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Research report

Evidence for a role of endogenous cannabinoids in the modulation of acute and tonic pain sensitivity

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Abstract

The competitive CB1 receptor antagonist SR141716A was used to test the hypothesis that endogenous cannabinoids modulate tonic pain sensitivity. Pretreatment with the antagonist significantly enhanced the response to a chemical nociceptive stimulus in the formalin test. Postreatment with the antagonist 5 min following the induction of tonic pain produced hyperalgesia during the tonic phase only. These findings suggest that endogenous cannabinoids serve naturally to modulate the maintenance of pain following repeated noxious stimulation.

Keywords: Formalin; Pain; Hyperalgesia; Cannabinoid; SR141716A

1. Introduction

In rodents, the antinociceptive action of cannabinoid agonists has been documented using many different types of noxious stimuli [3,19,22,29]. Together with the identification of the CB1 cannabinoid receptor and the putative endogenous ligand anandamide [6,7], these findings raised the possibility that endogenous cannabinoids serve normally to dampen pain sensitivity and prompted efforts to elucidate the neural substrates of cannabinoid antinociception. Tsou et al. [31] demonstrated that cannabinoids suppress the expression of FOS in the spinal cord in response to a noxious chemical stimulus (intraplantar formalin). Cannabinoids also decreased the responses of wide dynamic range neurons in the spinal cord to noxious thermal and mechanical stimuli [13,14] and suppressed responses to noxious mechanical stimuli in the ventral posterolateral thalamic nucleus, a major termination zone of the lateral spinothalamic tract [18]. These findings demonstrate the presence of appropriate neural substrates for cannabinoid antinociception, but they provide only indirect evidence for a role of endogenous cannabinoids in pain modulation.

Rinaldi-Carmona et al. [27] reported the development of a competitive CB1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl) -1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride (SR141716A). This compound antagonized all the known effects of cannabinoid agonists in vivo including the antinociceptive effects of WIN 55,212-2 and Δ^9-tetrahydrocannabinol (Δ^9-THC) [5,26]. SR141716A is an important tool, in part because the effects of this compound in otherwise untreated animals can be attributed to the blockade of endogenous cannabinoids. For example, the improvement of memory in rodents receiving SR141716A [30] suggested that endogenous cannabinoids serve naturally to inhibit memory formation.

Richardson et al. reported that intrathecal injection of SR141716A [24,25] or CB1 antisense treatment [25] in otherwise untreated mice produces hyperalgesia in the hot plate test. Herzberg et al. [12], found that intraperitoneal injection of this compound increases thermal hyperalgesia and mechanical allodynia in untreated rats with chronic constriction injury of the sciatic nerve. These findings suggest that endogenous cannabinoids may tonically dampen sensitivity to an acute thermal noxious stimulus

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and suppress the enhancement of pain sensitivity that occurs in persistent pain. In order to further explore the hypothesis that endogenous cannabinoids modulate responses to persistent as well as acute pain, we examined the effect of systemic injection of SR141716A on pain induced by a prolonged noxious chemical stimulus in the formalin test. Preliminary reports of the findings presented herein have appeared [20].

2. Materials and methods

All protocols were reviewed and approved by the Brown University Institutional Animal Care and Use Committee. Sprague–Dawley rats were used for all experiments.

2.1. Drug preparation and administration

WIN 55, 212-2 (WIN2) was dissolved in a 1:9 solution of emulphor and 0.9% saline and injected i.p. (10 mg/kg). SR141716A was dissolved in a 1:1:18 solution of ethanol, emulphor and 0.9% saline and injected i.p. (1 mg/kg). Dilute formaldehyde (0.5%) was prepared in a solution of mono and dibasic potassium phosphate (pH 7.4) and injected s.c. (150 μl) into the plantar surface of the left hindpaw.

