Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 474 (2003) 269–272
www.elsevier.com/locate/ejphar

Short communication

Antitussive effect of WIN 55212-2, a cannabinoid receptor agonist

Kayo Morita, Junzo Kamei*

Department of Pathophysiology and Therapeutics, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-chome, Shinagawa-ku, Tokyo 142-8501, Japan

Received 7 April 2003; received in revised form 17 June 2003; accepted 24 June 2003

Abstract

Several lines of evidence indicate that the opioid and cannabinoid systems produce synergistic interactions. The present study examined the opioid receptors involved in the antitussive effect of WIN 55212-2 ((*R*)-(+)-[2,3-dihydro-5-methyl-3-[4-morpholinylmethyl]-pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl) methanone mesylate), a high-affinity cannabinoid receptor agonist, in mice. WIN 55212-2, at doses of 0.3–3 mg/kg ip, produced a dose-dependent antitussive effect. This antitussive effect of WIN 55212-2 was antagonized by pretreatment with either methysergide (3 mg/kg ip), a 5-HT receptor antagonist, or naloxone (1 mg/kg ip), an opioid receptor antagonist. Furthermore, pretreatment with *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR141716A, 3 mg/kg ip), a cannabinoid CB_1 receptor antagonist, also significantly reduced the antitussive effect of WIN 55212-2. Blockade of μ-opioid receptors by pretreatment with β-funaltrexamine (40 mg/kg sc) significantly reduced the antitussive effect of WIN 55212-2. However, pretreatment with nor-binaltorphimine (20 mg/kg sc), a κ-opioid receptor antagonist, did not affect the antitussive effect of WIN 55212-2. Pretreatment with naloxonazine (35 mg/kg sc), a μ_1-opioid receptor antagonist, also did not affect the antitussive effect of WIN 55212-2. These results indicate that the antitussive effect of WIN 55212-2 is mediated by the activation of cannabinoid CB_1 receptors and μ_2 (naloxonazine-insensitive)-opioid receptors, but not μ_1 (naloxonazine-sensitive)- or κ-opioid receptors.

Keywords: Antitussive effect; Cough; cannabinoid CB_1 receptor; WIN 55212-2; SR141716A

1. Introduction

Δ^9-Tetrahydrocannabinol has a wide range of central and peripheral actions, including hypothermia, antinociception, catalepsy, sedation, memory disruption and anxiolytic-like effects (see Martin, 2002). Two subtypes of cannabinoid receptors, CB_1 and CB_2, mediate these cannabinoid-induced effects. Cannabinoid CB_1 receptors are located centrally (Matsuda et al., 1990), whereas cannabinoid CB_2 receptors are expressed almost exclusively by peripheral immune cells (Munro et al., 1993). Cannabinoid receptor agonists produce several characteristic symptoms in rodents and share several actions with opioid receptor agonists. Indeed, Welch et al. suggested that the mechanisms by which the cannabinoids produce antinociception involve the modulation of endogenous opioid systems (Welch, 1993; Smith et al., 1994; Pugh et al., 1996). On the other hand, opioids, such as morphine and codeine, are well-known antitussive agents. We previously indicated that the antitussive effects of opioids are mediated predominantly by μ- and κ-opioid receptors. Although the pharmacological interaction between cannabinoid and opioid systems strongly suggests the possibility that cannabinoids have antitussive effects, the antitussive effects of cannabinoids have not yet been examined.

The availability of the synthetic cannabinoid agonist (+)-WIN 55212-2 has facilitated the characterization of cannabinoid receptor subtypes and their pharmacological profiles. WIN 55212-2 is highly selective for cannabinoid receptors and interacts negligibly with other neurotransmitter systems and ion channels (Martin et al., 1991; Compton et al., 1992). Several reports have indicated that WIN 55212-2 elicits hypothermia and antinociception in rodents via a cannabinoid CB_1 receptor mechanism (Compton et al., 1992; Fan et al., 1994; Fox et al., 2001).

The aim of the present study was to investigate the antitussive effect of the cannabinoid receptor agonist WIN 55212-2, and to explore the possible involvement of the endogenous opioid system in the antitussive effect of WIN 55212-2.

* Corresponding author. Tel.: +81-3-5498-5030; fax: +81-3-5498-5029.
E-mail address: kamei@hoshi.ac.jp (J. Kamei).

