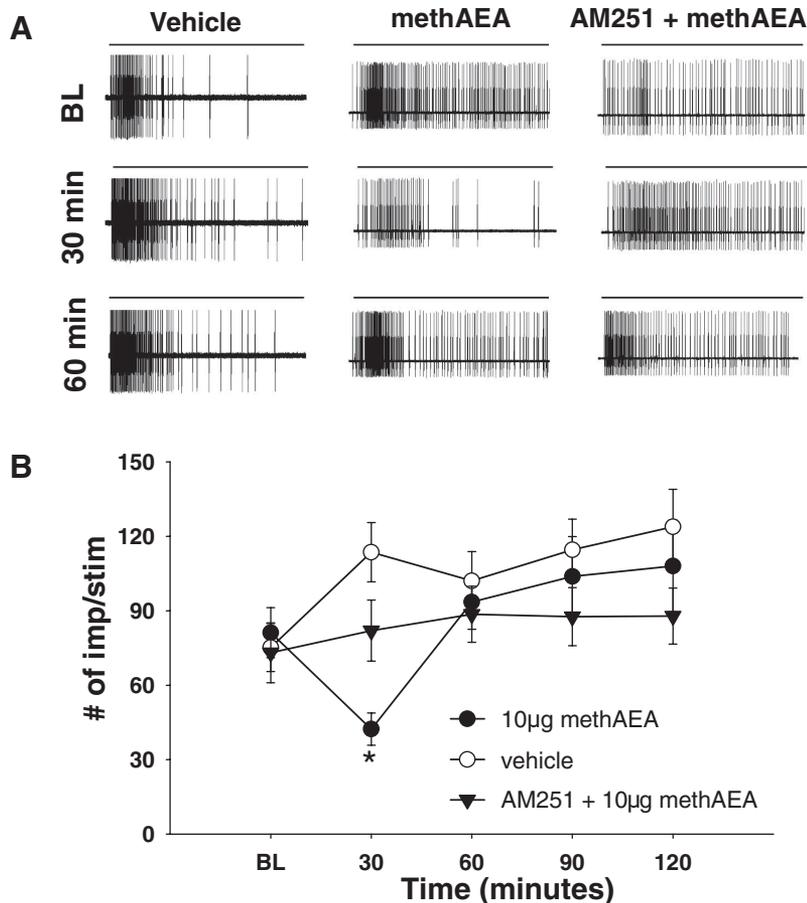[1] The online version of this article contains supplemental data.

FIG. 6. Local administration of methAEA decreases mechanically evoked responses of Aδ nociceptors from inflamed skin by activation of CB₁ receptors. *A*: examples of mechanically-evoked responses via stimulation with a 26 g von Frey filament before and after administration of methAEA (10 μg), vehicle, or AM251 (30 μg) followed by methAEA (10 μg) are shown. Each column represents responses of different Aδ nociceptors, and each row represents a different time point indicated on the *left*. The line above each trace represents the time of stimulation (5 s). *B*: the mean number impulses evoked by stimulation with a 26 g von Frey filament before and after administration of methAEA (10 μg), vehicle, or AM251 (30 μg) followed by methAEA (10 μg) are shown. BL, mean baseline predrug number of impulses; Time, time after administration of drug; # of imp/stim, number of impulses elicited by stimulation with a von Frey monofilament for 5 s; *, significant difference from vehicle ($P < 0.05$). $n = 15–20$ units per group.

fects were 3- to 10-fold greater than the highest doses of ACEA and methAEA used in the present study (Abadji et al. 1994; Hillard et al. 1999; Meybohm et al. 2008). No antiallodynia or antihyperalgesia occurred when the doses of cannabinoids used in the present study were injected into the paw contralateral to the inflamed paw, demonstrating that the antihyperalgesic effects of cannabinoids occurred through peripheral mechanisms. The antiallodynia and antihyperalgesia produced by ACEA and methAEA were blocked by co-administration with the CB₁ receptor antagonist AM251 but not with the CB₂ receptor antagonist AM630. The contribution of CB₁ receptors to the antiallodynia/antihyperalgesia produced by ACEA and methAEA is consistent with their higher affinity for CB₁ over CB₂ receptors, 1,400- and 40-fold, respectively (Abadji et al. 1994; Hillard et al. 1999).

