



Local application of the cannabinoid receptor agonist, WIN 55,212-2, to spinal trigeminal nucleus caudalis differentially affects nociceptive and non-nociceptive neurons

Alex M. Papanastassiou, Howard L. Fields, Ian D. Meng*

Department of Neurology, University of California, San Francisco, CA 94143-0114, USA

Received 7 March 2003; received in revised form 25 August 2003; accepted 10 November 2003

Abstract

Cannabinoid receptor agonists produce analgesia for pains of non-cranial origin. However, their effectiveness for craniofacial pains is currently unclear. In the present study, the cannabinoid CB1/CB2 receptor agonist, WIN 55,212-2 (WIN), was bath applied to the brainstem while activity of spinal trigeminal nucleus caudalis (Vc) neurons evoked by transcutaneous electrical stimulation was recorded in isoflurane anesthetized rats. Neurons were characterized using mechanical and electrical stimulation of the face, and were classified as either low-threshold mechanoreceptive (LTM) or wide dynamic range (WDR). LTM neurons responded to light brushing of the receptive field and received only A β primary afferent fiber input. WDR neurons showed a graded response to mechanical stimulation, responding maximally to noxious stimuli, and demonstrated both A- and C-fiber evoked activity. In addition, WDR neurons displayed longer latency, C-fiber mediated post-discharge (PDC) activity after repetitive stimulation. Local bath application of 2.0 mg/ml WIN significantly reduced PDC activity ($3 \pm 1\%$ control, $P < 0.01$), C-fiber evoked activity ($58 \pm 9\%$ control, $P < 0.01$), and A β evoked activity ($57 \pm 10\%$ control, $P < 0.01$) in WDR neurons. In contrast, LTM A β -fiber evoked activity increased after local administration of WIN ($204 \pm 52\%$ control, $P < 0.01$). SR141716A, a CB1 receptor antagonist, prevented the effects of WIN on WDR PDC and LTM A β evoked activity. These results indicate that cannabinoid receptor agonists may be effective agents for craniofacial pain. Furthermore, the particular sensitivity of PDC activity, a measure of neuronal hyperexcitability, to cannabinoid receptor agonists may be relevant to the treatment of persistent craniofacial pain. © 2003 International Association for the Study of Pain. Published by Elsevier B.V. All rights reserved.

Keywords: Cannabinoid receptor agonist; Craniofacial pain; Wide dynamic range

1. Introduction

Cannabinoid compounds are effective analgesics for cancer and post-operative pain (Campbell et al., 2001; Jain et al., 1981; Noyes et al., 1975a,b). Understanding the mechanisms by which cannabinoid compounds produce analgesia has advanced significantly since two G $_{i/o}$ -protein coupled cannabinoid receptors, CB1 and CB2, were cloned (Devane et al., 1988; Matsuda et al., 1990; Munro et al., 1993), and receptor-selective agonists and antagonists were developed (Palmer et al., 2002). Although both CB1 and CB2 agonists are antinociceptive in animals,

only CB1 receptors are present on neurons throughout the central and peripheral nervous systems, whereas CB2 receptors are primarily located on immune cells (Pertwee, 2001).

Behavioral studies in animals indicate that cannabinoids produce analgesia for pains of non-cranial origin (Buxbaum et al., 1969; Herzberg et al., 1997; Martin and Lichtman, 1998; Moss and Johnson, 1980; Sofia et al., 1973). Furthermore, cannabinoid analgesia likely is due in part to direct actions at the level of the dorsal horn. Intrathecal administration of cannabinoid receptor agonists produces antinociception and reduces hypersensitivity in acute and chronic pain tests, respectively (Fox et al., 2001; Johaneck et al., 2001; Lichtman et al., 1992; Mao et al., 2000; Martin et al., 1999; Richardson et al., 1998; Welch and Stevens, 1992; Welch et al., 1998; Yaksh, 1981).

Electrophysiological studies have demonstrated that cannabinoid receptor agonists also inhibit nociceptive

* Corresponding author. Present address: Department of Physiology, School of Osteopathic Medicine, University of New England, 11 Hills Beach Road, Biddeford, ME 04005, USA. Tel.: +1-207-283-0170x2195; fax: +1-207-294-5931.

E-mail address: imeng@une.edu (I.D. Meng).

neurons in the lumbar spinal cord dorsal horn (SCDH). Local bath application of cannabinoid receptor agonists to the SCDH decreases heat- and electrically evoked nociceptive neuronal activity (Chapman, 2001; Drew et al., 2000; Hohmann et al., 1998; Kelly and Chapman, 2001). Additionally, SCDH neuronal hyperexcitability, as measured by the long latency discharge following repetitive low-frequency electrical stimulation, is maximally suppressed, indicating a possible therapeutic use of cannabinoids in the treatment of persistent pain states (Chapman, 2001; Drew et al., 2000; Kelly and Chapman, 2001).

The direct action of cannabinoid receptor agonists in the SCDH suggests that they may also affect trigeminal neurons, suppressing nociceptive inputs from the head and face. However, the effectiveness of cannabinoids for craniofacial pains is currently unclear. There is additional anecdotal evidence that marijuana, which contains the CB1/CB2 receptor agonist, Δ^9 -tetrahydrocannabinol (THC), relieves cranial pains such as migraine and trigeminal neuralgia (Dunn and Davis, 1974; Noyes and Baram, 1974; Russo, 1998). However, the only clinical study assessing the effect of THC on orofacial pain produced ambiguous results in a small number of patients (Raft et al., 1976). The spinal trigeminal nucleus caudalis (Vc) is the primary relay for craniofacial pain, and as such represents a likely target for the possible analgesic action of cannabinoids. CB1 receptors are present in the trigeminal ganglia and trigeminal nucleus, consistent with their location in dorsal root ganglion and SCDH neurons (Herkenham et al., 1991; Richardson et al., 1998; Tsou et al., 1998). The experiments presented here were conducted to determine the effects of a locally applied cannabinoid receptor agonist in vivo on the electrically evoked activity and hyperexcitability of nociceptive and non-nociceptive Vc neurons. Preliminary results have been presented (Papanastassiou et al., 2001).

