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CB_2 cannabinoid receptor-mediated peripheral antinociception

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Abstract

Cannabinoid receptor agonists diminish responses to painful stimuli. Extensive evidence implicates the CB_1 receptor in the production of antinociception. However, the capacity of CB_2 receptors, which are located outside the central nervous system (CNS), to produce antinociception is not known. Using AM1241, a CB_2 receptor-selective agonist, we demonstrate that CB_2 receptors produce antinociception to thermal stimuli. Injection of AM1241 in the hindpaw produced antinociception to a stimulus applied to the same paw. Injection of an equivalent dose of AM1241 into the paw contralateral to the side of testing did not. The antinociceptive actions of AM1241 were blocked by the CB_2 receptor-selective antagonist AM630, but not by the CB_1 receptor-selective antagonist AM251. AM1241 also produced antinociception when injected systemically (intraperitoneally). The antinociceptive actions of systemic AM1241 were blocked by injection of AM630 into the paw where the thermal stimulus was applied, but not the contralateral paw. These findings demonstrate the local, peripheral nature of CB_2 cannabinoid antinociception. AM1241 did not produce the CNS cannabinoid effects of hypothermia, catalepsy, inhibition of activity or impaired ambulation, while this tetrad of effects was produced by the mixed CB_1/CB_2 receptor agonist WIN55,212-2. Peripheral antinociception without CNS effects is consistent with the peripheral distribution of CB_2 receptors. CB_2 receptor agonists may have promise clinically for the treatment of pain without CNS cannabinoid side effects.

Keywords: Cannabinoid receptor; CB_1 receptor; Antagonists

1. Introduction

Cannabinoid receptor agonists produce relief of pain in a variety of animal models (Richardson, 2000). The majority of these effects appear to be mediated by CB_1 cannabinoid receptors, as they are blocked by CB_1 receptor-selective antagonists (Richardson, 2000). CB_1 receptors are found in the central nervous system and have also been suggested to lie on peripheral nerve terminals (Hohman and Herkenham, 1999a,b). Administration of the endogenous CB_1 receptor agonist, anandamide into the ipsilateral hindpaw of the rat reduced carrageenan-induced hyperalgesia (Richardson et al., 1998a) or formalin-induced nociception (Calignano et al., 1998), indicating that activation of peripheral CB_1 receptors produces antinociception.

CB_2 receptors are located outside of the central nervous system (Munro et al., 1993; Facci et al., 1995; Galiegue et al., 1995; Griffin et al., 1999). It is not known whether activation of peripheral CB_2 receptors results in relief of pain. In this regard, administration of the endocannabinoid palmitoylethanolamide (PEA) reduced edema and inflammatory hyperalgesia (Mazzari et al., 1996). Furthermore, administration of PEA into the ipsilateral paw decreased the behavioral response to formalin injection into the hindpaw (Calignano et al., 1998). This effect was sensitive to the CB_2 receptor-selective antagonist SR144528, but insensitive to the CB_1 receptor-selective antagonist SR141716A. Intraperitoneal PEA was inactive and intravenous PEA was much less potent than was the intraplantar-administered drug. These findings suggest that intraplantar palmitoylethanolamide exerted its antinociceptive effect by activation of peripheral CB_2 receptors. However, PEA may not be a direct CB_2 receptor agonist, as it does not bind to CB_2 receptors transfected into host cells (Showalter et al., 1996). Therefore, it has been suggested that PEA activates CB_2 receptors indirectly, by initiating a chain of events resulting in CB_2 receptor activation. For example, it has therefore been proposed that, rather than being a direct CB_2 receptor

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agonist, PEA may enhance the effects of other endocannabinoids, perhaps by inhibiting their inactivation (Lambert and DiMarzo, 1999).

The present studies test the hypothesis that direct and selective activation of peripheral CB_2 receptors can inhibit responses to acute noxious stimuli. They also test the prediction that, because CB_2 receptors are not found in the CNS, selective CB_2 receptor activation will not produce central nervous system cannabinoid effects. These experiments utilize AM1241, a CB_2 receptor-selective agonist.

2. Methods

2.1. Animals

All procedures were approved by the University of Arizona Animal Care and Use Committee and followed the guidelines of the International Association for the Study of Pain (Zimmermann, 1983). Male Sprague–Dawley rats (Harlan, Indianapolis, IN), 250–350 g at the time of testing, were maintained in a climate-controlled room on a 12-h light/dark cycle and allowed food and water ad libitum.