We found that intraplantar administration of ACEA and methAEA produced antinociception to mechanical stimuli only during inflammation. Similarly, the ability of ACEA to attenuate behavioral responses to radiant heat was greater during inflammation (Amaya et al. 2006). This enhancement of ACEA's antinociceptive effects was related to increased CB₁ receptor labeling in both nociceptive DRG neurons and their peripheral nerve terminals (Amaya et al. 2006). Similarly, upregulation of CB₁ receptors also occurred in DRG neurons 2 wk after spinal nerve ligation (SNL) (Mitrirattanakul et al. 2006) and was related to the enhanced antinociception produced by locally administered cannabinoids in this model of neuropathic pain (Fox et al. 2001). These studies suggest that peripherally mediated antinociception produced by locally ad-

ministered cannabinoids results from increased CB₁ receptor expression on nociceptive DRG neurons and presumably their peripheral endings; however, the specific nociceptor subtypes involved have not been determined. It is possible that acute changes also exist to regulate CB₁ receptor activity because locally administered cannabinoids also produce peripherally mediated antinociception in animal models of acute pain such as intraplantar injection of capsaicin (Johanek et al. 2001), cutaneous heat injury (Johanek and Simone 2004), and intraplantar injection of formalin (Calignano et al. 1998; Guindon et al. 2006) through activation of peripheral CB₁ receptors.

We found that administration of CB₁ receptor antagonist AM251 or CB₂ receptor antagonist AM630 alone did not alter mechanical allodynia or hyperalgesia after inflammation evoked by CFA. These results suggest that there were no changes in endocannabinoid tone after inflammation produced by CFA. A similar observation was noted by Gutierrez et al. (2007) after inflammation evoked by carrageenan and using the CB₁ receptor antagonist SR141716A or the CB₂ receptor antagonist SR144528. Additional studies are needed to delineate the potential role of endocannabinoids during inflammation and their relevance to changes in hyperalgesia.

Sensitization of nociceptors during inflammation

Under pathological conditions, nociceptors can become sensitized, which is characterized by a decrease in response threshold, increased responses to suprathreshold stimuli, and ongoing activity (Bessou and Perl 1969; for review, see Raja
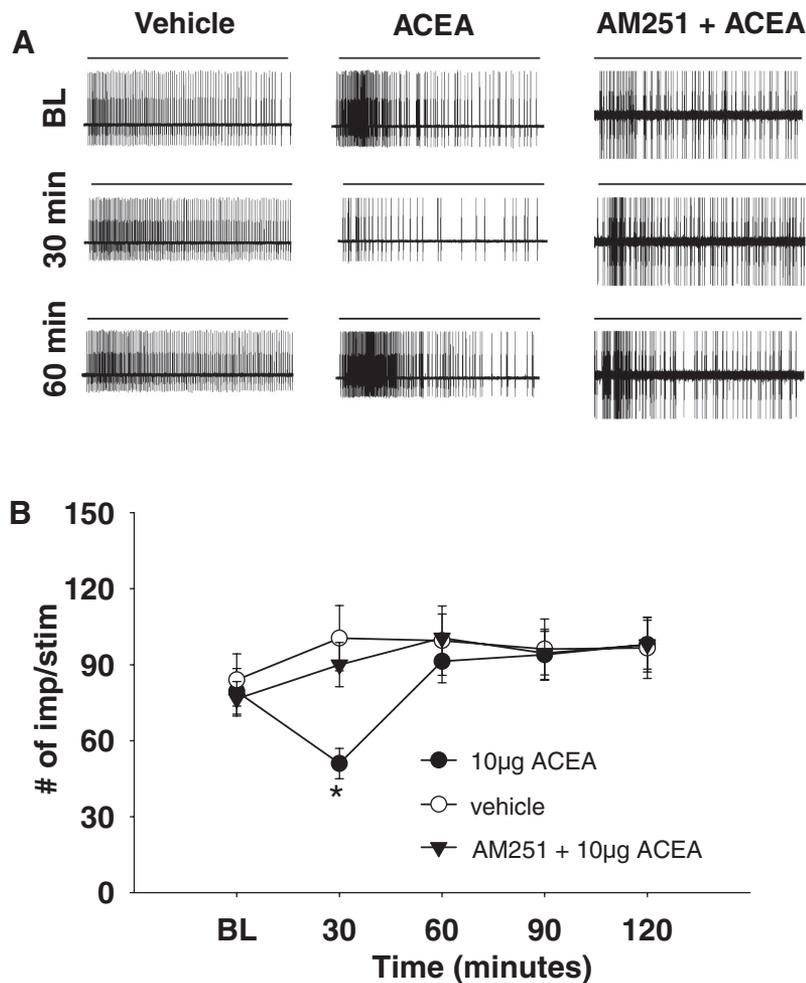
FIG. 7. Local administration of ACEA decreases mechanically evoked responses of Aδ nociceptors from inflamed skin by activation of CB$_1$ receptors. *A*: examples of mechanically evoked responses via stimulation with a 26 g von Frey filament before and after administration of ACEA (10 μg), vehicle, or AM251 (30 μg) followed by ACEA (10 μg) are shown. Each column represents responses of different Aδ nociceptors, and each row represents a different time point indicated on the *left*. The line above each trace represents the time of stimulation (5 s). *B*: the mean number impulses evoked by stimulation with a 26 g von Frey filament before and after administration of ACEA (10 μg), vehicle, or AM251 (30 μg) followed by ACEA (10 μg) are shown. BL, mean baseline predrug number of impulses; Time, time after administration of drug; # of imp/stim, number of impulses elicited by stimulation with a von Frey monofilament for 5 s; *, significant difference from vehicle ($P < 0.05$). $n = 15–20$ units per group.