2. Methods

2.1. Animals and surgical preparation

The experimental protocols were approved by the Committee on Animal Research at University of California San Francisco, and were in accordance with the policies and recommendations of the NIH guidelines for the handling and use of laboratory animals. Experiments were conducted using 47 male Sprague–Dawley rats (300–540 g, B&K Universal). Rats were anesthetized initially with sodium methohexital (70 mg/kg i.p.), and a tracheotomy was performed. Animals were then artificially ventilated, and anesthesia was maintained with isoflurane (1.25–2.25%). End-tidal CO₂ was maintained at 3.5–4.5%, and body temperature was maintained using a heating blanket. The right external jugular and femoral artery were cannulated, and mean arterial blood pressure (MAP) was monitored.

After placement of animals in a stereotaxic frame, an occipital craniectomy exposed the dorsal brain stem and a C1 laminectomy exposed the rostral cervical cord. A constant volume bath of 40–80 μ l over the dorsal surface of the brain stem was created by caudal insertion through the trapezius muscle of a 20-gauge by 1-inch inflow catheter, and placement at the level of obex of an L-shaped 16-gauge outflow needle. Fluid flow was maintained using a push-pull pump (Stoelting), and flow throughout the bath was verified by perfusing Evans Blue dye through the bath after experiments. Animals were paralyzed with pancuronium (0.6 mg/kg per h, i.v.) or gallamine triethiodide (20 mg/kg per h, i.v.) before recording.

2.2. Recording methods

Electrophysiological recording began no sooner than 45 min after completion of surgery. Tungsten microelectrodes (3–12 M Ω , FHC, Bowdoinham, ME) were used to record extracellularly from single neurons located in Vc, from approximately 1.5 mm caudal to obex to the junction between Vc and C1. Neurons were identified using light brush of the face, and receptive fields were mapped. Neurons were classified as low-threshold mechanoreceptive (LTM) units or wide dynamic range (WDR) neurons according to their responses to light brush, non-noxious pressure, and noxious pinch (Hu, 1990). No nociceptive-specific or cells with deep receptive fields were studied. LTM neurons responded maximally to innocuous stimuli, and showed no increased response with pressure or noxious pinch. WDR neurons were sensitive to non-noxious and noxious stimuli, and showed an increase in discharge as the intensity of stimulation increased.

Transcutaneous electrical stimulation of the center of the receptive field evoked only short-latency (0–30 ms) A β primary afferent activity in LTM neurons. For WDR neurons, low intensity stimulation evoked short-latency A-fiber activity, while high intensity stimulation evoked both A-fiber (0–30 ms latency), as well as C-fiber evoked activity (30–150 ms latency; Dickenson et al., 1980; Hu, 1990). In addition, repetitive, high intensity stimulation also generated C-fiber-dependent post-discharge (PDC) activity (150–800 ms latency). An example of the electrically evoked activity of one WDR neuron in response to high-intensity stimulation is shown in Fig. 2A.

2.3. Test stimulation

LTM neurons were stimulated electrically at 1.5–3 times threshold (0.10 ms, 0.20–2.50 mA) to elicit A β responses. For WDR neurons, two separate intensities were used to evoke low threshold A β -fiber activity and high threshold C-fiber activity. The duration of low intensity electrical stimuli was set at 0.10 ms, with a current 1.5–3 times threshold for A-fiber evoked activity (0.10–5.00 mA). High intensity stimuli were set at a 2.0 ms duration, with a current

1.5–3 times threshold C-fiber evoked activity (0.25–4.5 mA). For both LTM and WDR neurons, each trial consisted of a series of 16 stimuli delivered at 0.5 Hz and consecutive trials were separated by 7.5 min. In WDR neurons only, high-intensity stimulation was delivered 2 min after low intensity stimulation. After two stable baseline trials, neuronal activity was determined during two or three trials each after low- and high-dose drugs or vehicle control. Drugs were infused into the bath over 10 min, and effects were measured 5, 12.5 and in some cases 20 min after the bath solution was changed. Three treatment groups each for LTM and WDR neurons were studied: (1) successive administration of WIN 55,212-2 (WIN) at 0.2 and 2.0 mg/ml; (2) co-administration of SR 141716A (SR, 1 mg/ml) with WIN (0.2 and 2.0 mg/ml); (3) administration of vehicle only. In most cases, only one neuron was studied per animal, and no additional neurons were studied after drugs were administered.

2.4. Histology

Electrolytic lesions were performed at the end of each experiment to mark recording sites (10.0 μ A, 20 s). Animals were euthanized with pentobarbital overdose and perfused through the heart with normal saline followed by 10% formalin. The brain stem was removed and stored in formalin, and then 30% sucrose solution at 4 °C for at least 48 h prior to cutting. Frozen coronal sections were cut on a sliding microtome, mounted, and stained with 0.3% cresyl violet.

2.5. Drugs

WIN (Sigma) was dissolved in 2-hydroxypropyl- β -cyclodextrin (Sigma) or solutions of 9:1 normal saline to alkamuls solution. No difference was observed with use of either vehicle, so data were pooled together. SR 141716A (SR, generous gift from NIDA) and SR with WIN were dissolved in 9:1 normal saline to alkamuls solution.

2.6. Statistical analysis

In preliminary experiments, three trials were conducted following each dose of WIN. Since no further reduction in activity occurred during the third trial, activity during the second trial was analyzed in all experiments. For each trial, the sum of A β -fiber evoked, C-fiber evoked, and PDC activities in response to the 16 consecutive stimuli was calculated. Data were analyzed as percent of baseline. Statistical significance was assessed by two-way ANOVA using time as a repeated measurement. Post-hoc tests were performed using the Newman Keuls test. Probabilities of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. General properties

WDR neurons ($n = 27$) and LTM neurons ($n = 21$) recorded at depths of 319–1252 and 130–952 μ m, respectively, from the surface of the brain stem were studied (Fig. 1). These cells were uniformly located in the deep laminae of the Vc/C1 transition 2–3.5 mm caudal to obex, and receptive fields were evenly distributed between the trigeminal subdivisions, V1-3.