2.2. Time course of SR141716A effect

In order to determine the latency to onset of the antagonist's effect following i.p. injection, the time course of the reversal of WIN2-induced catalepsy by SR141716A was examined. The injection intervals for the formalin test were chosen based upon on the results of this experiment.

Seven rats received an i.p. injection of WIN2 (10 mg/kg) at time zero and were tested for catalepsy in the ring immobility test at subsequent 3 min intervals. The latency to descend from the ring was recorded as a catalepsy score (a 30 s cutoff was imposed). Three rats received an intraperitoneal injection of SR141716A (1 mg/kg) 15 min after the injection of WIN2. Testing continued for an additional 45 min.

2.3. Formalin test

Prior to testing, the rat was acclimated to the test chamber and experimenter on two occasions. Each rat was placed individually in a clear plexiglass cage for testing. Mirrors were positioned underneath the cage and on three sides to permit unobstructed viewing of the injected paw.

Forty-eight rats were divided into six groups of eight. Two groups of rats received an i.p. injection of SR141716A

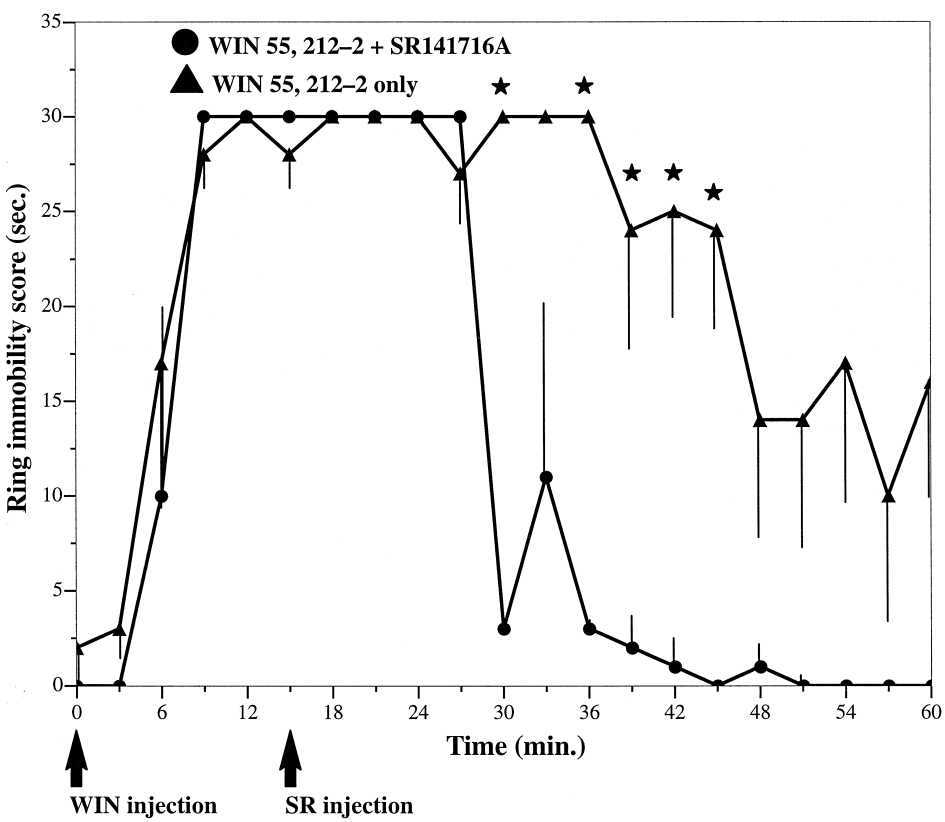

Fig. 1. Production of ring catalepsy by WIN2 and its reversal by SR141716A. Fifteen minutes after the injection of WIN2 (10 mg/kg i.p.), each rat received an intraperitoneal injection of SR141716A (1 mg/kg i.p.). SR141716A significantly antagonized WIN2-induced catalepsy within 15 min of injection (Fisher's Protected t:$P < 0.05$). ∗Significant at $P < 0.05$.

or vehicle 15 min prior to the formalin injection. Two additional groups of rats received vehicle or SR141716A injection 5 min following administration of formalin. The last two groups of rats received a sham injection (i.p. puncture using an empty syringe) prior or subsequent to formalin injection.