2.2. Drug administration

AM1241, is a potent ($K_i = 2$nM) cannabinoid receptor agonist with 95–340-fold selectivity for the CB_2 receptor in vitro. AM630 is a CB_2 receptor-selective antagonist with 70–165-fold selectivity for binding to the CB_2 receptor in vitro (Ross et al., 1999; Hosohata et al., 1997). AM251 is a 300-fold selective CB_1 receptor antagonist (Gatley et al., 1996, 1997). WIN55,212-2, a mixed CB_1/CB_2 receptor agonist, was purchased from Sigma (St. Louis, MO). All drugs were dissolved in dimethyl sulfoxide (DMSO). Drugs were injected subcutaneously in the dorsal surface of the paw (i.paw, 50 μl) or intraperitoneally (i.p., 0.5 ml). Measurements were taken 25 min after i.paw injection or 15 min after i.p. injection. In preliminary experiments, these were determined to be the times of maximal drug effect.

2.3. Testing of antinociception

Assessment of antinociceptive actions of AM1241 was made by evaluating the response latency to removal of the paw from a focused source of thermal stimulation, as described by Hargreaves et al. (1988). Rats were allowed to acclimate within plexiglass enclosures on a clear glass plate maintained at 30°C. A radiant heat source (a high-intensity projector lamp) was focused onto the plantar surface of the hindpaw. Activation of the heat source activated a timer which stopped when withdrawal of the paw was detected with a photodetector. A maximal cut-off of 40 s was utilized to prevent tissue damage.

2.4. Testing of CNS effects

Cannabinoid-induced hypothermia was determined by measuring rectal temperature using a thermistor probe (Digital Thermometer 100, VWR Scientific). AM1241 (3.3 mg/kg), WIN55,212-2 (3.3 mg/kg) or vehicle was injected i.p. Twenty-five minutes after injection, rectal temperature was again measured.

Ring immobility was determined as described by Martin et al. (1991). CB_1 cannabinoid agonists cause animals to become cataleptic. They display no voluntary movements in a setting (e.g. perched on a ring) where they would otherwise be active. Rats were acclimated to the testing room overnight. Animals were placed on the test apparatus, a 12.5 cm ring attached at a height of 16 cm to a ring stand. Each animal was observed for a 5 min period and the sum of all times during which the rat was motionless was determined to the nearest second. The criterion for immobility was the absence of all voluntary movements, including whisker and snout movements. AM1241 (3.3 mg/kg), WIN55,212-2 (3.3 mg/kg) or vehicle (DMSO) was injected i.p. and the measurement was repeated 25 min after injection. Immobility is expressed as the percent of the 5-min period that the rat was immobile.

For measurement of the inhibition of locomotor activity by cannabinoids, AM1241 (3.3 mg/kg), WIN55,212-2 (3.3 mg/kg) or vehicle (DMSO) was injected i.p. Rats were placed in individual photocell activity chambers (AccuScan Instruments, Inc., Columbus, OH). Spontaneous activity was measured as the number of interruptions of photocell beams over a 90-min period. The percent inhibition of locomotor activity by AM1241 or WIN55,212-2 was calculated compared to the activity of a separate group of rats that had received vehicle alone.

Ambulation was tested using a rotarod device (Columbus Instruments International, Columbus, OH). Animals were trained until they could remain on the device for a duration of 180 s at a speed of 10 revolutions per minute. They were again tested 25 min after i.p. administration of AM1241, WIN55,212-2 or vehicle and the time they were able to remain on the device until falling was recorded. A maximal cut-off time of 180 s was used.

2.5. Data analysis

Antinociception is expressed as the percent of the maximum possible effect (%MPE), using the formula: $\%MPE = (WL - CL) / (CO - CL)$, where WL is the withdrawal latency obtained experimentally, CL is the control (baseline) value before drug administration, and CO is the cut-off value (40 s). Dose-response curves were generated and the A_{50} values (doses producing 50% MPE) were calculated (Tallarida and Murray, 1987). In other experiments, groups were compared using ANOVA, followed by pairwise comparisons using Student's *t*-test with a Bonferroni correction. Significance was defined as $P < 0.05$.