0014-2999/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0014-2999(03)02009-0

2. Materials and methods

2.1. Animals

Male ICR mice (6 weeks old; Tokyo Laboratory Animals Science, Tokyo, Japan), weighing about 30 g, were used. They had free access to food and water in an animal room, which was maintained at 24 ± 1 °C with a 12-h light–dark cycle. This study was carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals as adopted by the committee on the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Antitussive assay

The cough reflex was induced as previously described (Kamei et al., 1993a,b). Briefly, animals were exposed to a nebulized solution of capsaicin (30 μmol/l) under conscious and identical conditions using a body plethysmograph. The coughs produced during a 3-min exposure period were counted. Capsaicin was dissolved to a concentration of 30 mg/ml in a 10% ethanol and 10% Tween 80 saline solution. The solution was further diluted with saline. The mice were exposed for 3 min to capsaicin 30 min before the injection of drugs to determine the frequency of control coughs. The animals were again exposed to capsaicin aerosol for 30 min after intracerebroventricular administration of drugs. Each animal was used only once. The number of coughs produced after drug injection (Ct) was compared with the number of control coughs (Cc). The antitussive effect was expressed as the % inhibition of the number of coughs= (Cc − Ct)/Cc × 100.

2.3. Drugs

(+)-WIN 55212-2 ((*R*)-(+)-[2,3-dihydro-5-methyl-3-[4-morpholinylmethyl]-pyrrolo-[1,2,3-de]1,4-benzoxazin-6-yl](1-naphthyl) methanone mesylate), β-funaltrexamine hydrochloride, a μ_1/μ_2-opioid receptor antagonist, naloxonazine dihydrochloride, a selective μ_1-opioid receptor antagonist, nor-binaltorphimine dihydrochloride, a selective κ-opioid receptor antagonist and methysergide maleate, a serotonin receptor antagonist, were purchased from Sigma-Aldrich. SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride), a selective cannabinoid CB_1 receptor antagonist, was generously supplied by Sanofi-Synthelabo Recherche (Montpellier, France). WIN 55212-2 was dissolved in 2.5% dimethyl sulfoxide (DMSO). All other drugs were dissolved in saline. WIN 55212-2 was injected intraperitoneally 30 min before the antitussive assay. β-Funaltrexamine (40 mg/kg sc) and naloxonazine (35 mg/kg sc) were injected 24 h before testing. Nor-binaltorphimine (20 mg/kg sc) was injected 3 h before testing. Methysergide or SR141716A was injected intraperitoneally 15 min before the injection of WIN 55212-2.

2.4. Data analysis

Data are expressed as means ± S.E. The statistical significance of differences was assessed by the Mann–Whitney *U*-test to evaluate the antitussive effect. A level of probability of 0.05 or less was considered significant.

3. Results

3.1. Effects of intraperitoneal administration of WIN 55212-2 on capsaicin-induced coughs

WIN 55212-2, at doses of 0.3, 1 and 3 mg/kg ip, dose-dependently inhibited the number of capsaicin-induced coughs when the antitussive effect was examined 30 min after administration (Fig. 1).

3.2. Effects of SR141716A and methysergide on the antitussive effect of WIN 55212-2

As shown in Fig. 2, pretreatment with SR141716A (3 mg/kg ip), a selective cannabinoid CB_1 receptor antagonist, significantly reduced the antinociceptive effect of WIN 55212-2. Furthermore, the antitussive effect of WIN 55212-2 was also significantly reduced by pretreatment with methysergide (3 mg/kg ip), a selective serotonin receptor antagonist (Fig. 2A).

3.3. Effects of opioid receptor antagonists on the antitussive effect of WIN 55212-2

Pretreatment with β-funaltrexamine (40 mg/kg ip), a μ_1/μ_2-opioid receptor antagonist, significantly reduced the antitussive effect of WIN 55212-2 (Fig. 2B). However, as shown in Fig. 2B, the antitussive effect of WIN 55212-2

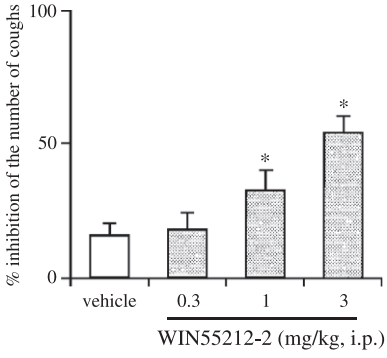

Fig. 1. Dose–response relationship of the antitussive effect of WIN 55212-2 in mice. The antitussive effects of WIN 55212-2 were assessed 30 min after intraperitoneal administration of the drug. The effects of WIN 55212-2 on the number of capsaicin-induced coughs were determined. Each column represents the mean with S.E. ($n = 10$). *$P < 0.05$ vs. vehicle-treated group.