Average electrically evoked activity and stimulation intensity thresholds for activating LTM and WDR neurons during baseline trials are shown in Table 1. In the pre-drug period, A β evoked activity in LTM neurons, as well as A-fiber, C-fiber, and PDC activity in WDR neurons, were similar between vehicle, WIN, and WIN/SR treatment groups. Electrical stimulation thresholds for WDR neurons were also comparable between all treatment groups. LTM A β thresholds were similar between WIN and WIN/SR groups, but vehicle cells had significantly higher thresholds ($P < 0.01$). Average evoked activity was equivalent between the two pre-drug baseline trials for all fiber types and cell types studied (data not shown).

3.2. Effects of WIN 55,212-2 on nociceptive neurons

Local bath application of WIN inhibited C-fiber mediated hyperexcitability of WDR neurons, as measured

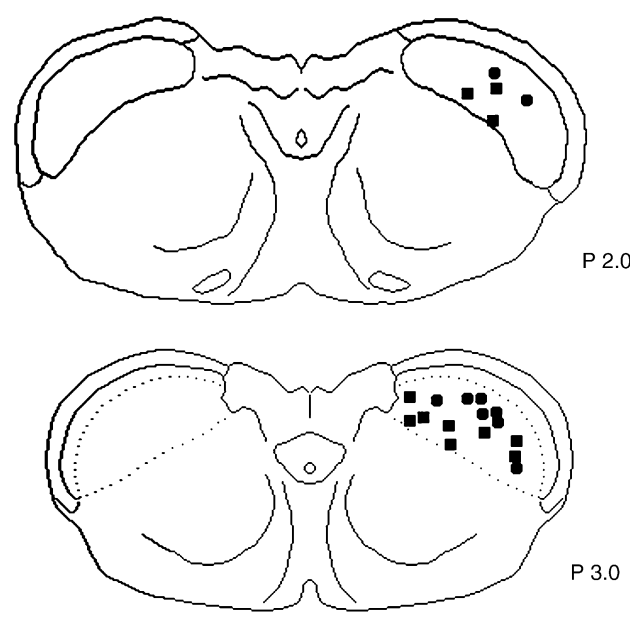


Fig. 1. Recording sites of neurons in spinal trigeminal nucleus caudalis as determined by electrolytic lesions. Neurons were classified according to receptive field properties and responses to electrical stimulation. Numbers to the right of outlines represent approximate distance from obex in millimeters. Squares, wide dynamic range (WDR) neurons; Circles, low-threshold mechanoreceptive (LTM) neurons. Calibration, 1 mm.

Table 1
Stimulation thresholds and neuronal evoked activity during baseline electrical stimulation trials

	Stimulation thresholds (mA)			Evoked activity (number of action potentials)			
	LTM, A β -fiber	WDR		LTM, A β -fiber	WDR		
		A-fiber	C-fiber		A $\bar{\nu}$ -fiber	C-fiber	PDC
Vehicle	1.7 \pm 0.4*	0.9 \pm 0.2	1.3 \pm 0.3	39 \pm 7	51 \pm 7	125 \pm 15	83 \pm 39
WIN	0.6 \pm 0.1	1.2 \pm 0.5	2.1 \pm 0.5	43 \pm 6	60 \pm 9	108 \pm 24	70 \pm 20
WIN/SR	0.7 \pm 0.3	1.4 \pm 0.7	1.2 \pm 0.4	47 \pm 5	50 \pm 12	122 \pm 27	64 \pm 18

Stimulation thresholds represent the minimum current required to activate the neuron in at least 50% of the trials. For both LTM and WDR neurons, A-fiber evoked activity was elicited by low intensity stimulation (0.1 ms pulse duration), and the number of action potentials in the first 30 ms were recorded. C-fiber evoked activity (2.0 ms pulse duration) represents number of action potentials with a latency of 30–150 ms, and post-discharge (PDC) activity represents action potentials with a latency of 150–800 ms. * $P < 0.01$.

by PDC activity. An example of the marked sensitivity of PDC activity to topical WIN is shown in Fig. 2. As a group (Fig. 3A), PDC activity decreased after both 0.2 ($n = 7$, $P < 0.01$) and 2.0 mg/ml WIN ($n = 6$, $P < 0.01$) compared with baseline activity. PDC activity did not decrease significantly after co-administration of SR with either 0.2 ($n = 4$, $P > 0.05$) or 2.0 mg/ml WIN ($n = 5$, $P > 0.05$). Following vehicle administration, PDC activity also remained unchanged ($n = 7$, $P > 0.05$). Post-hoc analysis following two-way ANOVA revealed a significant difference in PDC activity following administration of 0.2 mg/ml WIN alone and with SR ($P < 0.05$), and the vehicle control ($P < 0.05$). PDC activity after 2.0 mg/ml WIN was less than vehicle ($P < 0.01$), but not different from activity after co-administration of 2.0 mg/ml WIN with SR.

The administration of WIN also affected noxious C-fiber evoked activity, however to a lesser extent. Total C-fiber evoked activity (Fig. 3B) decreased after 2.0 ($n = 9$, $P < 0.01$), but not 0.2 mg/ml WIN ($n = 9$, $P > 0.05$) compared with baseline activity. Application of SR with WIN did not affect the reduction in C-fiber evoked activity. Total C-fiber evoked activity after co-administration of WIN and SR also decreased after 2.0 mg/ml WIN (Fig. 3B, $n = 7$, $P < 0.01$). After vehicle administration, total C-fiber evoked activity remained stable ($n = 10$, $P > 0.05$). Non-potentiated C-fiber evoked activity, defined as the evoked response to the first stimulus of each trial, was also analyzed since some neurons demonstrated an increase in C-fiber evoked discharge throughout the trial (i.e. wind-up). After 0.2 and 2.0 mg/ml WIN, non-potentiated C-fiber activity did not decrease significantly (105 \pm 39% of control and 74 \pm 16% of control, respectively; $n = 10$, $P = 0.08$). The effect of WIN on wind-up was not analyzed because wind-up scores were not consistent between baseline trials (> 15% difference).