et al. 1988; Treede et al. 1992). Nociceptor sensitization has been shown to correlate with psychophysical measures of hyperalgesia in humans (LaMotte et al. 1982, 1983; Meyer and Campbell 1981; Torebjörk et al. 1984). Previous studies have demonstrated that cutaneous Aδ and C nociceptors innervating glabrous skin are sensitized after intraplantar injection of CFA both in vivo (Andrew and Greenspan 1999; Djouhri et al. 2006) and in ex vivo preparations (Du et al. 2003, 2006; Wenk et al. 2006). Other studies of inflammatory pain using carrageenan have also demonstrated that Aδ and C nociceptors innervating non-glabrous skin exhibit enhanced responses to natural stimuli (Kirchhoff et al. 1990; Kocher et al. 1987; Koltzenburg et al. 1999).

We found that Aδ nociceptors innervating inflamed skin exhibited ongoing activity, a decrease in mechanical response thresholds, and enhanced responses to suprathreshold mechanical stimuli. Although we were unable to study Aδ nociceptors before and after the induction of inflammation, the functional classification of units in our study allowed for meaningful comparisons between Aδ nociceptors from non-inflamed and inflamed skin. Similar findings were reported by Andrew and Greenspan (1999) with the exception that they did not observe decreases in mechanical response thresholds, perhaps due to differences in sample size (40 vs. 145 in the present study). Similar to that study, we found no heat-responsive Aδ nociceptors from non-inflamed skin, whereas a small proportion of Aδ nociceptors from inflamed skin were sensitive to heat, as

also found by Wenk et al. (2006). However, a higher proportion of heat-responsive Aδ nociceptors were reported innervating the plantar surface of the hindpaw of naive rats as compared with our sample (Leem et al. 1993). The low proportion of heat-responsive Aδ nociceptors in our study was likely due to heat stimuli used (51°C for 5 s vs. 52°C for 20 s) that would have excluded Aδ nociceptors with higher heat response thresholds (Leem et al. 1993).