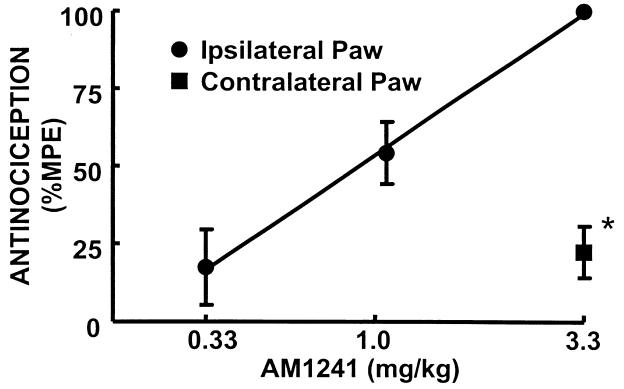

Fig. 1. Peripheral antinociceptive effect of AM1241. AM1241 was injected into the hindpaw ipsilateral or contralateral to the side of nociceptive testing. Data expressed as mean ± SEM. $N = 6$ per group.

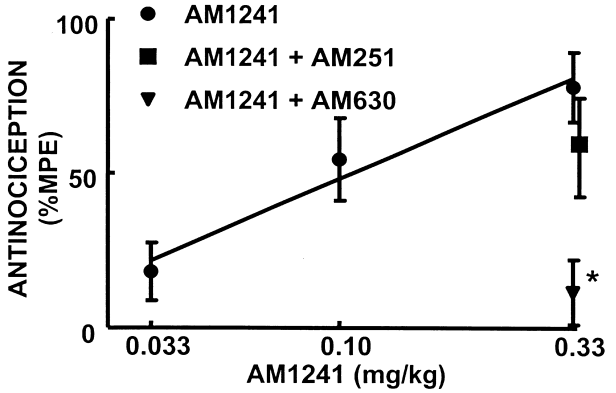

Fig. 3. Antinociceptive effects of intraperitoneal AM 1241. The antinociceptive effects of i.p. AM1241 were antagonized by i.p. AM630 (1 mg/kg) but not by i.p. AM251 (1 mg/kg). Data expressed as mean ± SEM. Groups were compared using ANOVA followed by pairwise comparisons using Student's *t*-test. $^{*}P < 0.05$ compared to AM1241 alone. $N = 6$ per group.

3. Results

The CB_2 receptor-selective agonist, AM1241 produced dose-dependent antinociception to a thermal stimulus applied to the hindpaw, when administered into the hindpaw on the side of testing (ipsilateral i.paw) (Fig. 1). AM1241 was much less active when given into the paw contralateral to the side of antinociceptive testing (Fig. 1). The A_{50} (analgesic dose yielding a 50% effect) was 847 μg/kg with the maximum possible effect (100% MPE) being achieved at 3.3 mg/kg. The CB_2 receptor-selective antagonist AM630 (100 μg/kg), administered in the ipsilateral paw, completely blocked the actions of 1 mg/kg i.paw AM1241 ($P < 0.05$ by ANOVA followed by Student's *t*-test with a Bonferroni correction), at doses of AM630 that produced no effect when given alone (Fig. 2). The CB_1 receptor-selective antagonist, AM251 (330 μg/kg) had no effect (Fig. 2).

AM 1241 also produced dose-dependent antinociception when administered intraperitoneally (i.p.), with an A_{50} of 103 μg/kg (Fig. 3). The effect of i.p. AM1241 (333 μg/kg) was completely blocked by i.p. AM630 (1 mg/kg), but not by i.p. AM251 (1 mg/kg) ($P < 0.05$ by ANOVA followed by Student's *t*-test with a Bonferroni correction) (Fig. 3). The effect of i.p. AM1241 was decreased by 86% by AM630 (100 μg/kg) administered in the paw ipsilateral to the side of testing ($P < 0.05$), while AM630 in the contralateral paw had no effect (Fig. 4). AM251 (330 μg/kg) had no effect when administered in either paw.