Activity of nociceptive neurons elicited by low intensity electrical stimulation that only elicited short latency activity was also decreased by WIN (Fig. 3C). The A-fiber evoked activity of WDR neurons decreased after both 0.2 ($n = 9$,

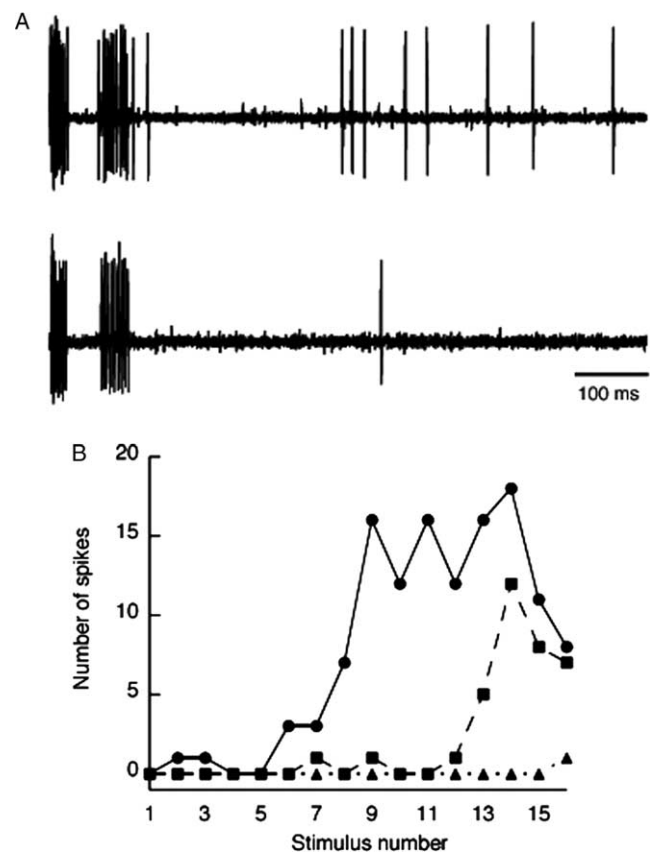


Fig. 2. A representative example demonstrating the effect of bath application of WIN 55,212-2 on the electrically evoked activity of a WDR neuron. (A) Example of discharge from a single neuron prior to (upper trace) and after 2.0 mg/ml WIN 55,212-2 (lower trace). Each trace recording is taken from the final stimulus in a series of 16 stimuli delivered at 0.5 Hz ($3 \times$ C-fiber threshold). Prior to drug administration, three distinct groups of evoked activity can be identified: A-fiber mediated activity (0–30 ms latency), C-fiber mediated activity (30–200 ms latency), and C-fiber mediated post-discharge (PDC) activity (200–800 ms latency). Following administration of WIN 55,212-2, PDC activity was almost completely eliminated. (B) Number of C-fiber mediated PDC action potentials of the same WDR neuron evoked by each of the 16 stimuli are plotted for control and post-WIN (0.2 and 2.0 mg/ml) trials. Circles, vehicle; Squares, 0.2 mg/ml WIN 55,212-2; Triangles, 2.0 mg/ml WIN 55,212-2.

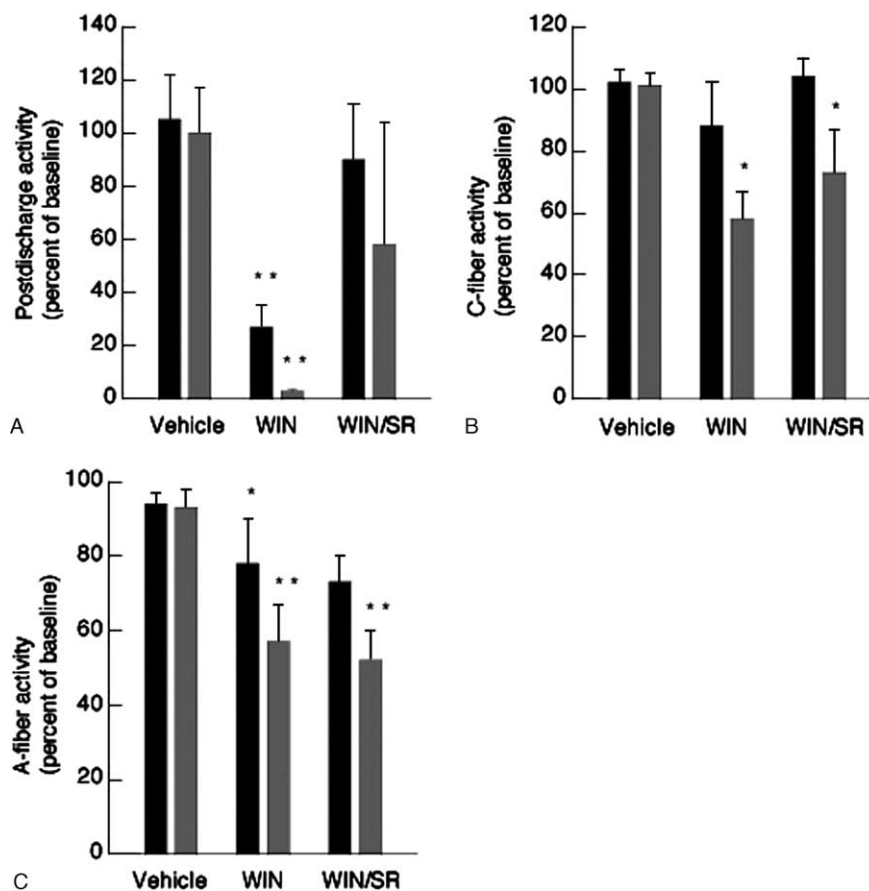


Fig. 3. Effect of WIN 55,212-2 on the electrically evoked responses of spinal trigeminal nucleus caudalis WDR neurons. (A) Application of both low- (black bar) and high-dose (gray bar) WIN 55,212-2 significantly attenuated C-fiber mediated post-discharge (PDC) activity. Inhibition of PDC activity was antagonized by co-administration of SR141716A with WIN 55,212-2. (B) C-fiber evoked activity was inhibited only after application of high-dose WIN 55,212-2. Inhibition of C-fiber evoked activity was unaffected by co-administration of SR141716A with WIN 55,212-2. (C) Both low- and high-dose WIN 55,212-2 inhibited A-fiber evoked activity. Inhibition was also observed after co-administration of SR141716A with WIN 55,212-2. In all graphs, data are presented as a percent of baseline. Black bars represent data from the stimulation trial after vehicle, 0.2 mg/ml WIN 55,212-2, or 0.2 mg/ml WIN 55,212-2 plus SR141716A (1.0 mg/ml). Gray bars represent data from the stimulation trial after vehicle, 2.0 mg/ml WIN 55,212-2, or 2.0 mg/ml WIN 55,212-2 plus SR141716A (1.0 mg/ml). * $P < 0.05$, ** $P < 0.01$ compared with baseline.

