

ANTINOCICEPTION AND MODULATION OF ROSTRAL VENTROMEDIAL MEDULLA NEURONAL ACTIVITY BY LOCAL MICROINFUSION OF A CANNABINOID RECEPTOR AGONIST

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Abstract—Systemic administration of a cannabinoid agonist produces antinociception through the activation of pain modulating neurons in the rostral ventromedial medulla (RVM). The aim of the present study was to determine how a cannabinoid receptor agonist acting directly within the RVM affects neuronal activity to produce behaviorally measurable antinociception. In lightly anesthetized rats, two types of RVM neurons have been defined based on changes in tail flick-related activity. On-cells increase firing (on-cell burst), whereas off-cells cease firing (off-cell pause), just prior to a tail flick. The cannabinoid receptor agonist WIN55,212–2 was microinfused directly into the RVM while monitoring tail flick latencies and on- and off-cell activity. Microinfusion of WIN55,212–2 (2.0 $\mu\text{g}/\mu\text{l}$ and 0.4 $\mu\text{g}/\mu\text{l}$) reduced the tail flick-related on-cell burst, decreased the duration of the off-cell pause, and increased off-cell ongoing activity. These changes were prevented by co-infusing the CB1 receptor antagonist, SR141716A (0.35 $\mu\text{g}/\mu\text{l}$), with WIN55,212–2 (0.4 $\mu\text{g}/\mu\text{l}$). Furthermore, 2.0 $\mu\text{g}/\mu\text{l}$ WIN55,212–2 delayed the onset of the off-cell pause and increased tail flick latencies. Microinfusion of WIN55,212–2 to brain regions caudal or lateral to the RVM had no effect on RVM neuronal activity or tail flick latencies. These results indicate that cannabinoids act directly within the RVM to affect off-cell activity, providing one mechanism by which cannabinoids produce antinociception. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: nucleus raphe magnus, WIN 55;212–2, on-cell, off-cell.

Systemic administration of cannabinoid receptor agonists, which include the major psychoactive ingredient of marijuana, Δ -9-tetrahydrocannabinol, reduce pain-related behaviors in a wide variety of animal models, including acute, inflammatory and neuropathic pain (Buxbaum et al., 1969; Buxbaum, 1972; Herzberg et al., 1997; Li et al., 1999; Lichtman and Martin, 1991; Mikuriya, 1973; Moss and Johnson, 1980; Sofia et al., 1973). Additional studies have demonstrated inhibition of nociceptive signals. Nociceptive

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Abbreviations: HBC, 2-hydroxypropyl- β -cyclodextrin; MOR, μ -opioid receptor; PAG, periaqueductal gray; RVM, rostral ventromedial medulla; %MPE, percent maximal possible effect.

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neurons recorded in the ventroposterolateral nucleus of the thalamus and spinal cord dorsal horn are suppressed by systemic administration of a cannabinoid receptor agonist (Hohmann et al., 1995, 1998; Martin et al., 1996). Cannabinoid-induced antinociception involves the activation of descending inhibitory pain pathways. Spinal transection in rats reduces systemic cannabinoid-induced antinociception in the tail flick test and eliminates inhibition of dorsal horn nociceptive neurons produced by i.v. administration of a cannabinoid receptor agonist (Hohmann et al., 1998; Lichtman and Martin, 1991).

Since Reynolds (1969) demonstrated that electrical stimulation of the midbrain periaqueductal gray region (PAG) produces antinociception in the rat, an enormous body of literature has been generated describing stimulation produced inhibition of pain related behaviors and spinal and medullary dorsal horn nociceptive neurons from several different brain regions (Fields and Basbaum, 1994). Probably the most studied pathway responsible for producing antinociception is the PAG–rostral ventromedial medulla (RVM)–dorsal horn circuit. Neurons in the RVM project directly to the dorsal horn and have reciprocal connections with PAG neurons (Fields and Basbaum, 1994). Activation of this circuit is critical for the production of morphine antinociception (for review, see discussion in Manning, 1998), and recent experiments have determined that cannabinoids activate this same descending inhibitory pathway. Microinjection of cannabinoid agonists into the PAG or RVM increase tail flick latencies, and inactivation of the RVM attenuates the antinociceptive effect of a systemically administered cannabinoid receptor agonist (Lichtman et al., 1996; Martin et al., 1998, 1999; Monhemius et al., 2001).

The neuronal activity of two types of RVM neurons is correlated with noxious stimulation-evoked withdrawal reflexes (Fields et al., 1983a). On-cells show a burst of activity just prior to withdrawal reflexes and off-cells are inhibited just prior to withdrawal reflexes. Several pharmacological differences between on- and off-cells have been demonstrated (Fields et al., 1991); the most thoroughly described difference is in their response to μ -opioid receptor (MOR) agonists. Systemic or local microinjections of MOR agonists that are sufficient to inhibit tail-flick latency inhibit on-cell activity and increase off-cell activity (Fields et al., 1983b; Heinricher et al., 1994). Iontophoresis of MOR agonists inhibit on-cells and have no effect on off-cells (Heinricher et al., 1992). Based on these results, it has been hypothesized that on-cells express MORs, and that direct MOR-mediated inhibition of on-cells disinhibits off-

cells (Fields and Basbaum, 1994; Fields et al., 1991). Consistent with these *in vivo* experiments, *in vitro* RVM slice recordings has revealed two types of neurons: primary cells and secondary cells (Pan et al., 1990). Secondary cells are directly hyperpolarized by MOR agonists and are likely equivalent to on-cells characterized *in vivo*.

