European Journal of Pharmacology 602 (2009) 36–40

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and Analgesia

Physiological evidence of a postsynaptic inhibition of the tail flick reflex by a cannabinoid receptor agonist

Liliane J. Dableh [a,*], Kiran Yashpal [a], James L. Henry [a,b]

[a] Department of Psychiatry, McGill University, 1033 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1
[b] Department of Physiology, McGill University, 1033 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1

ARTICLE INFO

Article history:
Received 4 July 2008
Received in revised form 9 October 2008
Accepted 31 October 2008
Available online 9 November 2008

Keywords:
Cannabinoid
WIN 55,212-2
Nociception
Analgesic
Tail flick test
Spinal cord
(Rat)

ABSTRACT

Current evidence indicates that cannabinoids are antinociceptive and this effect is in part mediated by spinal mechanisms. Anatomical studies have localized cannabinoid CB_1 receptors to pre- and postsynaptic sites within the spinal cord. However, behavioural tests have not clearly indicated the relative importance of each of these sites. In this study, the tail flick test was used as a model of acute pain in the rat to determine the site of action of WIN 55,212-2 ((*R*)-(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate), a synthetic cannabinoid receptor agonist. WIN 55,212-2 (3 mg/kg, i.p.) increased the latency of tail withdrawal from a noxious radiant heat source, indicating it is antinociceptive in this model. Using the same paradigm, WIN 55,212-2 was then tested against the synaptically-induced nociceptive hypersensitivity in response to noxious thermal stimulation of the tail (hot water tail immersion). WIN 55,212-2 blocked this hypersensitivity, confirming a spinal site of action of the cannabinoid receptor agonist. Further, WIN 55,212-2 blocked the nociceptive hypersensitivity induced by intrathecal administration of substance P. As substance P acts on postsynaptic tachykinin NK1 receptors in the dorsal horn of the spinal cord, the data are interpreted to suggest that WIN 55,212-2 expressed its anti-hypersensitivity effects at least partially via a postsynaptic site of action; the data do not rule out a presynaptic site of action. This study suggests that cannabinoids may induce analgesia via a postsynaptic site of action in the spinal cord, as well as the possibility that they may interact with substance P signaling.

1. Introduction

Though cannabinoids have been used therapeutically for thousands of years, it was not until relatively recently that rigorous and systematic studies started to elucidate their mechanisms of action. The principle psychoactive constituent of marijuana was discovered to be delta-9-tetrahydrocannabinol (Δ^9-THC) in the 1960s (Gaoni and Mechoulam, 1964), and early studies using animal models established its antinociceptive properties (Buxbaum, 1972; Sofia et al., 1975; Martin, 1985). To date, two receptor subtypes have been cloned: the cannabinoid CB_1 receptor (Matsuda et al., 1990), found mainly on neuronal cells, and the cannabinoid CB_2 receptor (Munro et al., 1993), localized predominantly outside of the nervous system. Subsequently, endogenous cannabinoid ligands, namely anandamide, palmitoylethanolamide and 2-arachidonoyl glycerol, were identified (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995).

Several studies have demonstrated the antinociceptive properties of cannabinoid receptor agonists in acute animal models of pain. Administration of cannabinoid receptor agonists increases tail flick and hot plate latencies (Yaksh, 1981; Martin, 1985; Smith et al., 1994; Stein et al., 1996) and the antinociceptive effects are reversible with the cannabinoid CB_1 receptor antagonist SR141716A (Welch et al., 1998). Evidence indicates that spinal mechanisms are an important component of cannabinoid-induced analgesia. Intrathecal administration of Δ^9-THC produces antinociception in the tail flick test in spinally transected animals (Smith and Martin, 1992). Intrathecal injection of a synthetic, non-selective, cannabinoid receptor agonist, WIN 55,212-2, produces a dose-dependent antinociceptive effect in the tail flick test (Dogrul et al., 2003), and decreases heat and mechanical hyperalgesia due to intraplantar capsaicin injection (Johanek et al., 2001). This latter effect is blocked by pretreatment with intrathecal SR171416A (Johanek et al., 2001). An immunocytochemical study showed that noxious stimulus-evoked *c-fos* expression in the spinal cord is attenuated by treatment with WIN 55,212-2 (Tsou et al., 1996). Electrophysiological studies have shown that the activity of spinal wide dynamic range neurons evoked by noxious heat stimulation of the receptive fields is suppressed by WIN 55,212-2 (Hohmann et al., 1998, 1999b), and that this effect is blocked by

* Corresponding author. Medical Sciences Program, Faculty of Health Sciences, Department of Psychiatry and Behavioural Neurosciences, McMaster University, Health Sciences Building, Room 4N35, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5. Tel.: +1 905 525 9140x24750; fax: +1 905 522 8844.
E-mail address: dablehl@mcmaster.ca (L.J. Dableh).