$P < 0.05$) and 2.0 mg/ml WIN ($n = 9$, $P < 0.01$) compared with baseline activity. Co-administration of SR with 0.2 and 2.0 mg/ml WIN did not affect the decrease in A-fiber evoked activity in WDR neurons (Fig. 3C); however, A-fiber evoked activity after vehicle administration remained stable ($n = 10$, $P > 0.05$).

3.3. Effect of WIN on non-nociceptive neurons

In contrast to the inhibition of nociceptive neurons, WIN consistently increased evoked activity of low threshold, non-nociceptive neurons. The A β -fiber evoked activity (Fig. 4) increased significantly after 2.0 mg/ml WIN ($n = 9$, $P < 0.01$) compared with baseline activity. Evoked activity of LTM neurons did not change after co-administration of SR with WIN ($n = 6$, $P > 0.05$) or vehicle administration ($n = 6$, $P > 0.05$). Post-hoc analysis following two-way ANOVA also revealed an increase in A β -fiber evoked activity after 2.0 mg/ml WIN when compared to activity

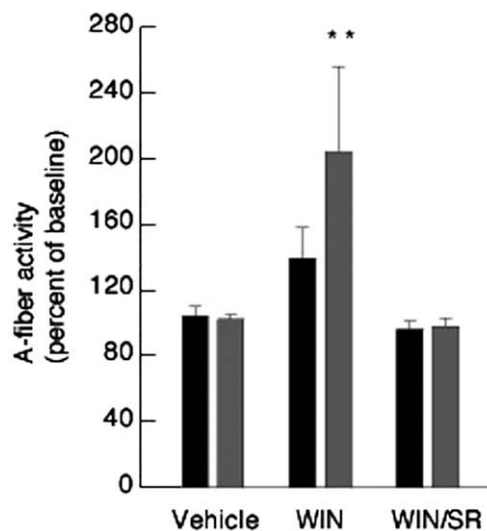


Fig. 4. Application of WIN 55,212-2 significantly increased A β -fiber evoked activity in LTM neurons. Activity remained unaffected after administration of vehicle and after co-administration of SR141716A with WIN 55,212-2. Refer to Fig. 3 for legend. ** $P < 0.01$ compared with baseline.

following co-administration of WIN with SR ($P < 0.01$) and vehicle ($P < 0.01$).

3.4. Mean arterial pressure measurements

Measurements of MAP remained stable in animals treated with vehicle, WIN, or WIN with SR, averaging between 100 and 105 mmHg. In the 10 animals that received locally applied WIN, MAP averaged 103 ± 5 , 104 ± 4 , and 102 ± 6 mmHg after vehicle, 0.2, and 2.0 mg/ml WIN, respectively.

4. Discussion

The main finding of this report is that local application of a cannabinoid receptor agonist to the surface of the caudal brain stem inhibits C-fiber mediated hyperexcitability as measured by PDC activity, and to a lesser extent A- and C-fiber evoked activity. This inhibition was partially antagonized by a CB1 receptor antagonist. In contrast, evoked activity of LTM neurons was increased by application of a cannabinoid receptor agonist, an effect that was antagonized by a CB1 receptor antagonist.

4.1. Technical issues

An important issue regarding this data relates to the inability of the CB1 receptor antagonist SR to attenuate WIN inhibition of A- and C-fiber latency inputs onto WDR neurons, even though SR blocked inhibition of PDC activity in these same neurons. Previous electrophysiological studies of lumbar dorsal horn nociceptive neurons also demonstrated only partial antagonism of cannabinoid receptor agonists with local SR (Drew et al., 2000; Kelly and Chapman, 2001). Specifically, the effects of WIN that were sensitive to SR in our study (PDC activity) were also shown to be sensitive to SR using arachidonyl-2-chloroethylamide (ACEA), a much more specific CB1 receptor agonist (Kelly and Chapman, 2001). SR, however, failed to significantly block the inhibition of A- δ and C-fiber produced by ACEA. Furthermore, in the tail flick assay i.t. administration of SR did not completely antagonize the antinociceptive effect of several CB1/CB2 receptor agonists given i.t. (Welch et al., 1998). In fact, delivered spinally, both the potency and efficacy of SR is reduced when compared to i.c.v. and i.p. routes of administration (Welch et al., 1998).

The consistent lack of antagonism raises the possibility of multiple cannabinoid receptor subtypes to which WIN and/or SR binds differentially (Welch et al., 1998). As further evidence, WIN has been shown to stimulate [35 S]GTP γ S binding and inhibit glutamatergic transmission in the CB1 receptor knockout mouse (Breivogel et al., 2001; Hajos et al., 2001). Whether both of these effects of WIN in the CB1 knockout represent actions on the same, possibly novel, receptor remains unknown. In both of these cases,

however, SR antagonized the effect of WIN. The ability of SR to antagonize all of the above-mentioned effects has led to the hypothesis that this potentially novel receptor is related to CB1. Although WIN inhibition of A- and C-fiber evoked activity was insensitive to SR, it remains possible that this inhibition is due to actions at a similar cannabinoid-like receptor, and that a higher dose of SR would have been effective, given the relatively lower affinity of SR for this receptor (Breivogel et al., 2001).

