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Research report

Endogenous cannabinoid, 2-arachidonoylglycerol, attenuates naloxone-precipitated withdrawal signs in morphine-dependent mice

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Abstract

In the present study, we examined the effects of endogenous ligand 2-arachidonoylglycerol (2-AG) on naloxone-precipitated withdrawal in morphine-dependent mice, in comparison with that of two cannabinoid agonists, an ingredient of *Cannabis sativa* Δ^8-tetrahydrocannabinol (Δ^8-THC) and the synthetic cannabinoid CB1 receptor agonist HU-210. 2-AG at a dose of 10 μg per mouse (i.c.v.) significantl inhibited both jumping and forepaw tremor as signs of withdrawal following naloxone challenge in morphine-dependent mice. Furthermore, both Δ^8-THC and HU-210 significantl attenuated these symptoms of withdrawal in morphine-dependent mice. Therefore, it is suggested that inactivation of the endogenous cannabinoid system is related to the induction of withdrawal syndrome in morphine-dependent mice. Moreover, hyperlocomotor activity in morphine-dependent mice was markedly increased by Δ^8-THC 10 mg/kg, which had no effect in naive mice. This findin suggested that in morphine dependence, upregulation of cannabinoid CB1 receptors occurred. Non-psychoactive CB1 receptor agonists or accelerators of endocannabinoid synthesis may be potential as therapeutic drugs for opiate withdrawal symptoms.

Theme: Neural basis of behavior

Topic: Psychopharmacological agents

Keywords: Endogenous cannabinoid; 2-Arachidnoylglycerol; Morphine; Opioid; Withdrawal sign

1. Introduction

Ever since Δ^9-tetrahydrocannabinol (Δ^9-THC) was isolated as the major psychoactive ingredient of *Cannabis sativa* [6], many hundreds of analogs of Δ^9-THC and of other natural and unnatural cannabinoids have been synthesized in the laboratory [19,20,31,33]. Cannabinoid CB1 receptor (CB1 receptor) was identifie in the brain [11], and cDNA encoding CB1 receptor was cloned from rat cerebellum [16]. Recently, anandamide (*N*-arachidonoylethanolamine) was isolated from porcine brain as endogenous cannabinoid (endocannabinoid) [3]. Moreover, 2-arachidonoylglycerol (2-AG) was identifie as a second endogenous ligand for CB1 receptors from rat brain [26]. 2-AG, which possesses the specifi binding site for CB1 receptors, has selective and effective synthetic pathways in the brain, especially in synaptosomes [26]. Also, the amount of 2-AG (0.23 nmol/g tissue) in rat brain is at least 50 times that of anandamide [28,29]. These finding suggest that 2-AG plays some physiological role as the major endocannabinoid and is suitable as endogenous ligand for CB1 receptors in the central nervous system [27].

Cannabinoids have psychotropic functions including the control of pain, cognition, emotional response and motor activity [4,18,34]. Furthermore, activation of CB1 receptor induces changes in the reward system, i.e., production of

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pleasure and/or euphoria [7,35]. On the other hand, the rewarding properties of cannabinoids are blocked by the opioid receptor antagonist naloxone [8], i.e. there are physiological links between the endocannabinoid and the opioid systems in the brain. In drug dependence, abnormal behavior like opioid-withdrawal signs were induced by naloxone in rats chronically treated with Δ^9-THC [13]. Conversely, withdrawal signs were precipitated by the CB1 receptor antagonist SR141716A in morphine-dependent rats [23]. These finding suggest that endocannabinoids have a role in opioid dependence, and the endocannabinoid system and opioid system modulate each other.

In this study, we investigated the role of the endocannabinoid system in morphine withdrawal using endocannabinoid 2-AG, in comparison with Δ^8-THC, an isomer of Δ^9-THC and ingredient of *C. sativa*, and the synthetic CB1 receptor agonist HU-210. It appears likely that CB1 receptors are activated during chronic morphine treatment. Therefore, further study was performed to examine whether or not CB1 receptors are functionally activated during the development of morphine dependence by utilizing Δ^8-THC-induced hyperlocomotion as an index.