Body temperature (38.1 ± 0.1°C) was not altered by i.p. injection of vehicle (DMSO) or AM1241 (3.3 mg/kg). In contrast, i.p. WIN55,212-2 (3.3 mg/kg) a mixed CB1/CB2 cannabinoid receptor agonist caused 2.1°C hypothermia ($P < 0.05$ by ANOVA followed by Student's *t*-test with a Bonferroni correction) (Fig. 5A). In the ring immobility test

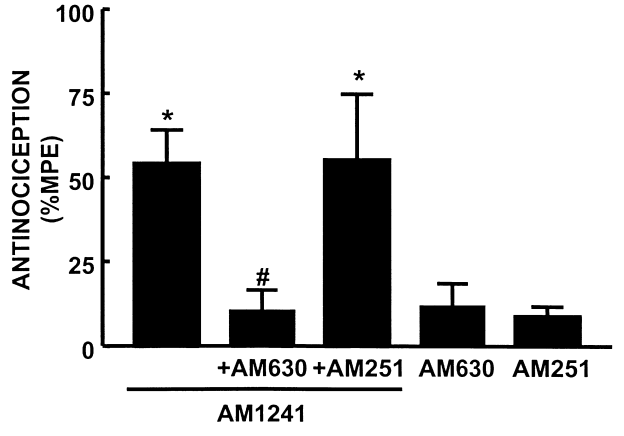

Fig. 2. Antagonism of the antinociceptive effects of i.paw AM1241 (1 mg/kg) by i.paw AM630 (100 μg/kg) but not by i.paw AM251 (330 μg/kg). AM630 is a CB_2 receptor-selective antagonist and AM251 is a CB_1 receptor-selective antagonist. Data are expressed as mean ± SEM. Groups were compared using ANOVA followed by pairwise comparisons using Student's *t*-test. $^{*}P < 0.05$ compared to baseline. $^{\#}P < 0.05$ compared to AM1241 alone. $N = 6$ per group.

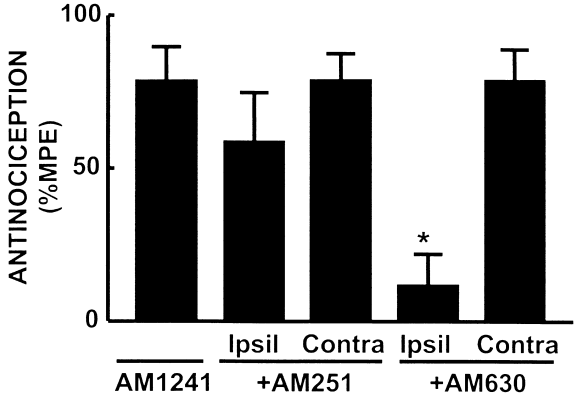

Fig. 4. Antagonism of the antinociceptive effects of intraperitoneal AM1241 (330 μg/kg) by 100 μg/kg AM630 or 330 μg/kg AM251 injected into the paw ipsilateral or the paw contralateral to the side of nociceptive testing. Data expressed as mean ± SEM. Groups were compared using ANOVA followed by pairwise comparisons using Student's *t*-test. $^{*}P < 0.05$ compared to AM1241 alone. $N = 6$ per group.