Systemic cannabinoids, like morphine, inhibit on-cell activity, increase off-cell activity, and increase tail flick latencies (Meng et al., 1998). Since inactivation of the RVM reduces systemic cannabinoid-induced analgesia (Meng et al., 1998), off-cell activation is the likely source of nociceptive inhibition. Still unknown, however, is the mechanism of cannabinoid actions on RVM neurons. Two cannabinoid receptors, CB1 and CB2, have been cloned (Devane et al., 1988; Matsuda et al., 1990) and putative endogenous ligands have been identified (Devane et al., 1992; Mechoulam et al., 1995). The CB1 receptor is located on neurons throughout the nervous system, whereas the CB2 receptor is located in peripheral tissues such as the spleen. Activation of G protein coupled CB1 receptors inhibits adenylate cyclase production in cell lines (Howlett, 1985). *In vitro* RVM slice experiments using whole cell patch clamp recording failed to reveal any direct postsynaptic effect of a cannabinoid receptor agonist (Vaughan et al., 1999). Cannabinoids, however, inhibited GABAergic inputs into all neuronal cell types (i.e. neurons that were hyperpolarized by MOR agonists and those that were not). Although microinjections of cannabinoid receptor agonists into the RVM increase tail flick latencies (Martin et al., 1998; Monhemius et al., 2001), their effect on the activity of RVM neurons remains unknown. The present study determined the effect of microinjecting a cannabinoid receptor agonist into the RVM on RVM neuronal activity and examined how these changes translate into behaviorally measurable antinociception.

EXPERIMENTAL PROCEDURES

Experimental animals and surgical preparation

All experiments were performed after the review and approval of the Institutional Animal Care and Use Committee at the University of California, San Francisco and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All possible efforts were made to minimize the number of animals used and their suffering. Experiments were conducted using 67 male Sprague–Dawley rats (325–450 g; Bantin and Kingman, Hayward, CA, USA) injected with sodium pentobarbital (60–70 mg/kg, i.p.) prior to surgery. The right external jugular vein was cannulated for maintaining anesthesia. After placing the rat into a stereotaxic frame, a hole was drilled in the interparietal bone to allow insertion of an electrode/cannula assembly into the medulla. Initiation of the tail flick reflex was recorded with a pair of needle electrodes inserted into the sacral longitudinal paraspinal muscles. The ventral surface of tail was blackened and body temperature was maintained at 37 °C with a hot water heating pad. Anesthesia was maintained with a constant, continuous infusion of sodium methohexital (30–80 mg/kg/h, i.v.). Anesthesia level was adjusted so that tail flicks could be elicited with a consistent latency (3.5–5.0 s) without any signs of discomfort. Electrophysiological recordings were initiated 45 min after completion of the surgery. Only one neuron was recorded per animal.

Analgesiometric testing

Tail flicks were evoked every 3 min using radiant heat applied 2–6 cm from the distal end of the tail. From a holding temperature of 35 °C, the temperature increased linearly to a plateau between 48 and 53 °C. The temperature plateau and rate of temperature rise was adjusted at the beginning of each experiment in order to elicit tail flicks with baseline latencies between 3.5 and 5.0 s. To prevent tissue damage, the stimulus was automatically terminated after 10 s in the absence of a tail flick.

Tail flicks were elicited every 3 min for at least 15 min prior to drug infusion and 15–60 min following drug infusion.

Extracellular recording and microinfusion

As previously described, an electrode/cannula assembly was constructed for extracellular recordings and drug infusions (Harasawa et al., 2000). A tungsten microelectrode (2–4 M Ω ; FHC, Bowdoinham, ME, USA) was glued parallel to a 30 gauge stainless steel cannula with a separation of 300–600 μ m between each tip. The assembly was inserted into the brain so that the electrode and cannula were in a rostro-caudal alignment with the electrode placed rostral to the microinfusion cannula. In placement control experiments, the distance between the recording electrode and the microinfusion cannula was increased to 1.2–1.8 mm. The infusion cannula was attached to a 50 μ l Hamilton microsyringe (Hamilton, Reno, NV, USA) by PE-10 tubing, and infusions were conducted over a period of 3–5 min using a syringe pump. Movement of a small air bubble in the PE-10 tubing was monitored to ensure drug delivery.

The electrode assembly was advanced into the medulla using a micromanipulator (DKI, Tujunga, CA, USA) until isolation of a single neuron was achieved. Action potentials were displayed on a digital oscilloscope and isolated using a window discriminator (BAK, Germantown, MD, USA). Data were acquired through a data acquisition board (National Instruments, Austin, TX, USA) interfaced with a Macintosh G4 computer programmed in LabVIEW (National Instruments; Budai, 1994). On- and off-cells were categorized according to their pattern of neuronal activity as related to the tail flick. On-cells were identified by an onset of activity that occurred just prior to the tail flick (on-cell burst), whereas off-cells ceased firing prior to the tail flick (off-cell pause).

Drug was injected (200 nl over 3–5 min) following at least five stable baseline tail flicks. The effect of WIN 55,212–2 (2.0 μ g/ μ l and 0.4 μ g/ μ l), WIN 55,212–2+SR 147161A (0.4 μ g/ μ l WIN 55,212–2, 0.35 μ g/ μ l SR 147161A), and vehicle (45% 2-hydroxypropyl- β -cyclodextrin, HBC) on the tail flick latency, on-cell burst, and off-cell pause was examined. The CB1 antagonist, SR147161A, was co-administered with only the low dose of WIN 55,212–2 because it did not dissolve with the higher dose.

Drugs

WIN 55,212–2, a CB1/CB2 receptor agonist, and SR 147161A, a CB1 receptor antagonist, were dissolved in 45% HBC (RBI, Natick, MA, USA).