2. Materials and methods

2.1. Animals

Male ddY mice (Nippon SLC Co., Hamamatsu, Japan) weighing 25–35 g were used for these studies. All animals were housed under constant temperature (23±2°C) and a 12:12-h light/dark cycle (lights on 07:00 h) with food and water available ad libitum.

2.2. Drugs

Morphine hydrochloride (Takeda Chemical Industries) and naloxone hydrochloride (Sigma Chemical Co.) were dissolved in saline. 2-AG was donated by Professor T. Sugiura, Department of Hygienic Chemistry and Nutrition, Faculty of Pharmaceutical Sciences, Teikyo University [26]. Δ^8-THC was donated by Professor Y. Shoyama, Department of Medicinal Resources Regulation, Faculty of Pharmaceutical Sciences, Kyushu University [25]. (−)-11-Hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU-210) was purchased from Tocris. 2-AG was dissolved in DMSO and diluted with saline (vehicle contained 5% DMSO at the most). Δ^8-THC and HU-210 were emulsifie in a 1% Tween-80 solution.

2.3. Development of morphine dependence

Morphine hydrochloride was administered s.c. twice daily in a volume of 1.0 ml/100 g body weight to mice. The dose of morphine was increased progressively from 8 to 45 mg/kg over a period of 5 days according to the method of Maldonado et al. [15].

2.4. Observation of withdrawal syndrome

The withdrawal syndrome was precipitated by administration of naloxone after the fina administration of morphine (45 mg/kg, i.p.). Immediately after naloxone administration, the animals were placed in an observant cylinder (15 cm in diameter and 50 cm in height). Jumping and forepaw tremor were used as experimental indices of withdrawal syndrome and counted during a 20-min period.

2.5. Measurement of locomotor activity

Locomotor activity was measured with an open-fiel and behavioral tracking analysis system (70 cm in diameter and 50 cm in height, video camera installed 2.5 m above apparatus; Neuroscience, Tokyo) for 10 min. The animals were placed in the center of the open fiel and then ambulation was measured 30 min after administration of Δ^8-THC. Δ^8-THC was administered 1.5 h after the fina administration of morphine (45 mg/kg, i.p.) to naive or morphine-dependent mice.

2.6. Drug treatment

2-AG was administered i.c.v. according to the method of Haley and McCormick [9] in a volume of 10 μl/mouse 10 min under slight anesthesia with halothane. Δ^8-THC and HU-210 were administered i.p. in a volume of 1.0 ml/100 g body weight to mice 30 min before the test.

2.7. Statistical analysis

Experimental data were presented as mean±S.E.M. of the numbers of withdrawal signs and ambulations. The data were evaluated by the Mann–Whitney *U*-test.

3. Results

3.1. Effects of 2-AG on naloxone-precipitated withdrawal in morphine-dependent mice

Jumping and forepaw tremor were dose-dependently precipitated by naloxone in morphine-dependent mice (Table 1), and the severity of these signs of withdrawal declined with time after the last administration of morphine (Table 2). At 2 h after the last administration of morphine, naloxone 3.2 mg/kg (i.p.) remarkably precipitated jumping (24.44±7.06) and forepaw tremor (24.56±3.79) (Table 1).

2-AG attenuated naloxone-precipitated withdrawal in morphine-dependent mice. Intracerebroventricular administration of 2-AG (10 μg/mouse) significantl at-

Table 1
Dose effects of naloxone on withdrawal in morphine-dependent mice

Dose of naloxone (mg/kg, s.c.)	Incidence	
	Jumping	Forepaw tremor
Saline	0.00±0.00	1.00±0.55
1.0	13.75±5.75	15.13±3.39*
3.2	24.44±7.06*	24.56±3.79**

The withdrawal syndrome was precipitated by administration of naloxone 2 h after the fina administration of morphine (45 mg/kg, i.p.). Immediately after naloxone administration, the animals were placed in an observant cylinder. Jumping and forepaw tremor used as experimental indices of withdrawal syndrome were counted during a 20-min period. Values are mean±S.E. *$P<0.05$, **$P<0.01$ compared with the saline-injected group (Mann–Whitney U-test).