Contributions of Aδ nociceptors to mechanical allodynia and hyperalgesia

Consistent with hyperalgesia observed 24 h after intraplantar injection of CFA, responses of Aδ nociceptors from inflamed skin evoked by the 26 g von Frey filament, the same filament used to characterize hyperalgesia in our behavioral studies, were enhanced compared with responses of Aδ nociceptors from non-inflamed skin. This enhanced responsiveness of Aδ nociceptors from inflamed skin suggests a contribution of Aδ nociceptors to mechanical hyperalgesia produced by CFA. Mechanical response thresholds of Aδ nociceptors from inflamed skin were lower than thresholds of Aδ nociceptors from non-inflamed skin; however, their respective thresholds were still below paw withdrawal thresholds in behavioral studies of non-inflamed and inflamed rats. The contribution of Aδ nociceptors to mechanical allodynia after intraplantar injection of CFA likely resides in their enhanced evoked responses rather
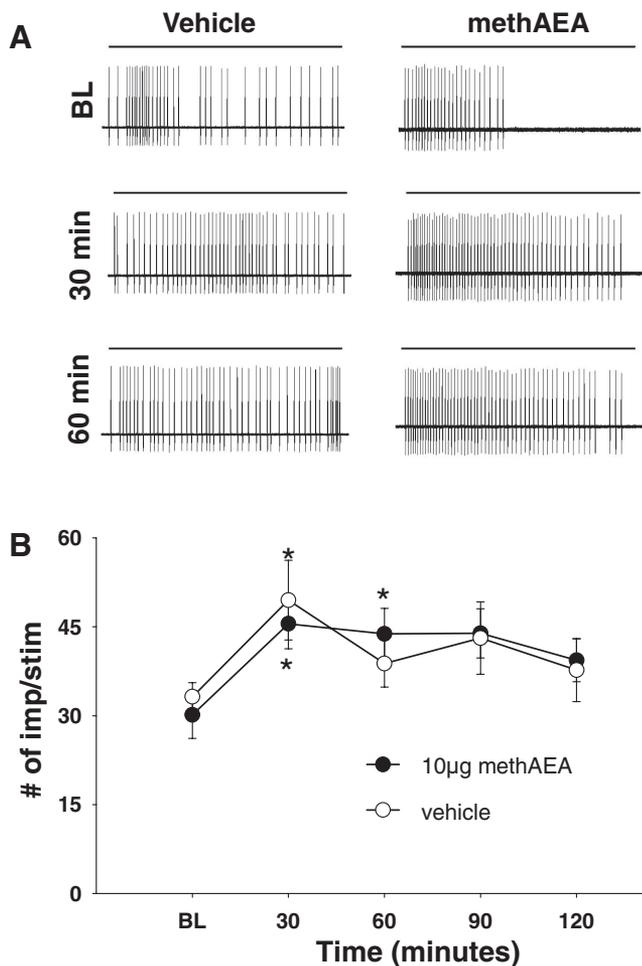
FIG. 8. Effect of methAEA on mechanically evoked responses of Aδ nociceptors from non-inflamed skin. *A*: examples of responses evoked by stimulation with the 26 g monofilament before and after administration of methAEA (10 μg) or vehicle. Each column represents responses of different Aδ nociceptors, and each row represents a different time point indicated on the *left*. The line above each trace represents the time of stimulation (5 s). *B*: the mean number impulses evoked by the monofilament before and after administration of methAEA (10 μg) or vehicle are shown. BL, baseline predrug number of impulses; Time, time after administration of drug; # of imp/stim, number of impulses elicited by stimulation with a von Frey monofilament for 5 s; *, significant difference from BL ($P < 0.05$). $n = 10$ units per group.

than changes in thresholds. In a similar study after an incision-injury to the plantar surface of the rat hindpaw, decreased mechanical response thresholds and increased responses to suprathreshold stimuli were also correlated to decreases in paw withdrawal thresholds and increases in paw withdrawal frequencies (Hämäläinen et al. 2002; Pogatzki et al. 2002).

Responses of Aδ nociceptors to intraplantar injection

Injection of cannabinoids or vehicles into mechanical RFs of Aδ nociceptors from both non-inflamed and inflamed skin produced excitation during the injection, termed injection responses. There were no within-group differences of injection responses exhibited by Aδ nociceptors regardless of the cannabinoid or vehicle injected. These results suggest that injection responses are a non-specific effect, possibly due to mechanical distention within RFs (Hilliges et al. 2002). Responses during injections were greater in magnitude in Aδ nociceptors

from inflamed skin than the injection responses of Aδ nociceptors from non-inflamed skin. This overall increase in the response during the injection is likely related to the enhanced sensitivity to mechanical stimulation during inflammation.