Data analysis

Once a unit was isolated, tail flick latencies were measured every 3 min for 15–18 min prior to and 21–45 min post-drug microinfusion. Tail flick latencies and neuronal responses were averaged for the three stimulation trials just before the microinfusion (pre-drug baseline), and the 9, 12 and 15 min trials following completion of the microinfusion (post-drug measurements). Responses from the 39, 42 and 45 min stimulation trials (post-drug recovery) were also averaged to obtain recovery data. Data from these same time points were used for the analysis of all experiments. The average of three data points was used to decrease variability,

since the time-course for drug effects that occur following drug microinfusion can vary between experiments, depending on the distance of the neuron with respect to infusion cannula.

Tail flick latencies were converted into the percent maximal possible effect (%MPE) using the formula:

$$\%MPE = [(post\text{-}drug\ latency - pre\text{-}drug\ latency) / (cutoff\ time - pre\text{-}drug\ latency)] \times 100$$

The effect of microinfusing HBC, WIN 55,212–2 and WIN 55,212–2+SR 141716A on tail flick latencies was determined by performing a two-way analysis of variance for repeated measures on the %MPE values. Post hoc comparisons for the three time points were made using the Tukey/Kramer test.

To determine the effect of drug microinfusion on on-cell activity, the peak height of the tail flick related burst (spikes/s), the latency to the onset of the burst (the time between the onset of heat application to the beginning of burst), and the ongoing activity (activity for 30 s prior to the onset of heat stimulation, spikes/s) were calculated. Pre- and post-microinfusion values for the tail flick related burst and ongoing activity were compared using the non-parametric Wilcoxon matched pairs signed rank test. The latency to the onset of the burst was analyzed with a two-way ANOVA for repeated measures with Tukey/Kramer test for post hoc comparisons.

Off-cell activity was evaluated by calculating the latency to onset of the pause (time between the onset of heat application and the last action potential prior to the tail flick), the duration of the tail flick related pause (time from the pause onset until the first action potential following the tail flick), and the ongoing firing (activity for 30 s prior to heat stimulation, spikes/s). Pre- and post-microinfusion values for the duration of the pause and ongoing activity were compared using the non-parametric Wilcoxon matched pairs signed rank test. The latency to the onset of the pause was analyzed with a two-way ANOVA for repeated measures with Tukey/Kramer test for post hoc comparisons. In order to make comparisons between treatment groups, data were normalized and percent controls were compared with a two-way ANOVA for repeated measures with Tukey/Kramer test for post hoc comparisons. In all cases, $P < 0.05$ was considered significant.

Histological verification

Electrolytic lesions (15 μ A negative current for 15 s) were performed at the conclusion of each experiment to mark the recording site. Animals were given an overdose of i.v. sodium pentobarbital and perfused transcardially with saline followed by 10% formalin. The brain was removed, postfixed in 10% formalin overnight, followed by 30% sucrose. Fifty-micron sections were cut on a freezing microtome, and sections were stained with Cresyl Violet (0.1%). The location of recording sites was determined according to the atlas of Paxinos and Watson (1986).

RESULTS

The effect of microinfusing vehicle, WIN 55,212–2 and WIN 55,212–2+SR141716A on tail flick latencies and neuronal activity was determined using a total of 67 animals. Neuronal activity and tail flick latencies were recorded for at least 20 min following the completion of each microinfusion. In some experiments, to determine the time-course of the drug effect, data were recorded for 45 min after the microinfusion. Recording sites were located within the RVM, including the nucleus raphe magnus, nucleus reticularis gigantocellularis pars α , and nucleus reticularis paragigantocellularis. A reconstruction of the recording sites for 13 on-cells and 14 off-cells revealed an even distribution throughout the RVM (Fig. 1). The cannula tip was also located within the RVM, caudal to the recording site. In

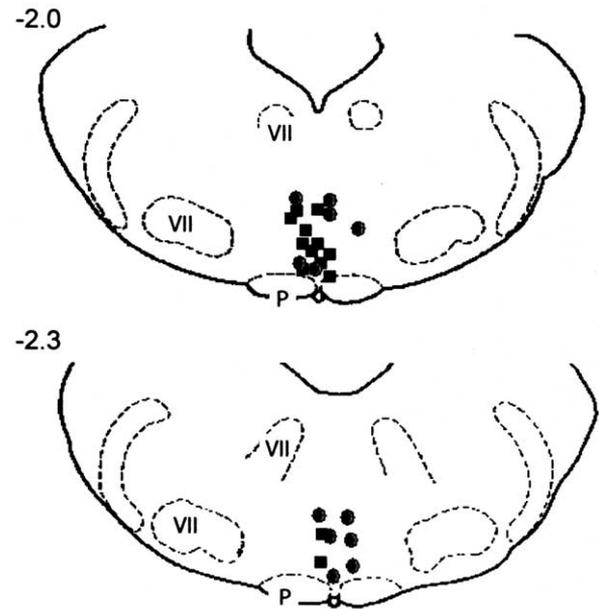


Fig. 1. Histologically verified locations of on- and off-cells based on electrolytic lesions performed at the end of each experiment. On-cells are represented by circles, and off-cells by squares. VII, facial nucleus and ascending fibers of the facial nerve; P, pyramidal tract. Numbers to the left of each section indicate distance caudal to interaural zero.

microinfusion placement control experiments, the cannula tip was located either lateral to the RVM in the facial nucleus or caudal to the RVM in ventral nucleus reticularis gigantocellularis (Fig. 2).

Antinociception following WIN 55,212–2 microinfusion

Comparison between groups revealed a significant effect of treatment on tail flick latencies (Fig. 3, $F(3,66) = 7.99$, $P < 0.01$). While microinfusion of HBC vehicle ($n = 12$) and low dose WIN 55,212–2 (0.4 μ g/ μ l, $n = 10$) were not antinociceptive, the high dose of WIN 55,212–2 (2.0 μ g/ μ l, $n = 11$) produced significant antinociception when compared with vehicle controls ($P < 0.01$). The increase in tail flick latencies produced by high dose WIN 55,212–2 recovered to control values within 45 min ($\%MPE = 2.4 \pm 4.8$).