Table 2
Time course of expression of naloxone-precipitated withdrawal in morphine-dependent mice

Time of naloxone injection after the last administration of morphine (h)	Incidence	
	Jumping	Forepaw tremor
0	0.00±0.00	6.63±1.77
2	24.44±7.06	24.56±3.79
8	4.50±3.84	10.75±2.17
24	3.83±3.83	11.83±2.23

The withdrawal syndrome was precipitated by administration of 3.2 mg/kg naloxone 0, 2, 8 or 24 h after the fina administration of morphine (45 mg/kg, i.p.). Immediately after naloxone administration, the animals were placed in an observant cylinder. Jumping and forepaw tremor used as experimental indices of withdrawal syndrome were counted during a 20-min period. Values are mean±S.E.

tenuated the increase in jumping (33.46±5.81 to 18.85±3.95, $P<0.01$) and forepaw tremor (23.15±3.32 to 9.88±2.85, $P<0.01$) in comparison with that in the vehicle-treated morphine-dependent group (Fig. 1).

3.2. Effects of Δ^8-THC and HU-210 on naloxone-precipitated withdrawal in morphine-dependent mice

Fig. 2 shows the effects of Δ^8-THC and HU-210 on naloxone-precipitated withdrawal. Administration of Δ^8-THC 10 mg/kg (i.p.) also significantl attenuated the increase in jumping (29.55±7.26 to 4.38±2.94, $P<0.05$) and forepaw tremor (26.64±4.23 to 12.25±4.85, $P<0.05$) in morphine-dependent mice. HU-210 at a low dose of 0.1 mg/kg (i.p.) also produced significan inhibitory effects on jumping (35.50±8.25 to 8.00±2.62, $P<0.05$) and forepaw tremor (34.00±8.43 to 7.33±2.88, $P<0.01$).

3.3. Effects of Δ^8-THC on locomotor activity in morphine-dependent mice

Δ^8-THC at doses of 3.2 and 10 mg/kg (i.p.) produced no significan changes in ambulation in naive mice (2151.5±419.5 and 2276.6±221.2 cm/10 min, respectively), but Δ^8-THC 32 mg/kg (i.p.) significantl increased ambulation (3880.2±636.3 cm/10 min, $P<0.05$). Δ^8-THC at 10 mg/kg by itself produced no significan changes in the increase of ambulation induced by single morphine 45 mg/kg in comparison to saline treatment (Fig. 3). However, Δ^8-THC at the same dose markedly increased the ambulation in morphine-dependent mice in comparison to saline (Fig. 3).

4. Discussion

Our results demonstrated that the endogenous ligand 2-AG as well as an ingredient of *Cannabis* Δ^8-THC and the synthetic CB1 receptor agonist HU-210 attenuate morphine-withdrawal signs in morphine-dependent mice like Δ^9-THC and anandamide [10,32].