Behavioral observation began immediately following the injection of formalin, and the rat's behavior was continuously monitored for the next 60 min. Time spent licking and/or lifting the injected hindpaw was recorded by a single, (unblinded) observer over successive 5 min intervals. This method of scoring [1], predicts formalin dose in a manner similar to that obtained using the weighted pain score of Dubuisson and Dennis [9]. Furthermore, because it necessitates only one observer, this scoring method reduces the variability introduced by multiple observers. Following testing, the animal was sacrificed by lethal injection of pentobarbital.

2.4. Data analysis

Ring catalepsy scores were compared between groups using analysis of variance and Fisher's protected *t*-test. Formalin pain scores were calculated as the sum of time spent licking and lifting the injected hindpaw and statistically compared using analysis of variance and Tukey's HSD procedure. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Time to effect of SR141716A

Intraperitoneal injection of WIN2 produced profound immobility in the ring catalepsy test within 9 min of injection (Fig. 1). Although there were signs of recovery between 40 and 60 min of injection, motor depression

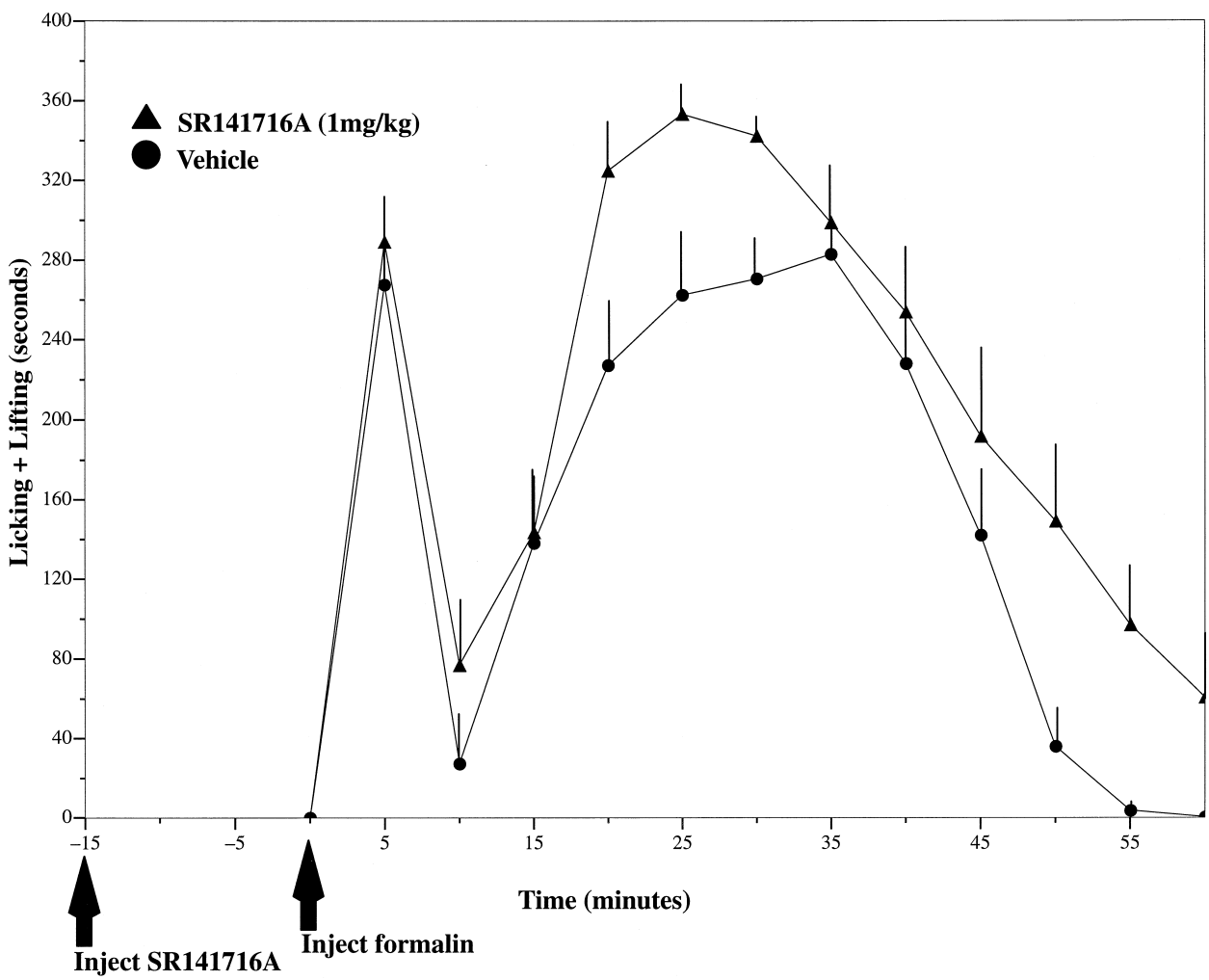

Fig. 2. The time course of formalin-induced pain behavior and the effect of pretreatment with SR141716A. SR141716A (1 mg/kg) or vehicle (1:1:18 ethanol/emulphor/saline) was administered i.p. 15 min prior to the formalin injection. Drug treatment significantly elevated the overall pain score ($P = 0.01$).