On-cell activity following WIN 55,212–2 microinfusion

Microinfusion of WIN 55,212–2 reduced the tail-flick-related neuronal activity of on-cells (see Fig. 4 for example). After infusing 0.4 μ g/ μ l WIN 55,212–2, the peak firing rate produced by thermal stimulation of the tail decreased from 26.6 ± 3.6 spikes/s to 7.1 ± 3.9 spikes/s (Wilcoxon test, $P < 0.05$, $n = 5$). The higher dose of WIN 55,212–2 also decreased the peak firing rate from 39.0 ± 11.0 spikes/s to 10.8 ± 6.4 spikes/s (Wilcoxon test, $P < 0.05$, $n = 6$). Co-administration of SR141716A with WIN 55,212–2 produced no change in the tail flick related burst activity (Wilcoxon test, 29 ± 7.2 – 28.6 ± 7.8 spikes/s, $n = 6$) and microinfusion of vehicle did not affect the tail flick related burst activity (Wilcoxon test, 25.1 ± 6.4 – 22.9 ± 6.7 spikes/s, $n = 6$). Data

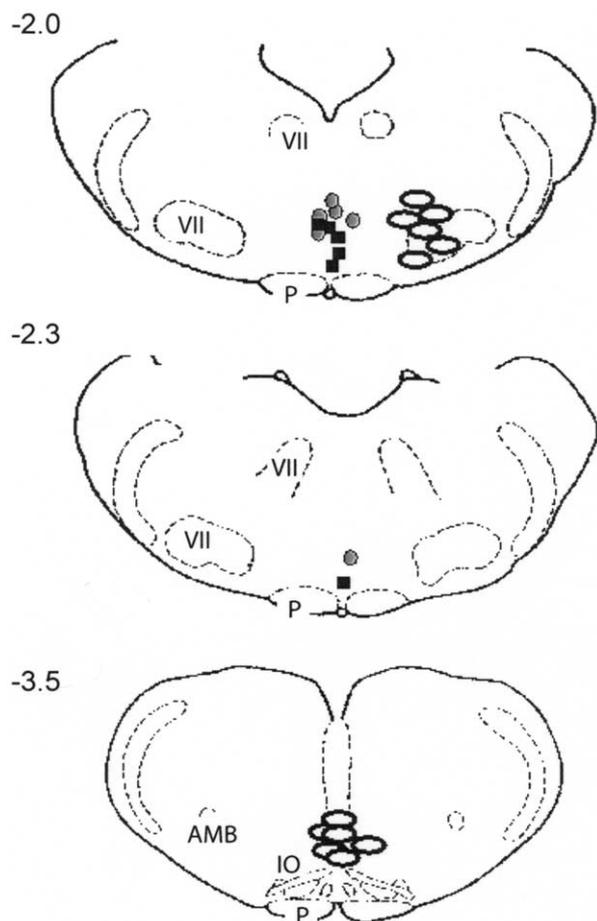


Fig. 2. Locations of on- and off-cells and corresponding microinfusion sites for off-site control experiments. Six of the microinfusions were located lateral to the recording site in and around the facial nucleus, and six were caudal to the recording site, located in ventral nucleus reticularis gigantocellularis. VII, facial nucleus and ascending fibers of the facial nerve; P, pyramidal tract; IO, inferior olive; AMB, nucleus ambiguus. Numbers to the left of each section indicate distance caudal to interaural zero.

were also analyzed as percent of baseline in order to compare between treatment groups. Microinfusion of both low and high dose WIN 55,212–2 inhibited the peak on-cell burst when compared with vehicle and co-administration groups (Fig. 5; $F(3,18)=24.46$, $P<0.01$).

The on-cell burst began to recover within 45 min following WIN 55,212–2 microinfusion. On-cell peak firing recovered from a post-drug low of 7.1 ± 3.9 – 20.8 ± 3.5 spikes/s after $0.4\ \mu\text{g}/\mu\text{l}$ WIN 55,212–2, and from 10.8 ± 6.4 – 24.5 ± 8.4 after $2.0\ \mu\text{g}/\mu\text{l}$ WIN 55,212–2. On-cell burst related activity remained unchanged 45 min after the microinfusion of vehicle (pre-drug= 24.3 ± 7.8 , post-drug= 21.9 ± 8.1 , recovery= 23.0 ± 6.9 spikes/s, $n=5$) and co-infusion of SR141716A with WIN 55,212–2 (pre-drug= 29.9 ± 8.8 , post-drug= 29.2 ± 9.5 , recovery= 27.5 ± 7.9 spikes/s, $n=5$). On-cell ongoing activity was not affected by WIN 55,212–2 microinfusion, which may simply reflect the low ongoing activity prior to drug administration (0.7 ± 0.4 – 0.02 ± 0.01 spikes/s after $0.4\ \mu\text{g}/\mu\text{l}$ WIN

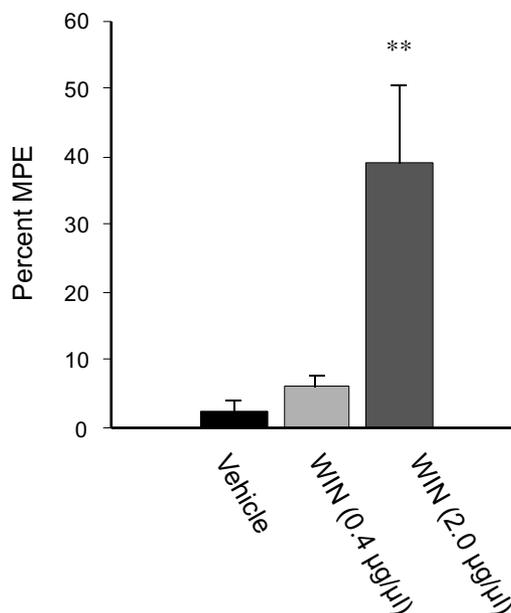


Fig. 3. Antinociception produced by WIN 55,212–2. Tail flicks were recorded prior to and following microinfusion of WIN 55,212–2 ($0.4\ \mu\text{g}/\mu\text{l}$, $n=10$; $2.0\ \mu\text{g}/\mu\text{l}$, $n=11$) or vehicle ($n=12$) into the RVM while recording from on- and off-cells. %MPE was calculated based on tail flick latencies recorded pre- and post- microinfusion (see text for details). Error bars are S.E.M. ** $P<0.01$ versus vehicle.