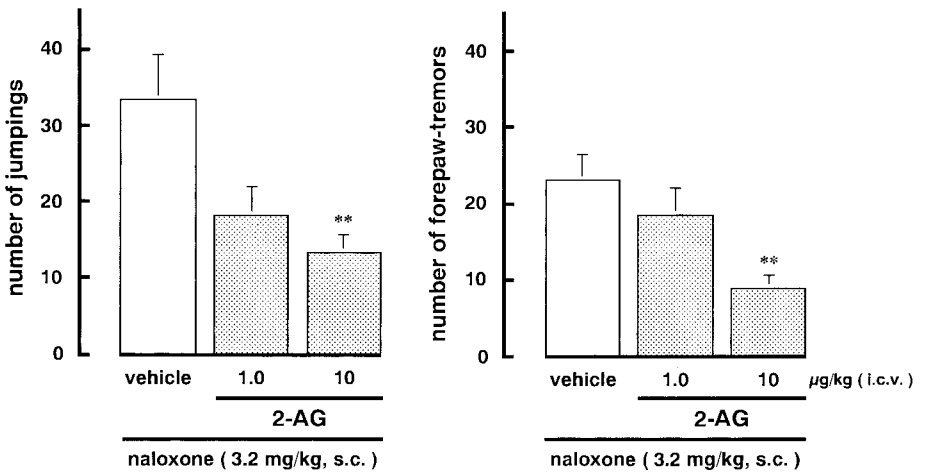

Fig. 1. Effects of 2-arachidonoylglycerol (2-AG) on naloxone-precipitated withdrawal in morphine-dependent mice. Each point represents the mean with vertical bars indicating the S.E. **$P<0.01$ compared with the vehicle-treated group (Mann–Whitney U-test).

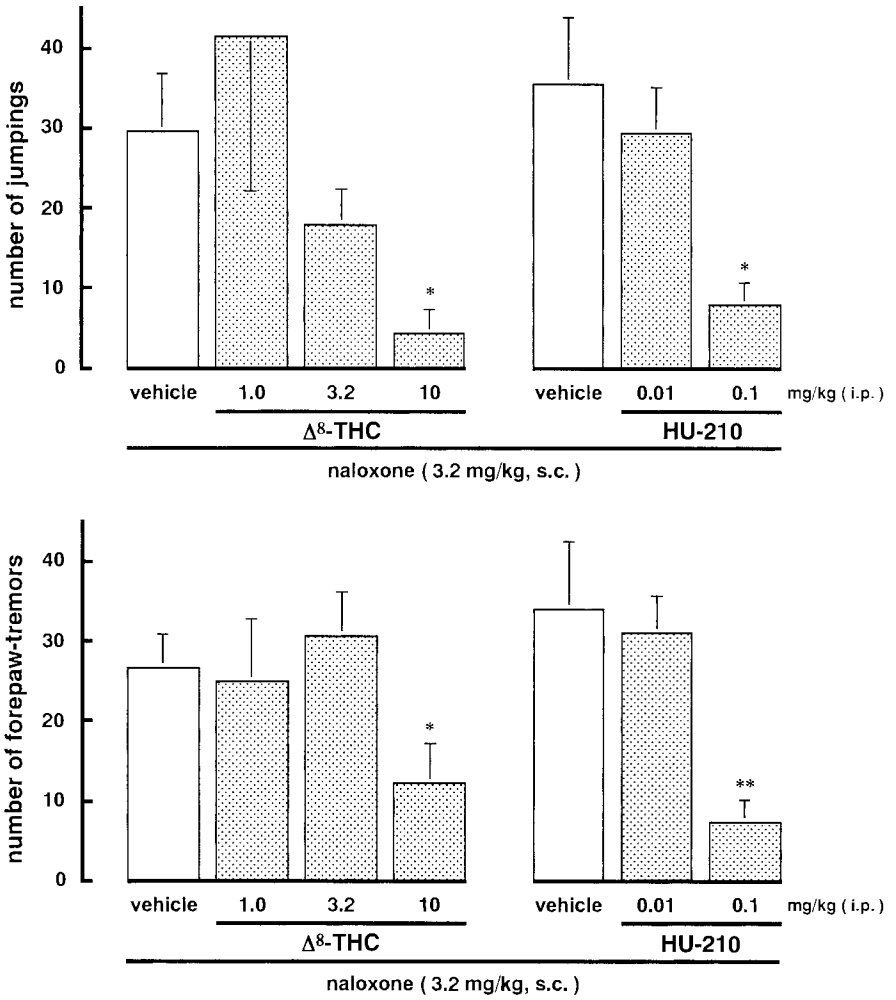

Fig. 2. Effects of Δ^8-THC and HU-210 on naloxone-precipitated withdrawal in morphine-dependent mice. Each point represents the mean with vertical bars indicating the S.E. $*P<0.05$ and $**P<0.01$ compared with the vehicle-treated group (Mann–Whitney *U*-test).