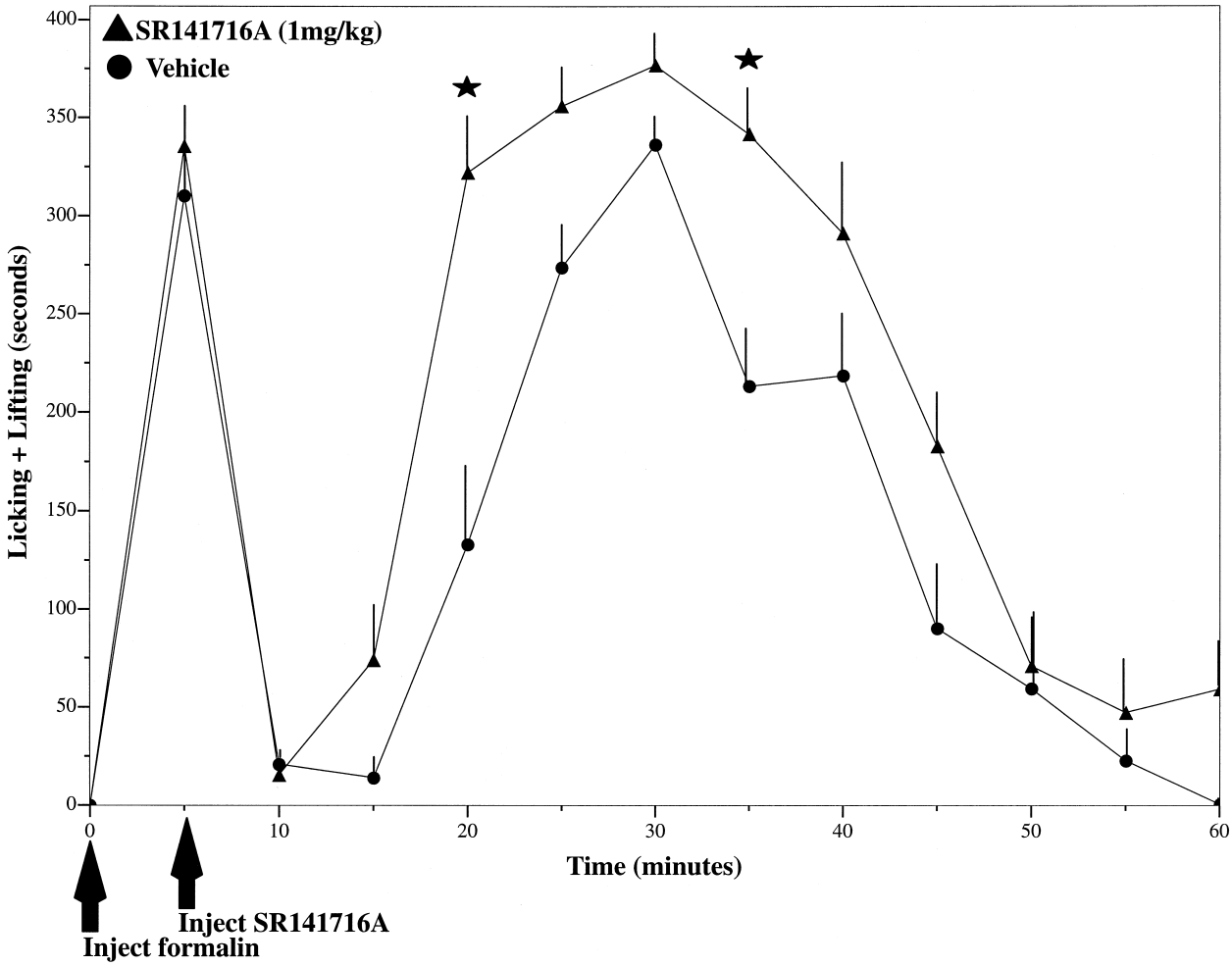

Fig. 3. The time course of formalin-induced pain behavior and the effect of delayed administration of SR141716A. SR141716A (1 mg/kg) or vehicle (1:1:18 ethanol/emulphor/saline) was administered i.p. 5 min subsequent to the formalin injection. Drug treatment significantly elevated the overall pain score ($P = 0.02$) and pain behavior during the peak of phase 2. $*$Significant at $P < 0.05$.

continued to be evident in control rats throughout the entire 60 min observation period.

Intraperitoneal injection of SR141716A significantly diminished the overall motor inhibition produced by the cannabinoid agonist ($P = 0.01$). This inhibition developed within 15 min of injection (Fisher's Protected t: $P < 0.05$) (the best estimate of latency to effect is 13.5 min) and lasted throughout the experiment.

3.2. Effect of SR141716A in the formalin test

As reported previously [9], intraplantar injection of formalin evoked a biphasic pattern of responses in both the experimental and control group (Figs. 2 and 3). Overall pain scores did not significantly differ between the groups that received vehicle and those that received sham injections.