55,212–2 and 1.6 ± 1.2 – 0.3 ± 0.2 spikes/s after $2.0\ \mu\text{g}/\mu\text{l}$ WIN 55,212–2, Wilcoxon test, $P>0.05$).

The time from onset of the thermal stimulation to the first action potential of the on-cell burst was unaffected by microinfusion of either vehicle or SR 141716A plus WIN 55,212–2, and showed a trend toward an increase with both low and high dose WIN 55,212–2 (Fig. 8A; $F(1,36)=0.14$, $P>0.05$). Consistent with their definition, prior to drug microinfusion all on-cells fired prior to the tail flick. However, in five of 11 on-cells microinfused with WIN 55,212–2 (both high and low doses), the burst started more than 2.5 s after occurrence of the tail flick following drug infusion. Microinfusion of vehicle or WIN 55,212–2 plus SR141716A did not affect the onset of the burst in relation to the tail flick.

Off-cell activity following WIN 55,212–2 microinfusion

Ongoing off-cell activity increased from 4.2 ± 1.2 – 9.0 ± 2.5 spikes/s after the microinfusion of $0.4\ \mu\text{g}/\mu\text{l}$ WIN 55,212–2 ($n=5$) and from 6.9 ± 2.6 – 22.9 ± 8.0 spikes/s after $2.0\ \mu\text{g}/\mu\text{l}$ WIN 55,212–2 ($n=5$, Wilcoxon test, $P<0.05$ for both doses). Vehicle and co-infusion of WIN 55,212–2 with SR 141716A did not affect ongoing activity (vehicle= 7.0 ± 2.2 – 9.2 ± 2.6 spikes/s, $n=6$; WIN 55,212–2+SR 141716A= 16.7 ± 5.2 – 18.9 ± 4.7 spikes/s, $n=6$). Microinfusion of WIN 55,212–2 also decreased the duration of the off-cell pause (see Fig. 6 for example). Following microinfusion of low dose WIN 55,212–2, the off-cell pause duration decreased from 16.0 ± 10.1 – 6.0 ± 4.0 s, and high dose WIN 55,212–2 decreased the pause duration from 38.8 ± 19.9 – 8.5 ± 3.4 s (Wilcoxon test, $P<0.05$). The duration of the off-cell pause

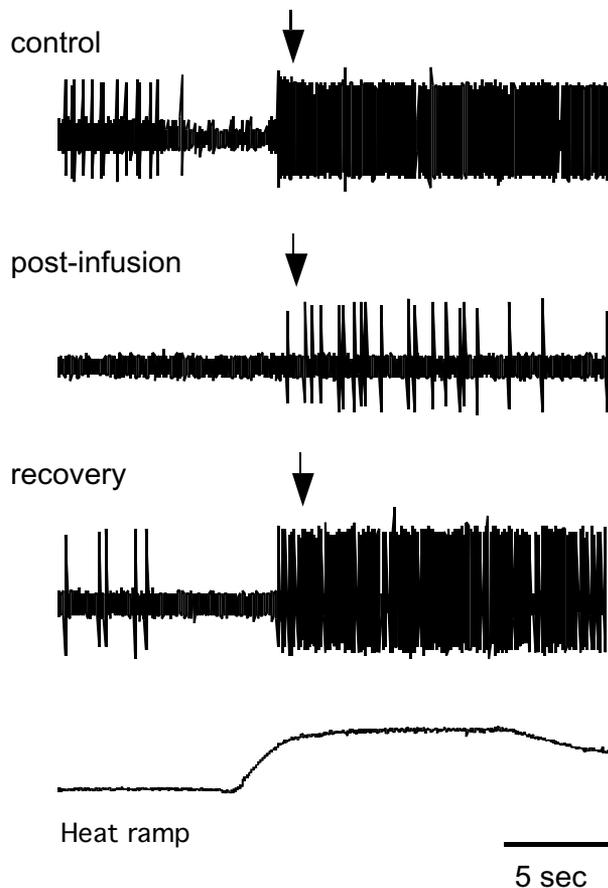


Fig. 4. Tail flick-related activity of an on-cell prior to and after microinfusion of WIN 55,212–2 (0.4 $\mu\text{g}/\mu\text{l}$). Within 15 min after the microinfusion (post-infusion), the baseline activity and on-cell burst were inhibited with no corresponding change in tail flick latency. The response recovered 45 min after drug infusion. Arrows indicate the time at which the tail flick occurred.

was not affected by microinfusion of vehicle (46.4 ± 16.5 – 48.8 ± 20.7 s) or WIN 55,212–2 plus SR 141716A (5.4 ± 1.2 – 6.2 ± 1.5 s). When analyzed as percent changes following drug microinfusion, both low and high dose WIN 55,212–2 decreased the duration of the off-cell pause compared with vehicle and co-administration groups (Fig. 7; $F(3,18)=12.33$, $P<0.05$). Only three of five off-cells microinfused with low dose WIN 55,212–2 and 1/5 neurons microinfused with high dose WIN 55,212–2 were held long enough to obtain recovery data. The off-cell pause duration remained reduced 45 min following infusion of low dose WIN 55,212–2 (control= 22.9 ± 16.8 s, post-infusion= 8.6 ± 6.7 s, recovery= 7.0 ± 3.6 s); however, the pause returned in the one cell recorded for 45 min after infusion of the high dose (control= 7.3 s, post-infusion= 2.0 s, recovery= 14.3 s).