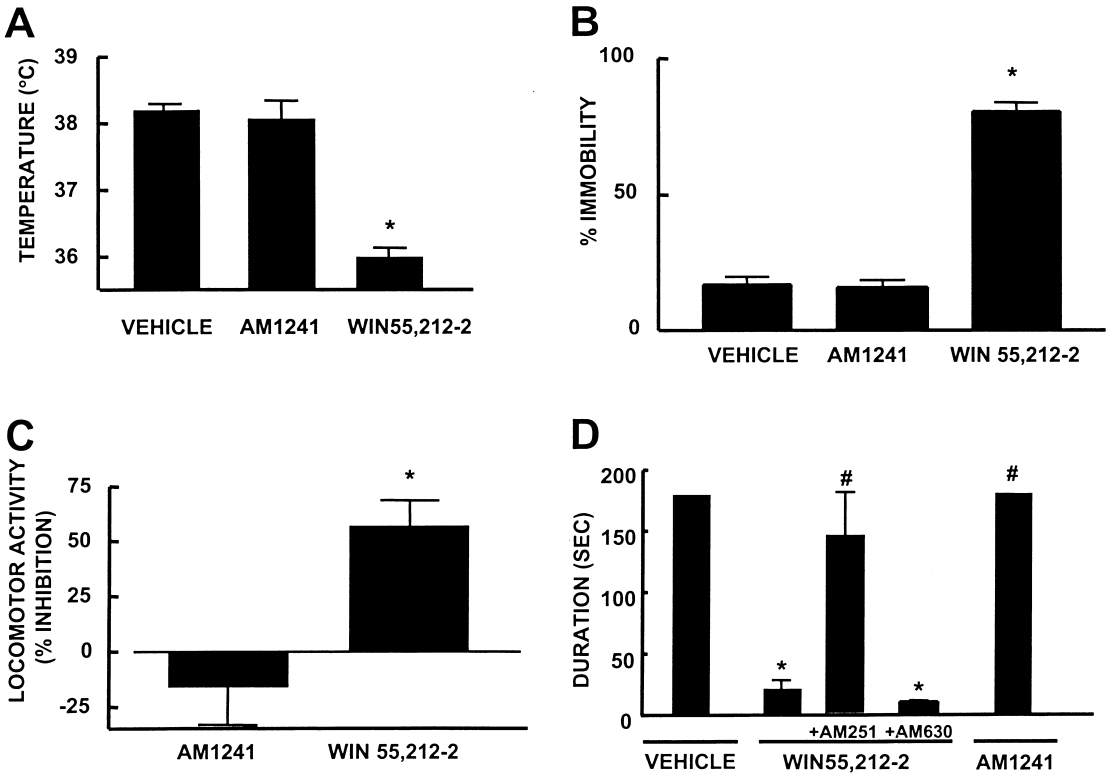

Fig. 5. Effect of AM1241 (3.3 mg/kg) and WIN,55212-2 (3.3 mg/kg) on CNS cannabinoid effects. (A) Hypothermia. (B) Ring immobility test of catalepsy. (C) Inhibition of locomotor activity. (D) Interference with ambulation, as assessed by performance on the rotarod apparatus. The WIN55,212 effect on ambulation is blocked by AM251, but not AM 630. All data expressed as mean ± SEM. $^{*}P < 0.05$ compared to vehicle (DMSO). $^{\#}P < 0.05$ compared to AM1241 alone. $N = 6$ per group.

of catalepsy, animals displayed no periods of immobility before injection. Injection of vehicle alone caused immobility for 17% of the observation period ($P < 0.05$ compared to baseline) (Fig. 5B). AM 1241 did not increase immobility compared to vehicle. In contrast, WIN55,212-2 produced 78% immobility ($P < 0.05$ compared to vehicle alone). Intraperitoneal AM1241 did not inhibit spontaneous activity compared to vehicle (Fig. 5C). WIN55,212-2 inhibited activity by 48% ($P < 0.05$ compared to vehicle). In the rotarod test of ambulation, all animals remained on the apparatus for the 180 min cut-off time before injection and after administration of vehicle alone (Fig. 5D). Intraperitoneal AM1241 (3.3 mg/kg) did not alter duration on the rotarod apparatus, while WIN55,212-2 (3.3 mg/kg i.p.), reduced the mean duration by 88% ($P < 0.05$ compared to vehicle alone). The effect of WIN55,212-2 on ambulation was prevented by AM251 (1 mg/kg i.p.), ($P < 0.05$ compared to agonist alone), but was not affected by AM630 (1 mg/kg i.p.).

4. Discussion

These experiments demonstrate that selective activation of peripheral CB_2 receptors is sufficient to produce antinociception. The CB_2 receptor-selective agonist, AM1241, injected into one hindpaw showed analgesic activity when a thermal stimulus was applied to the same paw. Taken alone, these data might be postulated to be due to drug diffusing into the bloodstream and acting at distant sites. For example, cannabinoid receptors are found at a variety of sites in the central nervous system (Herkenham et al., 1991) and activation of central cannabinoid receptors produces antinociception (Yaksh, 1981; Lichtman and Martin, 1991; Martin et al., 1993, 1995, 1996; Hohman et al., 1995; Lichtman et al., 1996; Richardson et al., 1998b,c). However, the inability of equivalent doses of AM1241 to produce antinociception when administered into the contralateral hindpaw supports a local site of action.

While both CB_1 and CB_2 receptors are found in peripheral tissues (Munro et al., 1993; Hohman and Herkenham, 1999a,b), the antinociceptive effects of AM1241 appear to be due to actions at the CB_2 receptor. AM1241 displays 95- to 340-fold selectivity for the CB_2 receptor in binding studies in vitro. Coadministration of the CB_2 receptor-selective antagonist AM630 in the same hindpaw blocked the analgesic effect of AM1241. In contrast the CB_1 receptor-selective antagonist AM251 did not block AM1241-induced antinociception.