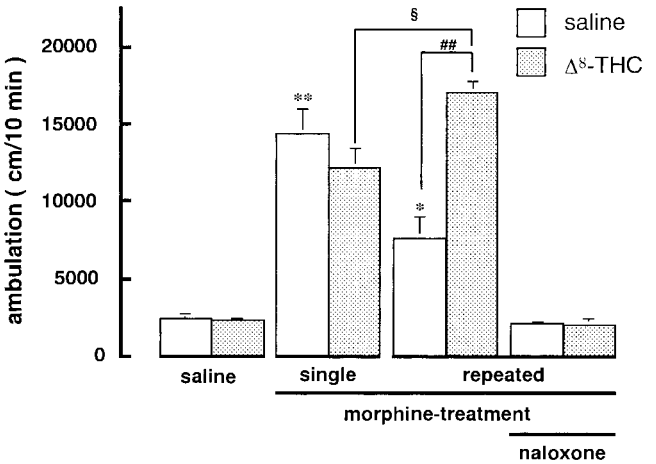

Fig. 3. Effects of Δ^8-THC (10 mg/kg) on locomotor activity in morphine-dependent and single morphine-treated mice. Each point represents the mean with vertical bars indicating the S.E. $*P<0.05$ and $**P<0.01$ compared with the saline-treated group. $^{\#\#}P<0.01$ compared with the saline-challenged group. $^{\S}P<0.05$ compared with the single Δ^8-THC-challenged group (Mann–Whitney *U*-test).

Intracerebroventricular administration of 2-AG attenuated morphine withdrawal, indicating that the activation of cannabinoid receptors in the brain prevents the expression of symptoms of withdrawal in morphine-dependent mice. HU-210 inhibited the expression of morphine withdrawal signs at a lower dose than Δ^8-THC, the difference between the groups seemingly due to the potency of HU-210 [12].

It has been reported that the CB1 receptor antagonist SR141716A-precipitated withdrawal signs in morphine-dependent rats [23]. Moreover, in the CB1 receptor knockout mice, the severity of withdrawal signs after chronic morphine treatment was markedly reduced [14]. According to these findings the endocannabinoid system may activate neuronal pathways involved in the development of morphine dependence.

It is reported that cannabinoid receptor agonists at low dose cause hyperlocomotion, but at high dose produce sedation and catalepsy-like behavior in rodents [1,2,4]. Hyperlocomotion induced by a cannabinoid receptor agon-

ist was considered to cause the dopamine release in nucleus accumbens [5,21,22]. Actually, both cannabinoids and opioids activate the release of dopamine in the nucleus accumbens [5] and an increase in extracellular dopamine in the nucleus accumbens produced by morphine was not observed in CB1 receptor knockout mice [17]. Therefore, the marked increase in locomotion induced by Δ^8-THC in morphine-dependent mice is due to the sharp increase of dopamine release via activated CB1 receptors following repeated morphine treatment.

Judging from the result that a CB1 receptor agonist attenuates the signs of morphine withdrawal, it is suggested that the appearance of the withdrawal syndrome in morphine-dependent mice is based on the inactivation of CB1 receptors and/or the inhibition of the release/synthesis of endocannabinoids. Additionally, it has been reported that CB1 receptor- and opioid μ receptor-mRNAs are co-localized in brain areas such as the nucleus accumbens, septum and the central amygdaloid nucleus, which are relevant to opiate withdrawal [23,30] and chronic morphine treatment significantl increased the expression of CB1 receptor mRNA and binding site in caudate-putamen [24]. These finding also support that some dramatic changes to the endocannabinoid system in the brain occurred in morphine-dependent animals. Moreover, considering the modulation between the endocannabinoid and opioid systems, it seems that the former is activated in opioid-dependent animals, and conversely, inactivated during withdrawal.

In conclusion, our results suggested that the functional upregulation of cannabinoid receptors that occurred during morphine dependence and withdrawal in morphine-dependent mice is due to the inactivation of CB1 receptors and/or the inhibition of the release/synthesis of endogenous cannabinoids. Furthermore, non-psychoactive CB1 receptor agonists or accelerators of endocannabinoid synthesis may have potential as therapeutic drugs to ameliorate opiate withdrawal symptoms.

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