The off-cell pause was significantly affected by drug microinfusion (Fig. 8B; $F(1,36)=5.17$, $P<0.05$). Post-hoc analysis revealed that only the high dose of WIN 55,212–2 increased the onset of the off-cell pause. Even after microinfusion of WIN 55,212–2, however, the off-cell pause onset always occurred prior to the tail flick. This result is in

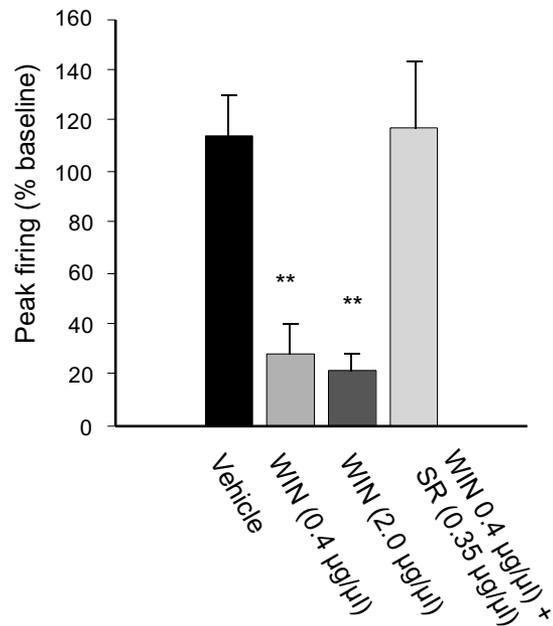


Fig. 5. Inhibition of on-cell tail flick related activity by WIN 55,212–2. Peak firing rate of on-cells decreased after both low ($n=6$) and high ($n=5$) doses of WIN 55,212–2, and co-infusion of SR 141716A with WIN 55,212–2 ($n=6$) blocked this decrease when compared with vehicle control ($n=6$). Error bars are S.E.M. ** $P<0.01$ versus vehicle.

contrast to the previously described effect of WIN 55,212–2 on the onset of the on-cell burst, in which the burst occurred only after the tail flick in several cells.

Microinfusion site controls

In 12 experiments, 2.0 $\mu\text{g}/\mu\text{l}$ WIN 55,212–2 was microinfused either caudal or lateral to the recording site (Fig. 2). A lateral position was chosen in some experiments because previous studies have demonstrated antinociception following the microinjection of a cannabinoid receptor agonist into the A5 noradrenergic cell group (Martin et al., 1999). An injection site further caudal was also tested to control for the rostro-caudal orientation of the electrode and microinfusion cannula. A site dorsal to the RVM was not examined since previous experiments did not reveal antinociception following microinjections of a cannabinoid receptor agonist dorsal to the RVM (Martin et al., 1998). Tail flick latencies remained unchanged following microinfusion of 2.0 $\mu\text{g}/\mu\text{l}$ WIN 55,212–2 either caudal or lateral to the RVM recording site, as did RVM on- and off-cell neuronal activity (data not shown).

DISCUSSION

Previous experiments from this laboratory demonstrated changes in RVM neuronal activity and tail flick latencies following i.v. administration of the cannabinoid receptor agonist, WIN 55,212–2 (Meng et al., 1998). The current study evaluated whether these effects on neuronal activity and tail flick latencies could be mediated by cannabinoid receptors located locally within the RVM. Results from the

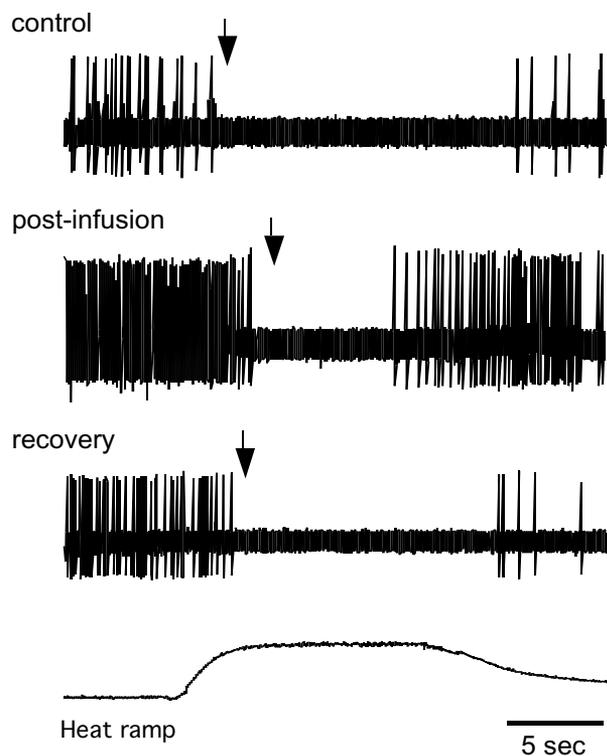


Fig. 6. Off-cell activity and tail flick-related pause before and after microinfusion of WIN 55,212–2 (2.0 $\mu\text{g}/\mu\text{l}$). Baseline activity increased and the pause was delayed and shortened 15 min after microinfusion. Recovery occurred within 45 min after drug infusion. Arrows indicate the time at which the tail flick occurred.

current study demonstrate that microinfusion of a cannabinoid receptor agonist into the RVM alters RVM neuronal activity and produces related changes in tail flick latencies in a manner similar to intravenously administered WIN 55,212–2. Specifically, WIN 55,212–2 increased the spontaneous activity of off-cells, decreased the duration and delayed the onset of the off-cell pause, inhibited and delayed the onset of the on-cell burst, and increased tail flick latencies. These effects were mediated by the CB1 receptor, since co-infusion of SR141716A with WIN 55,212–2 blocked the changes in neuronal activity. Furthermore, these effects were due to local actions within the RVM, since microinfusion of WIN 55,212–2 outside the RVM failed to produce any changes in neuronal activity or tail flick latencies.