Systemic (i.p.) administration of AM1241 also produced antinociception to a thermal stimulus applied to the paw. The antinociceptive actions of systemic AM1241 appear to

be mediated at peripheral sites, as injection of AM630 into the tested paw blocked the antinociceptive actions of intraperitoneal AM1241. These findings suggest that the primary site of action of AM1241 is local at the site of testing. In contrast, injection of AM630 into the paw contralateral to the side of testing did not affect the antinociceptive actions of systemic AM1241, demonstrating that the actions of i.paw AM630 were local and not due to uptake into the circulation and spread to distant sites.

AM1241 was more potent in producing antinociception when administered i.p. than when administered in the dorsal surface of the ipsilateral paw. This finding might be taken to suggest that the effect of i.paw AM1241 may be systemic rather than local. However, comparing the i.paw with the i.p. routes is complicated by the issue of drug distribution. Specifically, the systemic absorption of the drug may be much more efficient from the peritoneal cavity than from paw tissue, perhaps because the surface area available for absorption is much greater in the peritoneum. Administration of test drug into the paw ipsilateral to the side of nociceptive testing *vs.* administration into the contralateral paw is a more direct test of local *vs.* systemic effects because systemic absorption should be similar from both sites.

Further, the relatively low potency of i.paw AM1241 may be explained by the site of injection. AM1241 was injected in the dorsal surface of the paw, while the presumed site of action was in the plantar surface, where the test stimulus was applied. This method was necessary because injection of vehicle (DMSO) into the plantar surface prolonged withdrawal latency, while injection of vehicle into the dorsal surface had no effect. If AM1241 does not readily penetrate the tissue of the paw to reach the plantar surface, or if this lipophilic drug becomes entrapped in fatty tissue of the hindpaw, it would reduce the apparent potency of the drug.

Our results contrast with those of Hanuš et al. (1999) who studied the antinociceptive effects of the CB_2 receptor agonist HU-308, a compound that shows 500-fold selectivity for the CB_2 receptor. HU-308 resulted in decreased licking behavior in the late, but not the early phase after injection of formalin into the hindpaw. However, Hanuš *et al.* did not detect production of significant antinociception by HU-308 in mice on a 55°C hot plate. In contrast, we observed a profound antinociception to thermal stimuli.

Our data do not specifically address the mechanism by which activation of peripheral CB_2 receptors leads to antinociception. One possibility is that AM1241 activates CB_2 receptors on peripheral nerve endings, thereby decreasing sensitivity of primary afferent neurons. There is indirect evidence that CB_2 receptors may be present on peripheral neurons (Griffin et al., 1997; Pertwee, 1999). The CB_2 receptor-selective agonists 1-propyl-2-methyl-3-(1-naphthoyl) indole and 1-deoxy-11-hydroxy-Δ^8-THC-dimethylheptyl inhibit electrically-evoked contractions of mouse vas deferens at concentrations below their dissociation constant for displacement of CP-55940 from CB_2 binding sites. This inhibition was not blocked by the CB_1 receptor-selective antagonist SR141716A. A neuronal site of action was inferred by the finding that 1-propyl-2-methyl-3-(1-naphthoyl) indole inhibits electrically-evoked contractions at concentrations that do not inhibit contractions induced by exogenous administration of neurotransmitters. However, direct evidence for peripheral neuronal CB_2 receptors has not been obtained. CB_2 receptor messenger RNA was not found in dorsal root ganglion cells (Hohman and Herkenham, 1999b).

Alternatively, the CB_2 receptor is present on immune cells, including mast cells, macrophages, B-lymphocytes, T-lymphocytes and natural killer cells (Munro et al., 1993; Facci et al., 1995; Galiegue et al., 1995). It is possible that AM1241 activates the CB_2 receptor on mast or other immune cells, thereby decreasing the basal release of molecules that sensitize the peripheral nociceptor (e.g. prostanoids, cytokines, adenosine triphosphate, 5-hydroxytryptamine or histamine). It is not clear whether there is significant basal release of sensitizing molecules from mast or other immune cells in non-inflamed tissue. If there is, inhibition of this release could provide a mechanism for CB_2 receptor-mediated antinociception. Activation of CB_2 cannabinoid receptors results in inhibition of adenyl cyclase activity by a $G_{i/o}$ protein and stimulation of mitogen-activated protein kinase (for review see Pertwee, 1997). CB_2 receptors have also been reported to be coupled to G protein-coupled inwardly rectifying potassium channels (Ho et al., 1999). Interpretation of experimental data relating adenylate cyclase activity to immune cell function is complex. Importantly, however, the inhibitory effect of cannabinoids in several tests of immune cell function can be prevented by blocking or reversing the cannabinoid-induced decrease in intracellular cyclic AMP (Schatz et al., 1993; Kaminski et al., 1994). Mast cell degranulation may be linked to adenylate cyclase activity, as suggested by the observation that degranulation can be augmented by increasing adenylate cyclase activity or suppressed by decreasing adenylate cyclase activity (Holgate et al. 1980).