0014-2999/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.10.061

pretreatment with SR171416A (Hohmann et al., 1999b). Further, WIN 55,212-2 suppressed the wind-up of spinal wide dynamic range and nociceptive specific neurons induced by repetitive noxious electrical stimulation of the receptive fields (Strangman and Walker, 1999).

Anatomical studies have localized neuronal cannabinoid CB_1 receptors to both pre- and postsynaptic sites in the dorsal horn of the spinal cord. Cannabinoid CB_1 receptors have been observed in dorsal root ganglion cells (Hohmann et al., 1999a; Sañudo-Peña et al., 1999; Salio et al., 2002) and cannabinoid CB_1 receptor mRNA has been observed in medium and large-sized dorsal root ganglion cells using double-label *in situ* hybridization (Hohmann and Herkenham, 1999).

It has also been suggested that cannabinoid CB_1 receptors are present on spinal neurons. Cannabinoid CB_1 receptor immunoreactivity was localized to the superficial layers of the dorsal horn (lamina I and outer lamina II), and electron microscopy showed cannabinoid CB_1 receptors exclusively on postsynaptic sites (Salio et al., 2002). Cannabinoid CB_1 receptor immunoreactivity was colocalized with spinal markers of interneurons (Farquhar-Smith et al., 2000; Salio et al., 2002), and dorsal rhizotomy led to only minimal changes in cannabinoid CB_1 receptor immunoreactivity in the spinal cord dorsal horn (Farquhar-Smith et al., 2000). Rats given neonatal capsaicin to destroy ummyelinated nociceptive primary afferents showed moderately decreased cannabinoid CB_1 receptor radioligand binding in the spinal cord, suggesting that a large proportion of cannabinoid CB_1 receptors in the superficial dorsal horn may be located on cells other than unmyelinated primary afferents (Hohmann and Herkenham, 1998). Taken together, these data support a postsynaptic site of cannabinoid CB_1 receptors.

Functional studies have also addressed the issue of the synaptic site of action of cannabinoid receptor agonists, and it is generally accepted that they cause presynaptic depression (reviewed in Lovinger, 2008). Using intracellular recordings in substantia gelatinosa neurons, Morisset and Urban (2001) showed that both anandamide and WIN 55,212-2 decreased the frequency, but not the amplitude, of glutamatergic, dorsal root-evoked, miniature excitatory postsynaptic currents (mEPSCs). The cannabinoid receptor agonist HU210 inhibited a capsaicin-evoked calcium increase in rat dorsal root ganglion neurons, and this effect was reversed by the cannabinoid CB_1 receptor antagonist SR171416A (Millns et al., 2001). Anandamide administration inhibited capsaicin-induced release of immunoreactive calcitonin gene-related peptide (CGRP) in the spinal cord from primary afferent neurons in an SR171416A-reversible manner (Richardson et al., 1998). SR171416A increased capsaicin-evoked substance P release in the mouse spinal cord (Lever and Malcangio, 2002). These studies suggest that presynaptic inhibition of neurotransmitter release in the spinal cord may be a major mechanism of cannabinoid-induced antinociception.

The purpose of the present study was to examine the site of action of the cannabinoid receptor agonist WIN 55,212-2 in two experimental paradigms in the rat. In the first paradigm, WIN 55,212-2 was tested against presumed substance P-induced nociceptive hypersensitivity through sustained noxious stimulation of the tip of the tail by immersion in 50 °C water for 90 s.

In the second paradigm, WIN 55,212-2 was tested against substance P-induced nociceptive hypersensitivity, where substance P was administered by intrathecal injection and resulted in the typical transient decrease in reaction time in the tail flick test. A block of the effect of tail immersion but not of the intrathecal substance P-induced hypersensitivity would suggest a presynaptic site of action. A block by WIN 55,212-2 in both paradigms would suggest a postsynaptic site of action.