As previously described, i.v. administration of WIN 55,212–2 decreased on-cell and increased off-cell activity. The firing of neutral cells, neurons that do not demonstrate tail flick related changes in activity, were not consistently affected by systemic WIN 55,212–2 (Meng et al., 1998). Based on these findings, the present study focused on the effect of CB1 receptor activation in the RVM on on- and off-cell activity. The similar changes in RVM neuronal activity following i.v. and local administration raises the possibility that systemic administration of cannabinoids may produce analgesia in part through direct actions in the RVM. Microinjection of a CB1 receptor antagonist into the

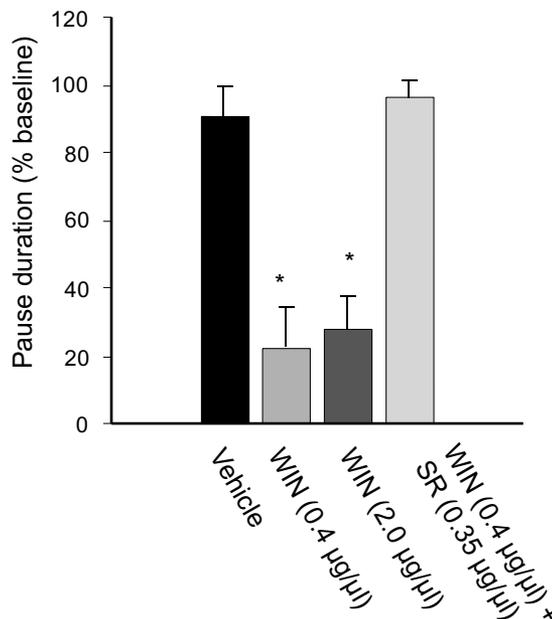


Fig. 7. Effect of WIN 55,212–2 on the off-cell pause. Duration of the off cell pause was shortened after WIN 55,212–2 (0.4 $\mu\text{g}/\mu\text{l}$, $n=5$; 2.0 $\mu\text{g}/\mu\text{l}$, $n=5$), and co-infusion of SR 141716A with WIN 55,212–2 ($n=6$) blocked the decrease when compared with vehicle control ($n=6$). Error bars are S.E.M. * $P<0.05$ versus vehicle.

RVM prior to systemic administration of a cannabinoid receptor agonist is required to determine the relative contribution of CB1 receptors in the RVM to cannabinoid analgesia.

Since inactivation of the RVM prevents systemic WIN 55,212–2 antinociception and off-cells are the only class of neurons that are activated by WIN 55,212–2, off-cell activation likely drives the behavioral antinociception (Meng et al., 1998). The increase in off-cell activity produced by high dose WIN 55,212–2 is consistent with these previous results and the notion that off-cell activation, and specifically a delay in the onset of the off-cell pause, is critical for the production of behavioral antinociception. Although off-cell baseline activity increased after the low dose of WIN 55,212–2, only the high dose delayed the onset of the off-cell pause and increased tail flick latencies. Furthermore, the off-cell pause both prior to and after microinfusion of WIN 55,212–2 always preceded the tail flick. In contrast, although the on-cell burst always preceded the tail flick prior to drug infusion, it often occurred after the tail flick following microinfusion of WIN 55,212–2.

A similar finding has been reported with the microinfusion of the μ opioid receptor agonist DAMGO into the RVM (Heinricher et al., 1994). After the microinfusion of DAMGO into the RVM, tail flick latencies increased only when off-cell activity increased and the onset of the pause delayed. Although strong inhibition of on-cell activity was found, this inhibition did not translate into increases in tail flick latencies. McGaraughty and Heinricher (2002) also suggested that a delay in the onset of the off-cell pause, and not an increase in off-cell baseline activity, is the critical factor leading to increased tail flick latencies. Microinjection of