At fully analgesic doses, systemic AM1241 did not produce hypothermia, catalepsy or inhibition of activity. These effects are believed to be mediated by CNS CB_1 cannabinoid receptors (Martin et al., 1991; Pertwee, 1997; Mechoulam et al., 1998). In contrast, approximately equianalgesic doses (A_{50} for antinociception produced by AM1241 was 103 μg/kg while A_{50} for WIN55,212-2 was 75 μg/kg, data not shown) of the mixed CB_1/CB_2 receptor agonist WIN55,212-2 produced this triad of cannabinoid effects. Similarly, analgesic doses of AM1241 produced no or small effects in the rotarod test of ambulation. We hypothesized that activation of CNS cannabinoid receptors would interfere with the ability to ambulate. This prediction is supported by the fact that systemic WIN55,212-2 interfered with ambulation in a manner sensitive to AM251, but not AM630. Taken together, these findings are consistent with the hypothesis that selective CB_2 receptor agonists may be free of CNS-mediated cannabinoid effects.

The experiments presented in this paper demonstrate that activation of peripheral CB_2 receptors is sufficient to produce antinociception to an acute thermal stimulus. They also demonstrate a lack of CNS cannabinoid effects by a selective CB_2 receptor agonist. These results have clear medical implications, since CB_2 receptor agonists would be predicted to be effective in treating acute pain without the central side effects of cannabinoid agonists with CB_1 receptor agonist activity. Central nervous system side effects, such as anxiety, sedation, and abuse potential have limited the development and use of cannabinoids as pain medications.

Acknowledgements

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References

Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature 1998;394:277–281.

Facci L, Dal Toso R, Romanello S, Buriani A, Skaper SD, Leon A. Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. Proc Natl Acad Sci USA 1995;92:3376–3380.

Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, Bouaboula M, Shire D, Le Fur G, Casellas P. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 1995;232:54–61.

Gatley SJ, Gifford AN, Volkow ND, Lan R, Makriyannis A. ^{123}I-labelled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid receptors. Eur J Pharmacol 1996;307:331–338.

Gatley SJ, Lan R, Pyatt B, Gifford AN, Volkow ND, Makriyannis A. Binding of the non-classical cannabinoid CP55,940, and the diarylpyrazole AM251 to rodent brain cannabinoid receptors. Life Sci 1997;61:PL191–PL197.

Griffin G, Fernando SR, Ros RA, McKay NG, Ashford ML, Shire D, Huffman JW, Yu S, Kainton JA, Pertwee RG. Evidence for the presence of CB2-like cannabinoid receptors on peripheral nerve terminals. Eur J Pharmacol 1997;339:53–61.

Griffin G, Wray EJ, Tao Q, McAllister SD, Rorrer WK, Aung MM, Martin BR, Abood ME. Evaluation of the cannabinoid CB2 receptor selective antagonist, SR144528: further evidence of cannabinoid CB2 receptor absence in the rat central nervous system. Eur J Pharmacol 1999;377:117–125.

Hanuš L, Breuer A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M, Pertwee RG, Ross RA, Mechoulam R, Fride E. HU-308: a specific agonist for CB2, a peripheral cannabinoid receptor. Proc Natl Acad Sci USA 1999;96:14228–14233.

Hargreaves KM, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988;32:77–88.

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;11:563–583.

Ho BY, Uezono Y, Takada S, Takase I, Izumi F. Coupling of cannabinoid CB_1 and CB_2 receptors to phospholipase C and G protein-coupled inwardly rectifying K^+ channels. Receptors Channels 1999;6:363–374.

Hohman AG, Herkenham M. Cannabinoid receptors undergo axonal flow in sensory nerves. Neuroscience 1999a;92:1171–1175.