4.2. WIN inhibition of neuronal hyperexcitability

The experiments presented here demonstrate that cannabinoid agonists applied directly to the brain stem have their greatest effect on neuronal hyperexcitability, as measured by C-fiber-mediated PDC activity. In contrast, non-potentiated C-fiber evoked responses were not significantly inhibited. The above findings are similar to those from electrophysiological experiments performed in the SCDH that showed decreased wind-up and PDC with systemic and spinal administration of cannabinoid receptor agonists, with smaller or non-significant effects on non-potentiated responses (Chapman, 2001; Drew et al., 2000; Kelly and Chapman, 2001; Strangman and Walker, 1999).

Cannabinoid inhibition of PDC is important because the same stimuli that produce PDC are sufficient to produce central sensitization, which may lead to features of persistent pain such as hypersensitivity, secondary hyperalgesia, and allodynia (Li et al., 1999; Wall and Woolf, 1986; Woolf, 1983; Woolf and Wall, 1986). Although PDC and wind-up are not necessary for the production of central sensitization, and are therefore not equivalent to central sensitization, both rely on an NMDA receptor-dependent mechanism (Woolf, 1996). That a cannabinoid receptor agonist had its greatest effect on neuronal hyperexcitability indicates that cannabinoids may most effectively counter pain due to prolonged nociceptor input. Behavioral studies have demonstrated suppression of inflammation-induced thermal hyperalgesia and mechanical allodynia (Martin et al., 1999a,b; Richardson et al., 1998), as well as mechanical hypersensitivity from chronic constriction nerve injury (Fox et al., 2001) with i.t. cannabinoid receptor agonist administration.

4.3. WIN inhibition of WDR neuronal activity

Inhibition of C-fiber evoked activity of Vc neurons observed here parallels findings in the SCDH. Inhibition of A-fiber evoked activity, however, conflicts with previous studies that did not find significant inhibition of A β evoked activity in SCDH neurons (Chapman, 2001; Drew et al., 2000; Hohmann et al., 1998; Kelly and Chapman, 2001). It is possible that the low intensity stimulation used in our study to activate A β -fibers also activated A δ -fibers. Since it was not possible to dissociate A δ - and A β -fibers due to the relatively short distance between the primary afferents in

the skin and their termination in Vc, a decrease in A δ -fiber evoked activity could account for the observed decrease in A-fiber evoked activity.

The location of CB1 receptors indicates that inhibition of WDR neuronal activity may occur at three possible sites: on primary afferent terminals, post-synaptically on WDR neurons, or indirectly through actions on local interneurons. CB1 receptors are present in rat trigeminal ganglion and nucleus (Herkenham et al., 1991; Richardson et al., 1998; Tsou et al., 1998). Studies of primary afferents and their cell bodies in the dorsal root ganglia indicate that CB1 receptors are located most frequently on A β and A δ primary afferents (Hohmann and Herkenham, 1998, 1999a,b). Furthermore, WIN and CP 55,940 reduced depolarization evoked increases in calcium in medium, but not small sized dorsal root ganglia neurons (Khasabova et al., 2002). The inhibition of transmitter release from A-fibers provides one possible explanation for the decrease in WDR neuronal A-fiber evoked activity observed in the experiments reported here.

Although CB1 receptors in the dorsal root ganglia are located mostly on A-fibers, other experiments support direct CB1 mediated actions on nociceptors. Richardson et al. (1998) demonstrated that anandamide inhibition of capsaicin evoked immunoreactive CGRP release from isolated rat spinal cord was CB1 receptor-dependent. Similarly, a cannabinoid receptor agonist reduced capsaicin-induced calcium responses in cultured adult rat dorsal root ganglion neurons (Millns et al., 2001). Taken together, these data indicate direct cannabinoid effects on C-fiber transmission, which could explain the decrease in C-fiber evoked activity observed in these experiments.

4.4. WIN facilitation of LTM neuronal activity

Cannabinoid activation of LTM neurons is consistent with previous electrophysiological experiments of non-nociceptive neurons in the ventroposterolateral nucleus of the thalamus. Martin et al. (1996) demonstrated a cannabinoid-induced reduction in spontaneous activity and increase in mechanically evoked responses of these neurons. Overall, however, no net change in the cumulative number of action potentials was observed in that study. Cannabinoid facilitation of LTM evoked activity is unexpected based on previous experiments conducted in the SCDH. Hohmann et al. (1995) found that the activity of three LTM neurons evoked by a non-noxious mechanical stimulus was unaffected by i.v. WIN. An additional study confirmed this result using the non-selective CB1/CB2 agonist CP 55,940 in an additional four cells (Hohmann et al., 1999a,b). It is possible that the results presented here differ because of the different stimulus or route of drug administration.

Cannabinoids could indirectly modulate the activity of Vc neurons. WIN has been shown to pre-synaptically inhibit GABAergic and glycinergic, but not glutamatergic,

neurotransmission of Vc substantia gelatinosa neurons, while no post-synaptic effects were observed (Jennings et al., 2001). Since direct actions of WIN on A-fiber afferents would be expected to primarily inhibit glutamatergic excitatory neurotransmitter release, a more likely mechanism for the observed facilitation is the inhibition of GABAergic inputs onto LTM neurons.

The physiological significance of increased non-nociceptive neuronal activity is unclear. Selective stimulation of primary afferent A-fibers inhibits C-fiber evoked activity of deep laminae WDR neurons in the SCDH (Mendell, 1966; Woolf and Wall, 1982). Facilitation of LTM neurons could enhance the A β -mediated inhibition of nociceptive neurons, a hypothesis currently under investigation. Alternatively, animal and human studies indicate that cannabinoid receptor agonists enhance the perception of somatosensory stimulation (Giuliani et al., 2000; Henriksson and Jarbe, 1971). Our findings may provide a neurophysiological basis for these observations.