Pretreatment with the cannabinoid receptor antagonist (1 mg/kg) significantly elevated the overall pain score ($P = 0.01$) (Fig. 2). Although it appears that the effect was confined to the tonic phase, the lack of a statistical interaction between drug and time ($P = 0.5$) indicates the absence of any differential effect of the drug during the acute and tonic phases of the test.

Delayed administration of SR141716A also produced hyperalgesia in the formalin test (Fig. 3). The latency to effect of the antagonist in this experiment coincided with that determined for its reversal of agonist-induced catalepsy (13.5 min). Animals injected with the antagonist displayed a significantly greater amount of overall pain behavior ($P = 0.02$) than did animals injected with the vehicle. The drug effect varied across time ($P = 0.04$) and post hoc tests revealed that the drug produced a significant enhancement of pain sensitivity only during the tonic phase of the response (Tukey's HSD = 143.78, $P < 0.05$) consistent with the delay in the injection time.

4. Discussion

The cannabinoid receptor antagonist SR141716A, but not vehicle or sham injection, enhanced responsiveness in

the formalin test when injected prior to or subsequent to formalin injection. These findings extend previous work [24,25], demonstrating a hyperalgesic effect of the antagonist on acute pain by providing evidence that the antagonist also enhances tonic pain sensitivity. These results suggest that endogenous cannabinoids serve naturally to suppress acute and tonic pain sensitivity.