Hohman AG, Herkenham M. Localization of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of dorsal root ganglia: a double-label in situ hybridization study. Neuroscience 1999b;90:923–931.

Hohman 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;56:2111–2118.

Holgate ST, Lewis RA, Austen KF. Role of adenylate cyclase in immunologic release of mediators from mast cells: agonist and antagonist effects or purine- and ribose-modified adenosine analogs. Proc Natl Acad Sci USA 1980;77:6800–6804.

Hosohata Y, Quock RM, Hosohata K, Makriyannis A, Consroe P, Roeske WR, Yamamura HI. AM630 antagonism of cannabinoid-stimulated [^{35}S]GTP -S binding in the mouse brain. Eur J Pharmacol 1997;321:R1–R3.

Kaminski NE, Koh WS, Yang KH, Lee M, Kessler FK. Supression of the humoral immune response by cannabinoids is partially mediated through inhibition of adenylate cyclase by a pertussis toxin-sensitive G protein-coupled mechanism. Biochem Pharmacol 1994;48:1899–1908.

Lambert DM, DiMarzo V. The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimemetic? Curr Med Chem 1999;6:757–773.

Lichtman AH, Martin BR. Spinal and supraspinal components of cannabinoid-induced antinociception. J Phamacol Exp Ther 1991;258:517–523.

Lichtman AH, Cook SA, Martin BR. Investigation of brain sites mediating cannabinoid-induced antinociception in rats: evidence supporting periaqueductal gray involvement. J Pharmacol Exp Ther 1996;276:585–593.

Mazzari S, Canella R, Petrelli L, Marcolongo G, Leon A. N-(2-hydroxyethyl) hexadecanamide is orally active in reducing edema formation and inflammatory hyperalgesia by down-modulating mast cell activation. Eur J Pharmacol 1996;300:227–236.

Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, Johnson MR, Melvin LS, Mechoulam R, Ward SJ. Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. Pharmacol Biochem Behav 1991;40:471–478.

Martin WJ, Lai NK, Patrick SL, Tsou K, Walker JM. Antinociceptive actions of cannabinoids following intraventricular administration in rats. Brain Res 1993;629:300–304.

Martin WJ, Patrick SL, Coffin PO, Tsou K, Walker JM. An examination of the central sites of action on cannabinoid -induced antinociception in the rat. Life Sci 1995;56:2103–2109.

Martin WJ, Hohman 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;16:6601–6611.

Mechoulam R, Fride E, DiMarzo V. Endocannabinoids. Eur J Pharm 1998;359:1–18.

Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–65.

Pertwee RG. Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol Ther 1997;74:129–180.

Pertwee RG. Evidence for the presence of CB1 cannabinoid receptors on peripheral neurons and for the existence of neuronal non-CB1 cannabinoid receptors. Life Sci 1999;65:597–605.

Richardson JD. Cannabinoids modulate pain by multiple mechanisms of action. J Pain 2000;1:2–14.

Richardson JD, Kilo S, Hargreaves KM. Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB1 receptors. Pain 1998a;75:111–119.

Richardson JD, Aanonsen L, Hargreaves KM. Antihyperalgesic effects of spinal cannabinoids. Eur J Pharmacol 1998b;345:145–153.

Richardson JD, Aanonsen L, Hargreaves KM. Hypoactivity of the spinal cannabinoid system results in NMDA-dependent hyperalgesia. J Neurosci 1998c;18:451–457.

Ross RA, Brockie HC, Stevenson LA, Murphy VL, Templeton F,

Makriyannis A, Pertwee RG. Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656, and AM630. Br J Pharmacol 1999;126:665–672.

Schatz AR, Koh WS, Kaminski NE. Delta 9-tetrahydrocannabinol selectively inhibits T-cell dependent humoral immune responses through direct inhibition of accessory T-cell function. Immunopharmacology 1993;26:129–137.

Showalter VM, Compton DR, Martin BR, Abood ME. Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. J Pharmacol Exp Ther 1996;278:989–999.

Tallarida RJ, Murray RB. Manual of pharmacological calculations with computer programs. 2nd edn. New York: Springer, 1987.

Yaksh TL. The antinociceptive effects of intrathecally administered levonantradol and desacetyllevonantradol in the rat. J Clin Pharmacol 1981;2:334S–340S.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–110.