The results of this study are relevant to the use of cannabinoid receptor agonists to treat craniofacial pain disorders. One study on the effect of cannabinoids on trigeminal nociception in animals found that topical application of WIN to cornea prior to mustard oil application reduced Fos-like immunoreactivity in the trigeminal nucleus interpolaris/caudalis transition region with no effect in the Vc/C1 transition region (Bereiter et al., 2002). Although few studies have addressed the use of cannabinoid compounds for craniofacial pain, anecdotal reports support their use to treat this type of pain (Dunn and Davis, 1974; Noyes and Baram, 1974; Raft et al., 1976; Russo, 1998). Previous clinical studies of non-craniofacial pain show that cannabinoid compounds are not effective monotherapy for acute pain because side effects occur at doses that produce mild to moderate analgesia (Campbell et al., 2001). However, when an inactive dose of oral Δ^9 -THC is administered to mice in combination with a variety of opioid compounds, analgesia is potentiated 2–25 times (Cichewicz et al., 1999). In a clinical context, increasing the potency of opioid analgesia with an ineffective dose of cannabinoid receptor agonist might minimize dose-limiting opioid side effects such as sedation.

Acknowledgements

This work was supported by a Howard Hughes Medical Institute Medical Student Fellowship (A.M.P.), the National Institutes of Health (DA14548 to I.D.M.), and the Center for Medicinal Cannabis Research (C00-SF-110 to I.D.M. and H.L.F.). The authors wish to thank Ritu Kapur for her technical assistance and Ichiro Harasawa for his helpful suggestions on an earlier version of this manuscript.

References

- Bereiter DA, Bereiter DF, Hirata H. Topical cannabinoid agonist, WIN55,212-2 reduces cornea-evoked trigeminal brainstem activity in the rat. *Pain* 2002;99:547–56.
- Breivogel CS, Griffin G, DiMarzo V, Martin B. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol Pharm* 2001;60:155–63.
- Buxbaum D, Sanders-Bush E, Efron D. Analgesic activity of tetrahydrocannabinol (THC) in the rat and mouse. *Fed Proc* 1969;28:735.
- Campbell FA, Tramer MR, Carroll D, Reynolds DJM, Moore RA, McQuay HJ. Are cannabinoids an effective and safe treatment option in the management of pain? A qualitative systematic review. *Br Med J* 2001;323:1–8.
- Chapman V. Functional changes in the inhibitory effect of spinal cannabinoid (CB) receptor activation in nerve injured rats. *Neuropharmacology* 2001;41:870–7.
- Cichewicz DL, Martin ZL, Smith FL, Welch SP. Enhancement of mu opioid antinociception by oral delta-9-tetrahydrocannabinol: dose–response analysis and receptor identification. *J Pharmacol Exp Ther* 1999;289:859–67.
- Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* 1988;34:605–13.
- Dickenson AH, Le Bars D, Besson JM. Diffuse noxious inhibitory controls (DNIC). Effects on trigeminal nucleus caudalis neurones in the rat. *Brain Res* 1980;200:293–305.
- Drew LJ, Harris J, Millns PJ, Kendall DA, Chapman V. Activation of spinal cannabinoid 1 receptors inhibits C-fiber driven hyperexcitable neuronal responses and the [35S]GTPgammaS binding in the dorsal horn of the spinal cord of noninflamed and inflamed rats. *Eur J Neurosci* 2000;12:2079–86.
- Dunn M, Davis R. The perceived effects of marijuana on spinal cord injured males. *Paraplegia* 1974;12:175.
- Fox A, Kesingland A, Gentry C, McNair K, Patel S, Urban L, James I. The role of central and peripheral cannabinoid₁ receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. *Pain* 2001;92:91–100.
- Giuliani D, Ferrari F, Ottani A. The cannabinoid agonist HU 210 modifies rat behavioural responses to novelty and stress. *Pharmacol Res* 2000;41:47–53.
- Hajos N, Ledent C, Freund TF. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience* 2001;106:1–4.
- Henriksson BG, Jarbe T. Cannabis-induced vocalization in the rat. *J Pharm Pharmacol* 1971;23:457–8.
- Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci* 1991;11:563–83.
- Herzberg U, Eliav E, Bennett GJ, Kopin IJ. The analgesic effects of R(+)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. *Neurosci Lett* 1997;221:157–60.
- Hohmann AG, Herkenham M. Regulation of cannabinoid and mu opioid receptors in rat lumbar spinal cord following neonatal capsaicin treatment. *Neurosci Lett* 1998;252:13–16.
- Hohmann AG, Herkenham M. Cannabinoid receptors undergo axonal flow in sensory nerves. *Neuroscience* 1999a;92:1171–5.
- Hohmann AG, Herkenham M. Localization of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridization study. *Neuroscience* 1999b;90:923–31.
- Hohmann AG, Martin WJ, Tsou K, Walker JM. Inhibition of noxious stimulus-evoked activity of spinal cord dorsal horn neurons by the cannabinoid WIN 55,212-2. *Life Sci* 1995;56:2111–8.
- Hohmann AG, Tsou K, Walker JM. Cannabinoid modulation of wide dynamic range neurons in the lumbar dorsal horn of the rat by spinally administered WIN55,212-2. *Neurosci Lett* 1998;257:119–22.
- Hohmann AG, Briley EM, Herkenham M. Pre- and postsynaptic distribution of cannabinoid and mu opioid receptors in rat spinal cord. *Brain Res* 1999a;822:17–25.
- Hohmann AG, Tsou K, Walker JM. Cannabinoid suppression of noxious heat-evoked activity in wide dynamic range neurons in the lumbar dorsal horn of the rat. *J Neurophysiol* 1999b;81:575–83.
- Hu JW. Response properties of nociceptive and non-nociceptive neurons in the rat's trigeminal subnucleus caudalis (medullary dorsal horn) related to cutaneous and deep craniofacial afferent stimulation and modulation by diffuse noxious inhibitory controls. *Pain* 1990;41:331–45.
- Jain AK, Ryan JR, McMahon GF, Smith G. Evaluation of intramuscular levonantradol and placebo in acute postoperative pain. *J Clin Pharmacol* 1981;21:320S–6S.
- Jennings EA, Vaughan CW, Christie MJ. Cannabinoid actions on rat superficial medullary dorsal horn neurons in vitro. *J Physiol* 2001;534(3):805–12.
- Johaneck LM, Heitmiller DR, Turner M, Nader N, Hodges J, Simone DA. Cannabinoids attenuate capsaicin-evoked hyperalgesia through spinal and peripheral mechanisms. *Pain* 2001;93:303–15.
- Kelly S, Chapman V. Selective cannabinoid CB₁ receptor activation inhibits spinal nociceptive transmission in vivo. *J Neurophysiol* 2001;86:3061–4.
- Khasabova I, Simone D, Seybold V. Cannabinoids attenuate depolarization-dependent Ca²⁺ influx in intermediate-size primary afferent neurons of adult rat. *Neuroscience* 2002;115:613–25.
- Li J, Simone DA, Larson AA. Windup leads to characteristics of central sensitization. *Pain* 1999;79:75–82.
- Lichtman AH, Martin BR. Cannabinoid induced antinociception is mediated by a spinal α noradrenergic mechanism. *Brain Res* 1991;559:309–14.
- Lichtman AH, Smith P, Martin BR. The antinociceptive effects of intrathecally administered cannabinoids are influenced by lipophilicity. *Pain* 1992;51:19–26.
- Mao J, Price DD, Lu J, Keniston L, Mayer DJ. Two distinctive antinociceptive systems in rats with pathological pain. *Neurosci Lett* 2000;280:13–16.
- Martin BR, Lichtman AH. Cannabinoid transmission and pain perception. *Neurobiol Dis* 1998;5:447–61.
- Martin WJ, Hohmann AG, Walker JM. Suppression of noxious stimulus-evoked activity in the ventral posterolateral nucleus of the thalamus by a cannabinoid agonist: correlation between electrophysiological and antinociceptive effects. *J Neurosci* 1996;16:6601–11.
- Martin WJ, Coffin PO, Attias E, Balinsky M, Tsou K, Walker JM. Anatomical basis for cannabinoid-induced antinociception as revealed by intracerebral microinjections. *Brain Res* 1999a;822:237–42.
- Martin WJ, Loo CM, Basbaum AI. Spinal cannabinoids are anti-allodynic in rats with persistent inflammation. *Pain* 1999b;82:199–205.
- Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990;346:561–4.
- Mendell LM. Physiological properties of unmyelinated fiber projection to the spinal cord. *Exp Neurol* 1966;16:316–32.
- Millns PJ, Chapman V, Kendall DA. Cannabinoid inhibition of the capsaicin-induced calcium response in rat dorsal root ganglion neurones. *Br J Pharmacol* 2001;132:969–71.
- Moss DE, Johnson RL. Tonic analgesic effects of delta-9-tetrahydrocannabinol as measured with the formalin test. *Eur J Pharmacol* 1980;61:313–5.
- Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993;365:61–5.
- Noyes R, Baram DA. Cannabis analgesia. *Comp Psychiatry* 1974;15:531–5.