The only known effect of SR141716A in vivo is the antagonism of CB1 cannabinoid receptors [28]. However, the possibility cannot be excluded that the effects we observed are the result of a non-specific action of the antagonist that has not yet been determined. Studies using in vitro systems that overexpress cannabinoid receptors suggested modest inverse agonist activity of SR141716A [2,15]. However, there is no corroborating evidence in more physiological systems where the receptor is not overexpressed. In rat cerebellar membranes, SR141716A antagonized the effects of WIN 55, 212-2 on GTPγS binding but alone produced no effect [23]. Hohmann et al. [14] did not observe an effect of the antagonist alone on either spontaneous or evoked activity of spinal nociceptive neurons. In view of the small magnitude of the effects observed in vitro and the lack of effects on spinal nociceptive neurons, it seems highly unlikely that inverse-agonism could account for the actions we observed.

The powerful motor effects of cannabinoids are a possible confound in the interpretation of behavioral measures of pain. Stimulation of locomotor behavior by SR141716A could produce increased licking and lifting that might falsely be attributed to hyperalgesia. However, SR141716A does not significantly alter locomotor activity at i.v. dosages less than 3 mg/kg [5]. The enhancement of locomotor activity by i.p. administration of a three-fold lower dosage seems extremely unlikely. Furthermore, the behavior was lateralized to the injured side which would not be expected if the effect were merely due to an increase in locomotor drive.

Different mechanisms mediate the acute and tonic response to formalin. Whereas the acute response to formalin mainly reflects the activation of primary afferents, the later response results jointly from inflammation and long term changes in the central nervous system [4,8,32] such as reduction of response thresholds and enlargement of receptive fields [33]. These changes are apparently initiated during phase one and completed relatively quickly [8,21]. As a consequence, their development can be modulated only when agents are administered prior to formalin [8]. The similar degree of hyperalgesia following pretreatment and posttreatment with the cannabinoid receptor antagonist is consistent with the hypothesis that endogenous cannabinoids contribute to the maintenance but not development of formalin-induced central sensitization.

The effects of SR141716A on formalin pain are similar to those produced by antagonists of voltage-sensitive calcium channels (VSCCs) [17]. Neodymium, a trivalent cation that inhibits calcium influx through VSCCs, suppresses formalin pain to an equal degree when administered prior to and subsequent to formalin. The N-type calcium channel antagonist SNX-111 is also an equally effective inhibitor of formalin pain following pretreatment and posttreatment, whereas antagonists of the L- and P-type calcium channels do not suppress formalin pain following postadministration. These findings are consistent with the hypothesis that VSCCs of the N-type participate in the maintenance of formalin pain. Inhibition of VSCCs may account for the effects of SR141716A on the maintenance of formalin pain, because cannabinoids interfere with Ca^{2+} entry through N- and Q-type Ca^{2+} channels [10,11,16].

The ability of cannabinoids to tonically inhibit the maintenance of pain evoked by repeated noxious stimulation suggests novel mechanisms for certain pathological states that involve central pain. For example, loss of activity in such a system would increase the likelihood that cutaneous injury would result in long lasting central hyperalgesia and allodynia.

Our studies suggest the existence of a non-opioid cannabinergic system that serves to modulate pain sensitivity and inhibit the maintenance of central sensitization in response to repeated or prolonged noxious stimulation. The identification of this endogenous non-opioid pain modulatory system may have clinical implications, especially with regard to the treatment of chronic pain. A number of similarities have been identified between formalin-evoked pain and human chronic pain. To the extent that formalin pain in rodents is an apt model of chronic pain in humans, our findings suggest that drugs that increase the synaptic concentration of endogenous cannabinoids or mimic their actions may be useful tools in managing pain in humans.