2. Material and methods

2.1. Animals

Male Sprague-Dawley rats weighing 300–350 g (Charles River, QC, Canada) were housed in pairs and maintained on a 12-hour light cycle, with food and water available *ad libitum*. All procedures complied with the "Guidelines for the Care and Use of Experimental Animals," Volumes I and II, of the Canadian Council on Animal Care. All procedures were approved by the McGill University Animal Care Committee.

2.2. Drugs

The non-selective cannabinoid receptor agonist WIN 55,212-2 ((*R*)-(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate; Sigma Chemical Co., St. Louis, MO, USA) was dissolved in 50% dimethyl sulfoxide (DMSO; Sigma Chemical Co.) and 50% physiological saline, and administered intraperitoneally (i.p.) at 3 mg/kg, as 0.1 ml/100 g body weight. For intrathecal injection, 20 µg of WIN 55,212-2 were given in 20 µl of vehicle (50% DMSO). Substance P (Peninsula Laboratories, Inc., San Carlos, CA, USA) was given as 6.5 nmol and delivered intrathecally. Substance P was dissolved in artificial cerebrospinal fluid (aCSF), an aqueous solution made up of 128.6 mM NaCl, 2.6 mM KCl, 1.0 mM $MgCl_2$ and 1.4 mM $CaCl_2$ and phosphate buffered to pH 7.33.

2.3. Surgical preparation (intrathecal catheters)

For administration of substance P and WIN 55,212-2 onto the spinal cord, each rat was implanted with an indwelling intrathecal catheter (Intramedic PE-10, Clay Adams, Division of Becton Dickinson, Parsippany, NJ, USA). Rats were anaesthetized with a combination of ketamine (70 mg/kg) and xylazine (7 mg/kg), i.p., and a catheter was inserted through an incision in the dura at the atlanto–occipital junction. The catheter was implanted such that the inner tip lay at the lower lumbar vertebral level. The external end of the catheter was fixed to the skull with dental cement to a screw embedded in the skull. The recovery period was one week and only animals without any neurological deficit were used. The viability of the catheter was tested the day before experimentation by injecting 20 µl of lidocaine; only animals exhibiting reversible sensory and motor blockade were used.

2.4. Tail flick test

The procedures followed are based on those described by Yashpal et al. (1993). Rats were lightly restrained while light from a projector bulb was focused on the tail, 5 cm from the distal end, on an area that was blackened to facilitate the absorption of light. Latency of tail withdrawal was measured by a timer set to stop when the tail moves and exposes a photodetector to the light beam. Reaction time was measured to 0.01 s. The intensity of the bulb and sensitivity of the detector were set so that a baseline reaction time of 10–12 s was achieved. Experimentation proceeded when three successive readings with a standard deviation of less than 10% of the mean were obtained. The average of the first three readings was taken as baseline, and all subsequent readings were expressed as a percentage of this baseline. Readings were taken every 3 min for all experiments.

After baseline measures were taken, the cannabinoid receptor agonist vehicle (50% DMSO in saline) was given i.p. such that the injection ended 2 min before the next reading, and seven additional readings were taken. The cannabinoid receptor agonist WIN 55,212-2 (3 mg/kg, i.p.) was then injected 2 min prior to the next reading, and 12 more readings were taken.

2.5. Tail immersion test

A light anaesthesia was induced by a solution consisting of sodium pentobarbital (20 mg/kg) and chloral hydrate (120 mg/kg) prepared on the day of use, and injected i.p. The level of anaesthesia allowed for stable reaction times, while preventing any signs of discomfort during

the course of the experiment. Noxious thermal stimulation was attained by immersing the tip of the tail in hot water.

After three consistent baseline readings were taken, the distal 4 cm of the tail were immersed in 50 °C water for 90 s. The immersion was timed to end 30 s before the next reading. Three subsequent readings were taken. WIN 55,212-2 (3 mg/kg, i.p.) or vehicle (50% DMSO, i.p.) was then given, and the injection ended 2 min before the next reading. Three further readings were taken, and the tail was immersed a second time, as described above. Five more readings were taken, the tail was immersed a third time, and a final five readings were taken. Upon completion of the tests, the rats were sacrificed.

2.6. Administration of substance P intrathecally

Substance P was given via injection into a surgically implanted intrathecal catheter (Section 2.3). An intrathecal injection of 6.5 nmol substance P in 10 µl of aCSF was made. This was immediately followed by an injection of 10 µl of aCSF to flush the catheter. The injections were timed to end 1 min prior to taking the next tail flick reading.