Injection of cannabinoid or vehicle into mechanical RFs of both Aδ nociceptors from non-inflamed and inflamed skin produced excitation that persisted after injection, termed post-injection responses. A greater proportion Aδ nociceptors from inflamed skin exhibited post-injection impulses compared with Aδ nociceptors from non-inflamed skin. Post-injection responses of Aδ nociceptors from inflamed skin did not differ in proportion and magnitude regardless of the cannabinoid or vehicle injected, also suggesting a nonspecific effect and probably reflects enhanced sensitivity to mechanical stimulation.

Cannabinoid modulation of Aδ nociceptors

After injection of either ACEA or methAEA into the RFs of Aδ nociceptors from inflamed skin, mechanically evoked re-
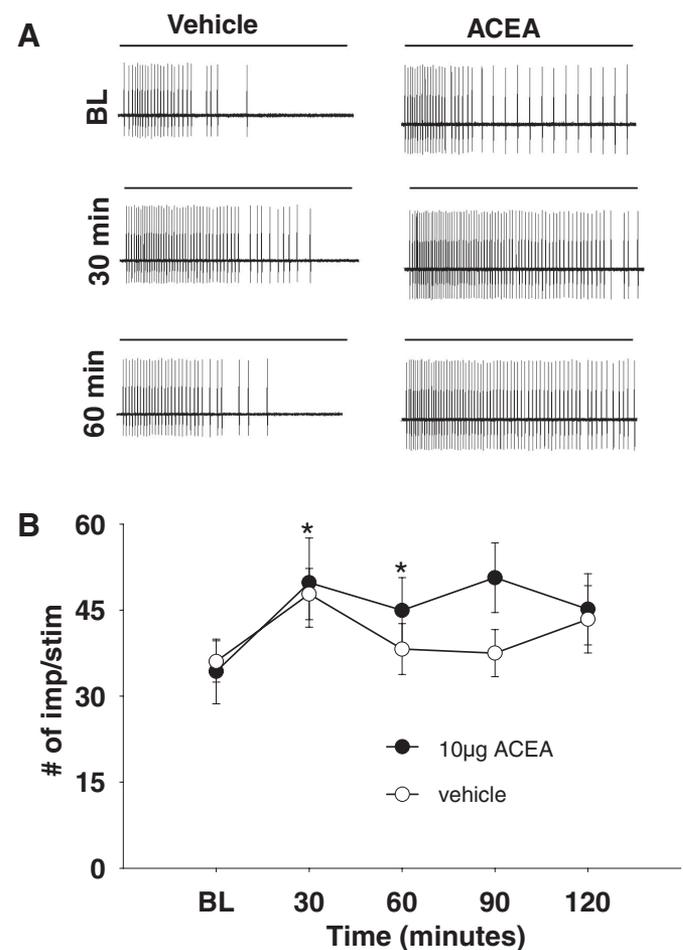

FIG. 9. Effect of ACEA on mechanically evoked responses of Aδ nociceptors from non-inflamed skin. *A*: examples of responses evoked by stimulation with the 26 g monofilament before and after administration of ACEA (10 μg) or vehicle. Each column represents responses of different Aδ nociceptors, and each row represents a different time point indicated on the *left*. The line above each trace represents the time of stimulation (5 s). *B*: the mean number impulses evoked by the monofilament before and after administration of ACEA (10 μg) or vehicle are shown. BL, predrug number of impulses; Time, time after administration of drug; # of imp/stim, number of impulses elicited by stimulation with a von Frey monofilament for 5 s; *, significant difference from BL ($P < 0.05$). $n = 10$ units per group.

sponses were attenuated and returned to baseline levels by 60 min after injection. This is similar to the behavioral time course of antiallodynia/antihyperalgesia after injection of ACEA or methAEA in our behavioral studies. Additionally, administration of the CB_1 receptor antagonist AM251 attenuated both the decrease in mechanically evoked responses and antiallodynia/antihyperalgesia produced by ACEA and methAEA, strongly suggesting that activation of peripheral CB_1 receptors underlies these effects. We did not administer the CB_2 receptor antagonist AM630 in the electrophysiological studies because AM630 did not alter the antihyperalgesic/antiallodynic effects of ACEA and methAEA in behavioral studies. Surprisingly, injection of cannabinoids or vehicles did not alter mechanical response thresholds of $A\delta$ nociceptors from inflamed skin at any time point tested. This lack of change in mechanical response thresholds likely reflects the greater contributions of the magnitude of evoked responses in mediating changes to mechanical sensitivity.