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References

[1] F.V. Abbott, K.B.J. Franklin, R.F. Westbrook, The formalin test: scoring properties of the first and second phases of the pain response in rats, Pain 60 (1995) 91–102.

[2] M. Bouaboula, S. Perrachon, L. Milligan, X. Canat, M. Rinaldi-Carmona, M. Portier, F. Barth, B. Calandra, F. Pecceu, J. Lupker, J. Maffrand, G. Le Fur, P. Casellas, A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin-like growth factor 1, J. Biol. Chem. 272 (1997) 22330–22339.

[3] D.M. Buxbaum, Analgesic activity of delta Δ^9-tetrahydrocannabinol in the rat and mouse, Psychopharmacol. Berlin 25 (1972) 275–280.

[4] T.J. Coderre, A.L. Vaccarino, R. Melzack, Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection, Brain Res. 535 (1990) 155–158.
[5] D.R. Compton, M.E. Aceto, J. Lowe, B.R. Martin, In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of Δ^9-tetrahydrocannabinol-induced responses and apparent agonist activity, J. Pharmacol. Exp. Ther. 277 (1996) 586–594.
[6] W.A. Devane, F.A. Dysarz III, M.R. Johnson, L.S. Melvin, A.C. Howlett, Determination and characterization of a cannabinoid receptor in rat brain, Mol. Pharmacol. 34 (1988) 605–613.
[7] E.A. Devane, L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, Isolation and structure of a brain constituent that binds to the cannabinoid receptor, Science 258 (1992) 1946–1949.
[8] A.H. Dickenson, A.F. Sullivan, Subcutaneous formalin-induced activity of dorsal horn neurones in the rat: differential response to an intrathecal opiate administered pre or post formalin, Pain 30 (1987) 349–360.
[9] D. Dubuisson, S.G. Dennis, The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats, Pain 4 (1977) 161–174.
[10] C.C. Felder, E.M. Briley, J. Axelrod, J.T. Simpson, K. Mackie, W.A. Devane, Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction, Proc. Natl. Acad. Sci. USA 90 (1993) 7656–7660.
[11] C.C. Felder, K.E. Joyce, E.M. Briley, J. Mansouri, K. Mackie, O. Blond, Y. Lai, A.L. Ma, R.L. Mitchell, Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors, Mol. Pharmacol. 48 (1995) 443–450.
[12] U. Herzberg, E. Eliav, G.J. Bennett, I.J. Kopin, The analgesic effects of R (+)-WIN 55, 212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain, Neurosci. Lett. 22 (1997) 157–160.
[13] A.G. Hohmann, W.J. Martin, K. Tsou, J.M. Walker, Inhibition of noxious stimulus-evoked activity of spinal cord dorsal horn neurons by the cannabinoid WIN 55, 212-2, Life Sci. 56 (1995) 2111–2118.
[14] A.G. Hohmann, K. Tsou, J.M. Walker, Electrophysiological evidence for cannabinoid-modulation of spinal nociceptive processing, Soc. Neurosci. Abstr. 22 (1996) 512.
[15] R.S. Landsman, T.H. Burkey, P. Consroe, W.R. Roeske, H.I. Yamamura, SR141716A is an inverse agonist at the human cannabinoid CB1 receptor, Eur. J. Pharmacol. 334 (1997) R1–R2.
[16] K. Mackie, B. Hille, Cannabinoids inhibit N-type calcium channels in neuroblastoma–glioma cells, Proc. Natl. Acad. Sci. USA 89 (1992) 3825–3829.
[17] A.B. Malmberg, T.L. Yaksh, Voltage-sensitive calcium channels in spinal nociceptive processing: blockade of N- and P-type channels inhibits formalin-induced nociception, J. Neurosci. 14 (1994) 4882–4890.