After three baseline readings, substance P was given (as described above), and three more readings were taken. WIN 55,212-2, or its vehicle, was given either by i.p. injection (3 mg/kg in 50% DMSO), or by intrathecal injection (20 µg in 20 µl of 50% DMSO, followed by 15 µl aCSF). Three more readings were taken, substance P was given a second time, and 5 final readings were taken.

2.7. Statistics

The data were analyzed by one-way (Fig. 1) or two-way (Figs. 2 and 3) analysis of variance (ANOVA) followed by Tukey's test for *post hoc* pairwise comparisons.

3. Results

3.1. Effects of WIN 55,212-2 on the tail flick test

In rats initially given vehicle (50% DMSO), the reaction time of tail withdrawal from the noxious radiant heat stimulus remained at baseline level following the injection (Fig. 1). However, immediately after injection of WIN 55,212-2 (i.p.), the latency of the tail withdrawal increased to approximately 140% ($P<0.01$; $n=4$) of baseline value, and remained significantly elevated for at least 15 min (Fig. 1). Thus, administration of WIN 55,212-2, increased tail withdrawal thresholds for approximately 15 min with the present dose.

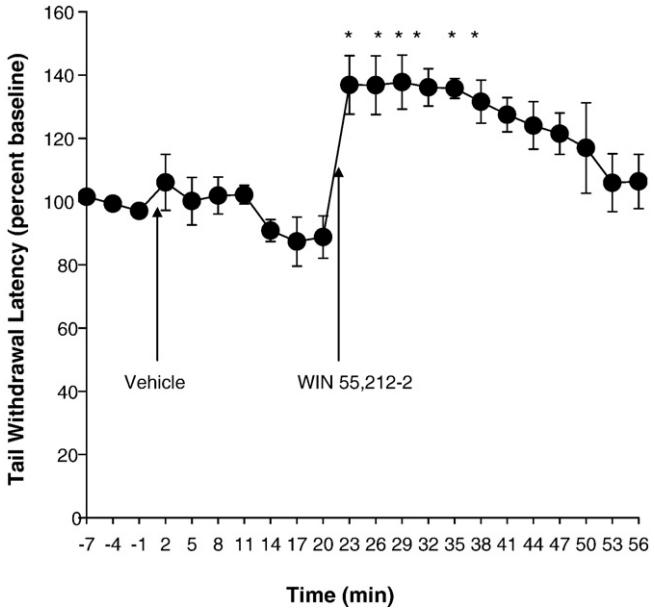

Fig. 1. Effect of administration of vehicle (50% DMSO) and WIN 55,212-2 (3 mg/kg; $n=4$), on the latency of tail withdrawal from a noxious radiant heat stimulus to the rat tail, 5 cm from the distal end. Arrows indicate time of vehicle/drug administration. Data are expressed as percentages of baseline value and shown as the means ± standard error of the mean (SEM). * $P<0.01$ compared to baseline value.

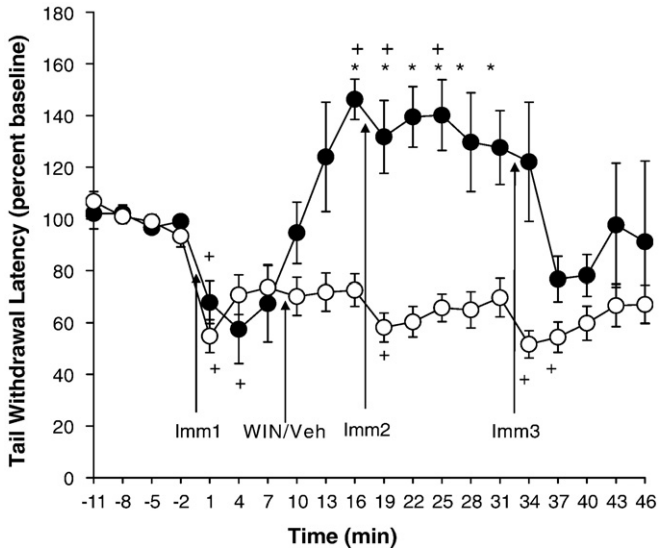

Fig. 2. Effect of immersion of the tip of the tail in 50 °C water on tail withdrawal latency from a noxious radiant heat stimulus in rats treated with WIN 55,212-2 (3 mg/kg; $n=4$; filled circles) or vehicle (50% DMSO; $n=6$; open circles), administered 7.5 min after the first tail immersion. Data are expressed as percentages of baseline value and shown as the means ± SEM. * $P<0.01$ compared to control group; + $P<0.05$ compared to baseline value.