- Noyes R, Brunk SF, Avery DH, Canter A. The analgesic properties of delta-9-tetrahydrocannabinol and codeine. *Clin Pharmacol Ther* 1975a;18:84–9.
- Noyes R, Brunk SF, Baram DA, Canter A. Analgesic effect of Delta-9-tetrahydrocannabinol. *J Clin Pharmacol* 1975b;15:139–43.
- Palmer S, Thakur G, Makriyannis A. Cannabinergic ligands. *Chem Phys Lipids* 2002;121:3–19.
- Papanastassiou A, Meng ID, Mitrovic I, Fields HL. Locally applied cannabinoid agonists differentially affect nociceptive and non-nociceptive trigeminal nucleus caudalis neurons. *Soc Neurosci Abstr* 2001;.
- Pertwee RG. Cannabinoid receptors and pain. *Prog Neurobiol* 2001;63:569–611.
- Raft D, Gregg J, Ghia J, Harris L. Effects of intravenous tetrahydrocannabinol on experimental and surgical pain. *Clin Pharmacol Ther* 1976;26–33.
- Richardson JD, Aanonsen L, Hargreaves KM. Anti-hyperalgesic effects of spinal cannabinoids. *Eur J Pharmacol* 1998;345:145–53.
- Russo E. Cannabis for migraine treatment: the once and future prescription? An historical and scientific review. *Pain* 1998;76:3–8.
- Sofia RD, Nalepa SD, Harakel JJ, Vassar HB. Anti-edema and analgesic properties of delta-9-tetrahydrocannabinol (THC). *J Pharmacol Exp Ther* 1973;186:646–55.
- Strangman NM, Walker JM. Cannabinoid WIN 55,212-2 inhibits the activity-dependent facilitation of spinal nociceptive responses. *J Neurophysiol* 1999;81:472–7.
- Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 1998;83:393–411.
- Wall PD, Woolf CJ. The brief and the prolonged facilitatory effects of unmyelinated afferent input on the rat spinal cord are independently influenced by peripheral nerve section. *Neuroscience* 1986;17:1199–205.
- Welch SP, Stevens DL. Antinociceptive activity of intrathecally-administered cannabinoids alone, and in combination with morphine in mice. *J Pharmacol Exp Ther* 1992;262:10–18.
- Welch SP, Huffman JW, Lowe J. Differential blockade of the antinociceptive effects of centrally administered cannabinoids by SR141716A. *J Pharmacol Exp Ther* 1998;286:1301–8.
- Woolf CJ. Evidence for a central component of post-injury pain hypersensitivity. *Nature* 1983;306:686–8.
- Woolf CJ. Windup and central sensitization are not equivalent. *Pain* 1996;66:105–8.
- Woolf CJ, Wall PD. Chronic peripheral nerve section diminishes the primary afferent A-fibre mediated inhibition of rat dorsal horn neurones. *Brain Res* 1982;242:77–85.
- Woolf CJ, Wall PD. The relative effectiveness of C primary afferent fibres of different origins in evoking a prolonged facilitation of the flexor reflex in the rat. *J Neurosci* 1986;6:1433–43.
- Yaksh TL. The antinociceptive effects of intrathecally administered levonantradol and desacetyllevonantradol in the rat. *J Clin Pharmacol* 1981;21:334S–40S.