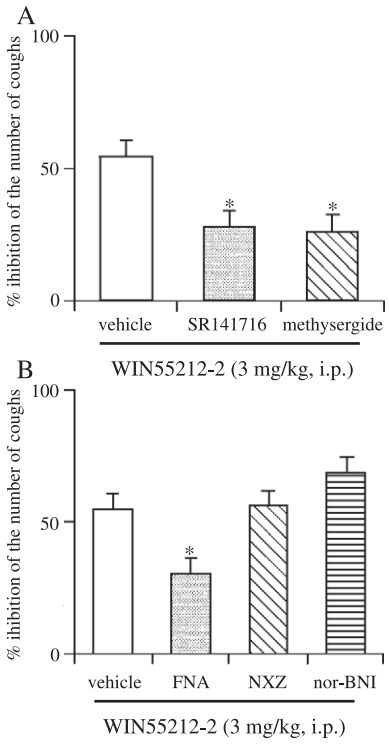

Fig. 2. Effects of SR141716A or methysergide (A), and opioid receptor antagonists (B) on the antitussive effect of WIN 55212-2. SR141716A (3 mg/kg) and methysergide (3 mg/kg) were each injected intraperitoneally 30 min before the administration of WIN 55212-2. β-Funaltrexamine (40 mg/kg sc) and naloxonazine (35 mg/kg sc) were injected 24 h before testing. Nor-binaltorphimine (20 mg/kg sc) was injected 3 h before testing. The antitussive effect of WIN 55212-2 (3 mg/kg) was assessed 30 min after intraperitoneal administration. Each column represents the mean with S.E. ($n = 10$). *$P < 0.05$ vs. respective vehicle-pretreated group.

was not antagonized by pretreatment with naloxonazine, a selective μ_1-opioid receptor antagonist. Moreover, pretreatment with nor-binaltorphimine (10 mg/kg ip), a κ-opioid receptor antagonist, also had no significant effect on the antitussive effect of WIN 55212-2 (Fig. 2B).

4. Discussion

In the present study, we observed that systemic administration of the cannabinoid receptor agonist WIN 55212-2 produced a dose-dependent antitussive effect in mice. The antitussive effect of WIN 55212-2 was significantly reduced by pretreatment with the cannabinoid CB_1 receptor antagonist SR141716A. These results indicate that the cannabinoid CB_1 receptor subtype play an important role in mediating the antitussive effect of this cannabinoid receptor agonist.

We also demonstrated that methysergide significantly antagonized the antitussive effect of WIN 55212-2. We previously demonstrated that a reduction in the level of serotonin (5-HT) in the whole brain decreased the potency of antitussive drugs that acted at the central nervous system, but not peripherally (Kamei et al., 1987). Neonatal treatment with 5,7-dihydroxytryptamine, which was sufficient to reduce whole brain levels of 5-HT to 19% of control levels, resulted in supersensitivity to the cough-depressant effect of dihydrocodeine (Kamei et al., 1988). Furthermore, the potentiation of the antitussive effect of dihydrocodeine observed in 5,7-dihydroxytryptamine-treated rats was abolished by pretreatment with methysergide, a 5-HT receptor antagonist. Therefore, the marked increase in the antitussive effect of dihydrocodeine might have been due to changes in the sensitivity of 5-HT receptors. Thus, we proposed that 5-HT receptors may play an important role in the cough-depressant activities of centrally acting, but not peripheral acting, antitussive drugs (Kamei et al., 1987, 1988). These results and those in the present study suggest that the antitussive effect of WIN 55212-2 may depend mainly on central mechanisms (modulation of serotonergic systems).