3.2. Effects of WIN 55,212-2 on the tail immersion test

Fig. 2 shows the effect of WIN 55,212-2 ($n=4$) or vehicle ($n=6$) on the facilitation of the tail withdrawal reflex induced by hot water tail immersion. After the first tail immersion and prior to any injection, the latency of tail withdrawal decreased to approximately 60% ($P<0.05$) of baseline in both groups. This response is similar to that reported earlier, which is blocked by a tachykinin NK1 receptor antagonist (Yashpal et al., 1993). Administration of WIN 55,212-2 significantly increased tail withdrawal latency to over 140% ($P<0.01$) of baseline, while vehicle administration had no effect on reaction times (Fig. 2).

A second tail immersion during the increased reaction time did not alter the values in the group treated with WIN 55,212-2. Values remained significantly higher than baseline for at least 12 min after the second tail immersion. After the third tail immersion, withdrawal latency returned to baseline value. In the control group, the second tail immersion caused a decrease in withdrawal latency ($P<0.05$), that was similar in magnitude to the response to the first tail immersion. After the third tail immersion, the control group showed another significant decrease in withdrawal latency ($P<0.05$), which returned towards baseline value after approximately 6 min. In summary, administration of WIN 55,212-2 blocked the effect of noxious stimulus-induced hypersensitivity due to tail immersion.

3.3. Effects of WIN 55,212-2 on substance P-induced hypersensitivity

The effect of WIN 55,212-2 on the decrease in reaction time in the tail flick test induced by intrathecal administration of substance P is shown in Fig. 3. In the series on intrathecal administration of WIN 55,212-2 (Fig. 3A), intrathecal substance P alone decreased tail flick latency to approximately 40% ($P<0.05$) of baseline in both groups.