The decrease in mechanically evoked responses after administration of ACEA and methAEA was likely due in part to direct activation of CB_1 receptors located on $A\delta$ nociceptors. Previous studies using immunohistochemical methods have localized CB_1 receptors to DRG neurons with nociceptive phenotypes that have either myelinated or unmyelinated fibers, indicating that at least a proportion of both $A\delta$ and C nociceptors express CB_1 receptors (Agarwal et al. 2007; Amaya et al. 2006; Bridges et al. 2003; Khasabova et al. 2002). Although we focused specifically on $A\delta$ nociceptors, the effects of cannabinoids on response properties of C nociceptors need to be determined. We cannot rule out potential actions of ACEA and methAEA on other cell types in the cutaneous environment, which could potentially affect nociceptive sensitivity and nociceptor activity. Fibroblasts (Ständer et al. 2005), endothelial cells (Liu et al. 2000), lymphocytes (Parolaro 1999), mast cells (Samson et al. 2003), keratinocytes (Maccarone et al. 2003), T-cells (Maccarrone et al. 2001), and dendritic cells (Matias et al. 2002) all express CB_1 receptors.

We did not find any evidence of CB_2 receptor-mediated antinociceptive effects in our study because the CB_2 receptor antagonist AM630 did not block the antinociceptive effects produced by ACEA and methAEA. This was likely due to the higher selectivity of ACEA and methAEA for CB_1 receptors over CB_2 receptors (1,400- and 40-fold, respectively). However, previous studies using selective CB_2 receptor agonists have demonstrated that these drugs produce antinociception to noxious heat (Ibrahim et al. 2005, 2006; Malan et al. 2001) and in a variety of pain models including hyperalgesia produced by carrageenan (Elmes et al. 2005; Gutierrez et al. 2007; Nackley et al. 2003; Quartilho et al. 2003), capsaicin (Hohmann et al. 2004), and neuropathic pain (Ibrahim et al. 2003). Locally administered CB_2 receptor agonists have also been shown to decrease evoked responses of nociceptive spinal cord neurons through activation of peripheral CB_2 receptors (Elmes et al. 2004; Nackley et al. 2004; Sokal et al. 2003). Although CB_2 receptors are mainly expressed on leukocytes, studies have demonstrated that nociceptive DRG neurons express functional CB_2 receptors (Anand et al. 2008; Sagar et al. 2005). Further studies are needed to determine how selective activation of peripheral CB_2 receptors affects the excitability and response properties of nociceptors.

In contrast to the effects on $A\delta$ nociceptors from inflamed skin, administration of methAEA or ACEA transiently increased mechanically evoked responses of non-inflamed $A\delta$ nociceptors. This small increase in evoked responses was related to decreased paw withdrawal thresholds and a trend for an increase in paw withdrawal frequencies in behavioral studies after intraplantar injection of either cannabinoid into non-inflamed hindpaws. This disparity between decreased paw withdrawal thresholds and a trend for an increase in paw withdrawal frequencies suggests that paw withdrawal threshold may be a more sensitive measure than withdrawal frequency using the 26 g von Frey monofilament. The enhanced responses of $A\delta$ nociceptors and increased mechanical sensitivity in behavioral studies likely resulted from irritation produced by the injection.

Summary

Local administration of cannabinoids, ACEA or methAEA, into inflamed hindpaws attenuated mechanical allodynia and hyperalgesia through activation of peripheral CB_1 receptors. In parallel studies, we found that local administration of either ACEA or methAEA decreased mechanically evoked responses of $A\delta$ nociceptors from inflamed skin that was also attributed to activation of CB_1 receptors. Overall these results suggest that attenuation of $A\delta$ nociceptors' responses contributes to the antiallodynia/antihyperalgesia produced by activation of peripheral CB_1 receptors after local administration of ACEA and methAEA during inflammation. Taken together, our data suggest that peripherally acting cannabinoids could be a potential therapeutic treatment for chronic inflammatory pain.

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