

Rapid communication

Attenuation of spontaneous opiate withdrawal in mice by the anandamide transport inhibitor AM404

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Abstract

The endogenous cannabinoid, anandamide, has been shown to attenuate naloxone-precipitated opiate withdrawal in rodents. Here we show that the spontaneous, but not the naloxone-precipitated withdrawal syndrome in morphine-dependent mice is attenuated by the inhibitor of carrier-mediated anandamide transport *N*-(4-hydroxyphenyl) arachidonylethanolamide (AM404) (2 and 10 mg/kg, i.p.). These results suggest that spontaneous but not opioid antagonist-precipitated withdrawal is associated with dynamic changes in endogenous cannabinoid signaling. © 2002 Elsevier Science B.V. All rights reserved.

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Opioid and cannabinoid systems cooperate in the regulation of physiological processes such as nociception and reward. Cannabinoid CB₁ receptors and μ -opioid receptors exhibit overlapping neuroanatomical distribution, convergent neurochemical mechanisms and similar functional neurobiological properties (Ledent et al., 1999; Manzanares et al., 1999; Navarro et al., 2001; Tanda et al., 1997). Theoretically, these functional interactions might be useful for the development of therapeutic strategies for several neuropsychiatric disorders, including opiate addiction. This hypothesis was recently supported by results of studies with the endogenous cannabinoid, anandamide (Vela et al., 1995), or the selective cannabinoid CB₁ receptor antagonist, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide, SR141716A (Ledent et al., 1999; Navarro et al., 2001), in animals models of opiate self-administration and dependence. These studies demonstrated that stimulation of cannabinoid CB₁ receptors attenuates opiate withdrawal, whereas cannabinoid CB₁

receptor blockade induces an opiate withdrawal syndrome in morphine-dependent rodents (Navarro et al., 1998; Vela et al., 1995). These studies also showed that blockade of CB₁ receptors results in a reduction of opiate self-administration in mice and rats.

Opiate dependence is associated with changes in cannabinoid CB₁ receptors in reward-related brain areas (Manzanares et al., 1999; Navarro et al., 2001). However little is known about the effects of chronic opiate dependence and withdrawal on anandamide release in the brain. Anandamide is a lipid mediator that is released from a membrane phospholipid precursor by stimulatory stimuli such as membrane depolarization or receptor activation. After its release, anandamide is eliminated by uptake into neurons and astrocytes, followed by enzymatic hydrolysis. Transport inhibitors may be used as pharmacological tools to determine the degree of anandamide release after pharmacological challenges—i.e. administration of dopaminergic agonists (Giuffrida et al., 1999)—by monitoring shifts in behavioral performance in the presence or absence of the carrier inhibitor (Beltramo et al., 2000).

In the present study we addressed the potential alterations in anandamide release associated with morphine withdrawal by characterizing the effects of the anandamide transport inhibitor, *N*-(4-hydroxyphenyl) arachidonylethanolamide

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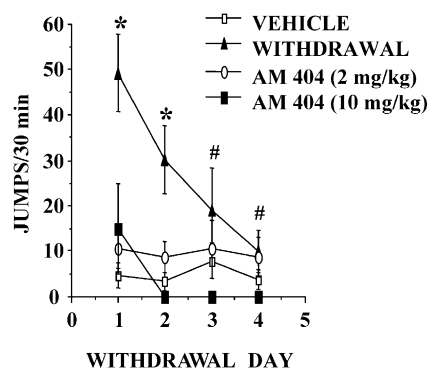


Fig. 1. Acute intraperitoneal injection of the anandamide transport inhibitor, AM404, reduced escape attempts (jumping behavior) by morphine-dependent mice during the first days after abrupt cessation of morphine administration. Values from independent experiments are means \pm S.E.M. of 8–10 determinations per group. * $P < 0.01$, “withdrawal” mice versus chronic vehicle or AM404-treated groups; # $P < 0.05$, “withdrawal” mice versus AM404 (10 mg/kg)-treated group, Student–Newman–Keul’s test.

(AM404, 2 and 10 mg/kg, i.p.), on either naloxone (1 mg/kg)-precipitated or spontaneous opiate withdrawal in mice. Naive C57/B6 mice from our colony were rendered morphine-dependent by intraperitoneal injection of increasing doses of morphine twice daily for 5 days (from 20 to 100 mg/kg). Control animals received daily injections of saline. On the sixth day, the animals were divided into two groups for naloxone-precipitated withdrawal and spontaneous withdrawal studies, respectively.

The first group received intraperitoneal injections of either vehicle (saline/dimethyl sulfoxide, 40:60, vol/vol) or AM404 (2 and 10 mg/kg) 30 min prior to an acute injection of naloxone (1 mg/kg, i.p.). Withdrawal signs were scored using the Gellert and Holtzman rating scale as described (Navarro et al., 2001). The administration of AM404 did not affect signs and symptoms of naloxone-precipitated opiate withdrawal such as escape attempts (jumps), wet-dog shakes, abdominal constrictions, weight loss, swallowing movements or piloerection. Jumping behavior during a 30-min period was the following: naloxone (1 mg/kg), 42 ± 4.8 ; AM404 (2 mg/kg) plus naloxone, 43.7 ± 2.8 ; AM404 (10 mg/kg) plus naloxone, 33.5 ± 6 ; $n = 10$ –12 animals per group).

The second group was treated in the same way (vehicle or AM404 30 min prior to withdrawal rating) but was studied 1, 2, 3 or 4 days after cessation of morphine administration. Spontaneous withdrawal mice exhibited a less intense set of withdrawal-associated behaviors, with the exception of escape reactions (jumps) that peaked 24 h after the last morphine injection, declining thereafter (Fig. 1). AM404 administration reduced in a dose-dependent manner the number of jump attempts: the 2 mg/kg dose reduced escape reactions during the first 48 h of withdrawal, whereas the 10 mg/kg dose of AM404 completely blocked escape reactions. Additionally, AM404 (10 mg/kg but not 2 mg/kg) reduced in a dose-dependent manner locomotor activity during withdrawal along the 4 days of the study, as measured in an open field (data not shown).

The lack of effect of AM404 on naloxone-precipitated withdrawal suggests that, after the induction of opiate dependence or during precipitated withdrawal, no anandamide is released in opiate dependence-related areas. The suggested decrease in anandamide release may underlie the increased expression of striatal cannabinoid CB₁ receptors in opiate-dependent rodents described recently (Navarro et al., 2001), reflecting the induction of compensatory mechanisms such as upregulation of the cannabinoid CB₁ receptor. In agreement with this hypothesis, the administration of low doses of anandamide (5 mg/kg) attenuated naloxone-precipitated opiate withdrawal (Vela et al., 1995).

Additionally, progressive neuroadaptation associated with spontaneous withdrawal will lead to normalization of anandamide release, as reflected by the attenuation of spontaneous withdrawal by the administration of the carrier inhibitor, AM404, which by curtailing anandamide uptake may enhance the inhibitory actions of this endogenous cannabinoid on locomotor activity and anxiety.

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