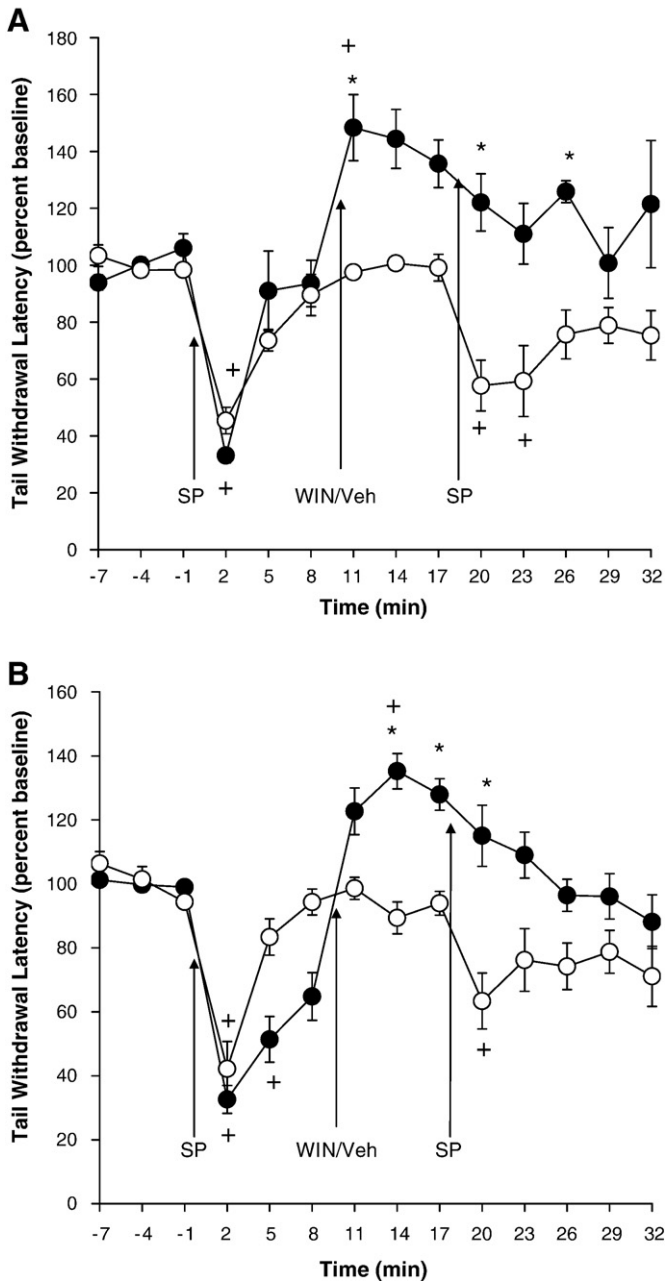

Fig. 3. Effect of intrathecal administration of substance P (6.5 nmol) on the latency of tail withdrawal from a noxious radiant heat stimulus in rats treated with: A, intrathecal WIN 55,212-2 (20 μg in 20 μl vehicle; $n=3$; filled circles) or vehicle (50% DMSO; 20 μl; $n=5$; open circles); or B, intraperitoneal WIN 55,212-2 (3 mg/kg; $n=5$; filled circles) or vehicle (50% DMSO; $n=5$; open circles). Drugs were administered 8 min after the first injection of substance P. Data are expressed as percentages of baseline value and shown as the means±SEM. * $P<0.01$ compared to control group; + $P<0.05$ compared to baseline value.

Intrathecal administration of WIN 55,212-2 increased tail withdrawal latency to over 140% ($P<0.05$; $n=3$) of baseline, which is significantly higher than reaction time observed in vehicle-treated rats ($P<0.01$; $n=5$). A second substance P injection, given during this effect of WIN 55,212-2, decreased tail flick latency to baseline. However, in vehicle-treated rats, the second injection of substance P caused tail flick latency to decrease to approximately 60% ($P<0.05$) of baseline value. The control group latency returned to baseline approximately 6 min after substance P injection.

Fig. 3B shows the effect of systemically administered WIN 55,212-2 to determine if it can inhibit the effect of intrathecal substance P administration. In the series on systemic administration of WIN 55,212-2, intrathecal substance P alone decreased tail flick latency to approximately 40% ($P<0.05$) of baseline in both groups. Administration of WIN 55,212-2 intraperitoneally increased tail withdrawal latency to over 130% ($P<0.05$; $n=5$) of baseline, which is significantly higher than reaction time observed in vehicle-treated rats ($P<0.01$; $n=5$). A second substance P injection decreased tail flick latency to approximately baseline value in WIN 55,212-2-treated rats. However in vehicle-treated rats, substance P caused tail flick latency to decrease to roughly 60% ($P<0.05$) of baseline value, and the latency returned to baseline after approximately 3 min. Therefore, substance P did not reduce latency of tail withdrawal to below baseline value after administration of WIN 55,212-2 when given either intrathecally or intraperitoneally.

4. Discussion

In the tail flick test as a model of acute pain in the rat, it was shown that WIN 55,212-2 increased tail flick withdrawal latency transiently for approximately 15 min, compared to baseline. The antinociceptive effect of WIN 55,212-2 was similar to that of other cannabinoid receptor agonists reported previously in the tail flick test (Yaksh, 1981; Lichtman and Martin, 1997; Welch et al., 1998). Neuronal cannabinoid CB_1 receptors in the spinal cord are thought to be involved in mediating the antinociceptive effects of cannabinoids (Smith and Martin, 1992), and they have been localized to both pre- and postsynaptic sites in the spinal dorsal horn (Hohmann et al., 1999a; Salio et al., 2002). Therefore, subsequent tail flick experiments tested the effect of WIN 55,212-2 against hypersensitivity caused by either noxious thermal stimulation of the tail, or intrathecal administration of substance P. The tail immersion paradigm has previously been shown to be a model of nociception-induced hypersensitivity, presumably via endogenous release of substance P in the spinal cord, as it causes a facilitation of the tail flick reflex which can be blocked by a tachykinin NK1 receptor antagonist (Yashpal et al., 1993). Therefore, WIN 55,212-2 was tested for its possible anti-hyperalgesic effects in this paradigm.

The initial decrease in tail withdrawal latency after tail immersion was similar to that reported previously (Yashpal et al., 1993). In the present study, administration of WIN 55,212-2 blocked the response to the second immersion of the tail. When tested later than the 15 min duration of the cannabinoid effect identified, the third tail immersion caused a decrease in the tail flick latency. Therefore, the return to baseline is interpreted as being due to the termination of the antinociceptive action of WIN 55,212-2.