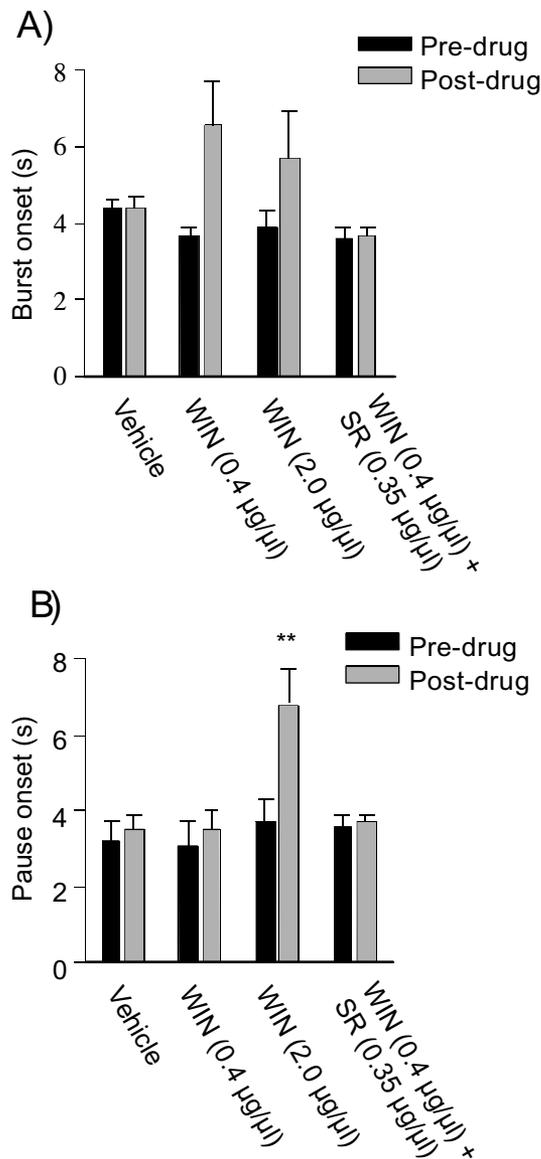


Fig. 8. Effect of WIN 55,212–2 on the onset of the on-cell burst and off-cell pause. (A) Onset of the on-cell tail-flick related activity was not affected by microinfusion of WIN 55,212–2. (B) Onset of the off-cell pause was delayed only by high dose WIN 55,212–2. Error bars are S.E.M. ** $P < 0.01$ versus pre-infusion controls.

morphine into the medial and cortical nuclei of the amygdala increased the off-cell baseline activity but did not affect the off-cell pause or tail flick latencies. Microinjections into the basolateral nucleus, however, increased off-cell baseline activity, delayed the onset of the off-cell pause, and increased tail flick latencies.

One possible explanation for the lack of antinociception produced by the low dose WIN 55,212–2 is that an insufficient number of off-cells were affected by the microinfusion. Using electrical stimulation, Hentall et al. (1984) estimated that activation of between 30 and 75 off-cells may be necessary to increase tail flick latencies. While diffusion of the higher dose of drug is likely to affect more

cells, it is unlikely that diffusion outside the RVM was responsible for producing antinociception, since microinfusions outside the RVM in control experiments did not affect tail flick latencies or RVM neuronal activity. A possible effect of WIN 55,212–2 on a subpopulation of neural cells that modulates nociception, however, cannot be ruled out.

The mechanism of off-cell activation and on-cell inhibition by local microinfusion of WIN 55,212–2 still needs to be elucidated. In whole cell recordings from RVM slices, WIN 55,212–2 inhibited GABA mediated inputs onto both primary and secondary (μ -responsive) cells, but did not produce any post-synaptic effects (Vaughan et al., 1999). Based on these *in vitro* results, activation of off-cells *in vivo* is likely due to inhibition of tonic GABA release. Consistent with this hypothesis are results from previous studies demonstrating increased off-cell activity correlating with increased tail flick latencies after microinfusion of the GABA_A receptor antagonist, bicuculline (Heinricher and Tortorici, 1994).

Inhibition of on-cells by WIN 55,212–2 is more difficult to explain when considering the *in vitro* results. It might be expected that inhibition of GABAergic inputs onto on-cells would produce an increase in neuronal activity. In an early RVM slice experiment using intracellular recording with sharp electrodes, neurons that were hyperpolarized by MOR agonists did not have any demonstrable GABAergic inputs (Pan et al., 1990). This observation suggests that the strength of GABAergic inputs may differ between on- and off-cells, which could contribute to results in the current study. However, no preference was reported in GABA-immunoreactive appositions to on- or off-cells (Skinner et al., 1997). Another possible explanation is that cannabinoid receptor agonists also inhibit excitatory inputs onto on-cells; activation of glutamate receptors is necessary to produce the heat evoked on-cell burst (Heinricher and McGaughy, 1998; Heinricher and Roychowdhury, 1997), and cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in slices from several brain regions (Auclair et al., 2000; Levenes et al., 1998; Robbe et al., 2001; Vaughan et al., 2000). Further investigations into the effects of cannabinoid receptor agonists on glutamatergic transmission in the RVM are required. Alternatively, the activation of off-cells by WIN 55,212–2 may inhibit on-cells, either directly through a local circuit or indirectly through the inhibition of nociceptive transmission at the spinal cord dorsal horn. The latter possibility seems unlikely, however, since inhibition of on-cells occurred even when tail flick latencies remained unchanged.

Cannabinoid and MOR agonists activate a similar descending pain modulating circuit, and several studies have demonstrated cannabinoid and opioid interactions. Recent experiments that have identified a possible role for the release of endocannabinoids in the hippocampus and cerebellum could give insight into cannabinoid and opioid interactions (Kreitzer and Regehr, 2001a,b; Maejima et al., 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). In the hippocampus, neuronal depolarization initiates the release of an endogenous cannabinoid receptor agonist that, acting as a retrograde signal, reduces GABA-

mediated transmission onto the depolarized neuron. The result is a positive feedback, further enhancing neuronal excitability. In the RVM, endocannabinoids may play a similar role. Activation of off-cells by MOR agonists might result in endocannabinoid release, providing a retrograde signal that inhibits GABAergic inputs onto off-cells, thereby causing an even greater increase in off-cell activity. Two predictions follow (1): inhibition of endocannabinoid reuptake in the RVM should potentiate morphine-induced increases in off-cell activity and analgesia, and (2) microinjections of a CB1 antagonist into the RVM should attenuate morphine-induced increases in off-cell activity and analgesia.

In summary, microinfusion of a cannabinoid agonist into the RVM increases tail flick latencies, inhibits on-cell activity and increases off-cell activity. These changes are similar to those previously reported following i.v. administration of a cannabinoid receptor agonist (Meng et al., 1998). The results of the present study, along with previous results in which inactivation of the RVM eliminated analgesia produced by i.v. cannabinoid administration (Meng et al., 1998), suggest that the analgesic effect of systemic cannabinoids could be produced at least partly by actions on CB1 receptors located within the RVM. The contribution of CB1 receptors within the RVM to systemic cannabinoid-induced analgesia, however, requires further investigation.

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