In the present study, we also observed that the antitussive effect of WIN 55212-2 was significantly reduced by pretreatment with the μ_1/μ_2-opioid receptor antagonist β-funaltrexamine, but not naloxonazine, a selective μ_1-opioid receptor antagonist. Furthermore, the κ-opioid receptor antagonist nor-binaltorphimine had no significant effect on the antitussive effect of WIN 55212-2. These results indicate that endogenous naloxonazine-resistant μ-opioid receptors, i.e., μ_2-opioid receptors, may be involved in the antitussive effect of WIN 55212-2. These results are consistent with our previous findings that naloxonazine-resistant μ-opioid receptors, i.e., μ_2-opioid receptors, but not μ_1-opioid receptors, play an important role in opioid receptor-mediated antitussive effects (Kamei et al., 1993a,b).

In conclusion, the antitussive effect of WIN 55212-2 is mediated by the activation of cannabinoid CB_1 receptors. Furthermore, this antitussive effect may be attributable to an increase in the production and/or release of endogenous opioid ligands for naloxonazine-insensitive opioid receptors (probably μ_2-opioid receptors), but not μ_1 (naloxonazine-sensitive)- or κ-opioid receptors.

Acknowledgements

We are grateful to Sanofi-Synthelabo Recherche for the gift of SR141716A. We also thank Ms. Y. Irie for her excellent technical assistance.

References

Compton, D.R., Gold, L.H., Ward, S.J., Balster, R.L., Martin, B.R., 1992. Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from delta 9-tetrahydrocannabinol. J. Pharmacol. Exp. Ther. 263, 1118–1126.

Fan, F., Compton, D.R., Ward, S., Melvin, L., Martin, B.R., 1994. Development of cross-tolerance between delta 9-tetrahydrocannabinol, CP 55,940 and WIN 55,212. J. Pharmacol. Exp. Ther. 271, 1383–1390.

Fox, A., Kesingland, A., Gentry, C., McNair, K., Patel, S., Urban, L.,

James, I., 2001. The role of central and peripheral Cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. Pain 92, 91–100.

Kamei, J., Ogawa, M., Kasuya, Y., 1987. Monoamines and the mechanisms of action of antitussive drugs in rats. Arch. Int. Pharmacodyn. Ther. 290, 117–127.

Kamei, J., Ogawa, M., Kasuya, Y., 1988. Supersensitivity of 5, 7-dihydroxytryptamine-treated rats to the respiratory depressant and antitussive effects of dihydrocodeine. Eur. J. Pharmacol. 153, 305–308.

Kamei, J., Iwamoto, Y., Kawashima, N., Suzuki, T., Nagase, H., Misawa, M., Kasuya, Y., 1993a. Possible involvement of μ_2-mediated mechanisms in μ-mediated antitussive activity in the mouse. Neurosci. Lett. 149, 169–172.

Kamei, J., Iwamoto, Y., Suzuki, T., Misawa, M., Nagase, H., Kasuya, Y., 1993b. The role of the μ_2-opioid receptor in the antitussive effect of morphine in μ_1-opioid receptor-deficient CXBK mice. Eur. J. Pharmacol. 240, 99–101.

Martin, B.R., 2002. Identification of the endogenous cannabinoid system through integrative pharmacological approaches. J. Pharmacol. Exp. Ther. 301, 790–796.

Martin, B.R., Compton, D.R., Thomas, B.F., Prescott, W.R., Little, P.J., Razdan, R.K., Johnson, M.R., Melvin, L.S., Mechoulam, R., Ward, S.J., 1991. Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. Pharmacol. Biochem. Behav. 140, 471–478.

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 (Lond.) 346, 561–564.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature (Lond.) 365, 61–66.

Pugh, G., Smith, P.B., Dombrowski, D.S., Welch, S.P., 1996. The role of endogenous opioids in enhancing the antinociception produced by the combination of Δ^9-tetrahydrocannabinol and morphine in the spinal cord. J. Pharmacol. Exp. Ther. 279, 608–616.

Smith, P.B., Welch, S.P., Martin, B.R., 1994. Interactions between Δ^9-tetrahydrocannabinol and *kappa* opioids in mice. J. Pharmacol. Exp. Ther. 268, 1382–1387.

Welch, S.P., 1993. Modulation of cannabinoid-induced antinociception by nor-binaltorphimine but not ICI 174,864 in mice. J. Pharmacol. Exp. Ther. 265, 633–640.