Though this test shows that WIN 55,212-2 blocked the response to tail immersion, presumably due to endogenously released substance P in the spinal cord, it does not indicate whether this was due to the block of the release of substance P, or the inhibition of its postsynaptic action. Thus, to determine if the block occurred pre- or postsynaptically, WIN 55,212-2 was tested on the response to exogenously administered substance P, which acts on postsynaptic tachykinin NK1 receptors. Intrathecal administration of substance P has been shown to facilitate the spinal tail flick reflex and is used as a model of hypersensitivity in the rat (Yashpal et al., 1993). We tested WIN 55,212-2 in this paradigm as well. WIN 55,212-2 given intrathecally increased the tail withdrawal latency, and subsequent administration of substance P did not produce the hypersensitivity seen prior to WIN 55,212-2 administration. As WIN 55,212-2 blocked the effect of exogenous substance P, this suggests a postsynaptic site of action.

A subsequent experiment was done to determine the effects of intraperitoneal administration of WIN 55,212-2 on the nociceptive hypersensitivity induced by intrathecal administration of substance P. Although substance P elicited the typical hypersensitivity when given prior to WIN 55,212-2, when it was given within the 15 min period of action of the cannabinoid receptor agonist, substance P administration simply reduced the duration of its analgesic effect. These data suggest

that WIN 55,212-2 crosses the blood brain barrier to act on the spinal cord.

Although anatomical and physiological studies have localized cannabinoid CB_1 receptors to pre- and postsynaptic sites within the spinal dorsal horn, physiological studies that specifically address the localization of these receptors are lacking. Using the tail flick test of acute nociception in these various interactions, this study provides evidence that postsynaptic sites are involved in the antinociception caused by the cannabinoid receptor agonist WIN 55,212-2. The data do not provide any evidence for or against an action of WIN 55,212-2 on presynaptic cannabinoid CB_1 receptors at the spinal level. Further, this study shows that a cannabinoid receptor agonist interfered with the action of substance P. This may have important implications, especially in light of the finding of Richardson et al. (1998) that cannabinoids are more effective in a hyperalgesic state, which is known to involve substance P. This may be of significance when targeting cannabinoid receptors to potentiate their effects. As several pain conditions remain inadequately treated, these studies emphasize the need for continued investigation into cannabinoids as a strategy for pain management.

Acknowledgement

This work was supported by a grant to J.L.H. from the Canadian Institutes of Health Research.

References

Buxbaum, D.M., 1972. Analgesic activity of delta-9-tetrahydrocannabinol in the rat and mouse. Psychopharmacologia 25, 275–280.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Dogrul, A., Gul, H., Akar, A., Yildiz, O., Bilgin, F., Guzeldemir, E., 2003. Topical cannabinoid antinociception: synergy with spinal sites. Pain 105, 11–16.

Farquhar-Smith, W.P., Egertova, M., Bradbury, E.J., McMahon, S.B., Rice, A.S.C., Elphick, M.R., 2000. Cannabinoid CB_1 receptor expression in rat spinal cord. Mol. Cell Neurosci. 15, 510–521.

Gaoni, Y., Mechoulam, R., 1964. Isolation, structure and partial synthesis of an active constituent of hashish. J. Am. Chem. Soc. 86, 1646–1647.

Hohmann, A.G., Herkenham, M., 1998. Regulation of cannabinoid and mu opioid receptors in rat lumbar spinal cord following neonatal capsaicin treatment. Neurosci. Lett. 252, 13–16.

Hohmann, A.G., Herkenham, M., 1999. Localization of central cannabinoid CB_1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label *in situ* hybridization study. Neuroscience 90, 923–931.

Hohmann, A.G., Tsou, K., Walker, J.M., 1998. Cannabinoid modulation of wide dynamic range neurons in the lumbar dorsal horn of the rat by spinally administered WIN 55,212-2. Neurosci. Lett. 257, 119–122.

Hohmann, A.G., Briley, E.M., Herkenham, M., 1999a. Pre- and postsynaptic distribution of cannabinoid and mu opioid receptors in rat spinal cord. Brain Res. 822, 17–25.

Hohmann, A.G., Tsou, K., Walker, J.M., 1999b. Cannabinoid suppression of noxious heat-evoked activity in wide dynamic range neurons in the lumbar dorsal horn of the rat. J. Neurophysiol. 81, 575–583.