[18] W.J. Martin, A.G. Hohmann, J.M. Walker, 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. 16 (1996) 6601–6611.
[19] W.J. Martin, N.K. Lai, S.L. Patrick, K. Tsou, J.M. Walker, Antinociceptive actions of cannabinoids following intraventricular administration in rats, Brain Res. 629 (1993) 300–304.
[20] W.J. Martin, J.M. Walker, N.M. Fortin, D. Salehani, G. Prestwich, D.G. Deutsch, Evidence for a role of endogenous cannabinoids in nociceptive processing, Soc. Neurosci. Abstr. 21 (1995) 651.
[21] K.E. McCarson, B.D. Goldstein, Time course of the alteration in dorsal horn substance P levels following formalin: blockade by naloxone, Pain 41 (1990) 95–100.
[22] D.E. Moss, R.L. Johnson, Tonic analgesic effects of Δ^9-tetrahydrocannabinol as measured with the formalin test, Eur. J. Pharmacol. 61 (1980) 313–315.
[23] F. Petitet, B. Jeantaud, M. Capet, A. Doble, Interaction of brain cannabinoid receptors with guanine nucleotide binding protein: a radioligand binding study, Biochem. Pharmacol. 54 (1997) 1267–1270.
[24] J.D. Richardson, L. Aanonsen, K.M. Hargreaves, SR141716A, a cannabinoid receptor antagonist, produces hyperalgesia in untreated mice, Eur. J. Pharmacol. 319 (1997) R3–R4.
[25] J.D. Richardson, L.M. Aanonsen, K.M. Hargreaves, Hypoactivity of the spinal cannabinoid system results in NMDA-dependent hyperalgesia, J. Neurosci. 18 (1998) 451–457.
[26] M. Rinaldi-Carmona, F. Barth, M. Héaulme, R. Alonso, D. Shire, C. Congy, P. Soubrié, J. Brelière, G. Le Fur, Biochemical and pharmacological characterization of SR141716A, the first potent and selective brain cannabinoid receptor antagonist, Life Sci. 56 (1995) 1941–1947.
[27] M. Rinaldi-Carmona, F. Barth, M. Héaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maruani, G. Néliat, D. Caput, P. Ferrara, P. Soubrié, J. Brelière, G. Le Fur, SR141716A, a potent and selective antagonist of the brain cannabinoid receptor, Fed. Eur. Biochem. Soc. 350 (1994) 240–244.
[28] M. Rinaldi-Carmona, F. Pialot, C. Congy, E. Redon, F. Barth, A. Bachy, J. Brelière, P. Soubrié, G. Le Fur, Characterization and distribution of binding sites for [^{3}H]-SR141716A, a selective brain (CB1) cannabinoid receptor antagonist, in rodent brain, Life Sci. 58 (1996) 1239–1247.
[29] R.D. Sofia, S.D. Nalepa, J.J. Harakal, H.B. Vassar, Anti-edema and analgesic properties of Δ^9-tetrahydrocannabinol (THC), J. Pharmacol. Exp. Ther. 186 (1973) 646–655.
[30] J.P. Terranova, J.J. Storme, N. Lafon, A. Pério, M. Rinaldi-Carmona, G. Le Fur, P. Soubrié, Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR141716, Psychopharmacology 126 (1996) 165–172.
[31] K. Tsou, K. Lowitz, A.G. Hohmann, W.J. Martin, C.B. Hathaway, D.A. Bereiter, J.M. Walker, Suppression of noxious stimulus-evoked expression of fos protein-like immunoreactivity in rat spinal cord by a selective cannabinoid agonist, Neuroscience 70 (1996) 791–798.
[32] A.L. Vaccarino, P. Marek, J.C. Liebeskind, Stress-induced analgesia prevents the development of the tonic, late phase of pain produced by subcutaneous formalin, Brain Res. 572 (1992) 250–252.
[33] C.J. Woolf, A.E. King, Dynamic alterations in the cutaneous mechanoreceptive fields of dorsal horn neurons in the rat spinal cord, J. Neurosci. 10 (1990) 2717–2726.