Johanek, L.M., Heitmiller, D.R., Turner, M., Nader, N., Hodges, J., Simone, D.A., 2001. Cannabinoids attenuate capsaicin-evoked hyperalgesia through spinal and peripheral mechanisms. Pain 93, 303–315.

Lever, I.J., Malcangio, M., 2002. CB_1 receptor antagonist SR141716A increases capsaicin-evoked release of substance P from the adult mouse spinal cord. Br. J. Pharmacol. 135, 21–24.

Lichtman, A.H., Martin, B.R., 1997. The selective cannabinoid antagonist SR141716A blocks cannabinoid-induced antinociception in rats. Pharmacol. Biochem. Behav. 57, 7–12.

Lovinger, D.M., 2008. Presynaptic modulation by endocannabinoids. Handb. Exp. Pharmacol. 184, 435–477.

Martin, B.R., 1985. Structural requirements for cannabinoid-induced antinociceptive activity in mice. Life Sci. 36, 1523–1530.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol. 50, 83–90.

Millns, P.J., Chapman, V., Kendall, D.A., 2001. Cannabinoid inhibition of the capsaicin-induced calcium response in rat dorsal root ganglion neurones. Br. J. Pharmacol. 132, 969–971.

Morisset, V., Urban, L., 2001. Cannabinoid-induced presynaptic inhibition of glutamatergic EPSCs in substantia gelatinosa neurons of the rat spinal cord. J. Neurophysiol. 86, 40–48.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of peripheral receptor for cannabinoids. Nature 365, 61–65.

Richardson, J.D., Aanonsen, L., Hargreaves, K.M., 1998. Antihyperalgesic effects of spinal cannabinoids. Eur. J. Pharmacol. 345, 145–153.

Salio, C., Fischer, J., Franzoni, M.F., Conrath, M., 2002. Pre- and postsynaptic localizations of the CB_1 cannabinoid receptor in the dorsal horn of the rat spinal cord. Neuroscience 110, 755–764.

Sañudo-Peña, M.C., Strangman, N.M., Mackie, K., Walker, J.M., Tsou, K., 1999. CB_1 receptor localization in rat spinal cord and roots, dorsal root ganglion, peripheral nerve. Acta Pharmacol. Sin. 20, 115–1120.

Smith, P.B., Martin, B.R., 1992. Spinal mechanisms of delta 9-tetrahydrocannabinol-induced analgesia. Brain Res. 578, 8–12.

Smith, P.B., Compton, D.R., Welch, S.P., Razdan, R.K., Mechoulam, R., Martin, B.R., 1994. The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. J. Pharmacol. Exp. Ther. 270, 219–227.

Sofia, R.D., Vassar, H.B., Knobloch, L.C., 1975. Comparative analgesic activity of various naturally occurring cannabinoids in mice and rats. Psychopharmacologia 40, 285–295.

Stein, E.A., Fuller, S.A., Edgemond, W.S., Campbell, W.B., 1996. Physiological and behavioural effects of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in the rat. Br. J. Pharmacol. 1, 107–114.

Strangman, N.M., Walker, J.M., 1999. Cannabinoid WIN 55,212-2 inhibits the activity-dependent facilitation of spinal nociceptive responses. J. Neurophysiol. 82, 472–477.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku, K., 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem. Biophys. Res. Commun. 215, 89–97.

Tsou, K., Lowitz, K.A., Hohmann, A.G., Martin, W.J., Hathaway, C.B., Bereiter, D.A., Walker, J.M., 1996. Suppression of noxious stimulus-evoked expression of Fos protein-like immunoreactivity in rat spinal cord by a selective cannabinoid agonist. Neuroscience 70, 791–798.

Welch, S.P., Huffman, J.W., Lowe, J., 1998. Differential blockade of the antinociceptive effects of centrally administered cannabinoids by SR141716A. J. Pharmacol. Exp. Ther. 286, 1301–1308.

Yaksh, T.L., 1981. The antinociceptive effects of intrathecally administered levonantradol and desacetyllevonantradol in the rat. J. Clin. Pharmacol. 21, 334S–340S.

Yashpal, K., Radhakrishnan, V., Coderre, T.J., Henry, J.L., 1993. CP-96,345, but not its stereoisomer, CP-96,344, blocks the nociceptive responses to intrathecally administered substance P and to noxious thermal and chemical stimuli in the rat. Neuroscience 52, 1039–1047.