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Lack of morphine-induced dopamine release in the nucleus accumbens of cannabinoid CB_1 receptor knockout mice

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Abstract

Morphine (10 and 20 mg/kg, s.c.) does not modify dopamine release in the nucleus accumbens of cannabinoid CB_1 knock-out mice under conditions where it dose-dependently stimulates the release of dopamine in the corresponding wild-type mice. These results demonstrate that cannabinoid CB_1 receptors, regulate mesolimbic dopaminergic transmission in brain areas known to be involved in the reinforcing effects of morphine.

Keywords: Cannabinoid CB_1 knock-out mice; Limbic dopamine; Morphine

Pharmacological evidence suggests that there are important interactions between the brain opioid and cannabinoid systems. The principal interactions are found in antinociception (Cichewicz et al., 1999) and drug reinforcement (Martellotta et al., 1998), and point to complex neuronal links between opioid and cannabinoid neurotransmission. Recently, Ledent et al. (1999), have generated cannabinoid CB_1 receptor knock-out (KO) mice that do not respond to delta-9-tetrahydrocannabinol and, interestingly, also show a reduction in the rewarding properties of morphine. Since the reinforcing properties of morphine appear to be mediated by an activation of the mesolimbic dopamine system (Di Chiara and Imperato, 1987; Koob, 1992), we have used cannabinoid CB_1 KO mice to investigate the role of cannabinoid CB_1 receptors in the ability of morphine to stimulate limbic dopamine release.

Cannabinoid CB_1 KO mice ($CB_1^{-/-}$) and wild-type mice ($CB_1^{+/+}$), 15–17 weeks old (Transgenic Alliance, France) were generated as described previously (Ledent et al., 1999). Animals were housed five per cage with free access to food and water. Room temperature was 22 ± 2°C and the light–dark cycle was 12:12 h (lights on at 0600 h).

Transversal microdialysis in mice was performed, as described by Imperato et al. (1996), and the dialysis tube was implanted at the level of the nucleus accumbens according to the atlas of Franklin and Paxinos (A +1 from bregma; V −4.7 from the dura). Experimental conditions and quantitative analysis by coulometric detection (Coulochem II, ESA) were as previously described (Imperato et al. 1996). The average concentration of dopamine in the last three predrug samples (0.17 ± 0.01 pmoles in 40 μl), were taken as 100% and all subsequent post-treatment values were expressed as a mean (± S.E.M.) percent variation of these basal values. Morphine–HCl (Sigma) was administered subcutaneously (s.c.) in a volume of 1 ml/100 g. Injections of distilled water (control groups) did not induce significant changes in the basal output of dopamine from the nucleus accumbens of cannabinoid CB_1 KO and WT mice (data not shown).

Between-groups comparisons were performed using a two-way analysis of variance (ANOVA) for repeated measures, factors being: treatment (two levels = two doses for drug) and time points (seven levels = 0–120 min). Post hoc analysis was performed by Student's *t*-test for paired and unpaired data.

Fig. 1(A,B) shows that injection of morphine, at the doses of 10 and 20 mg/kg, did not significantly modify dopamine release in cannabinoid CB_1 KO mice, but dose-

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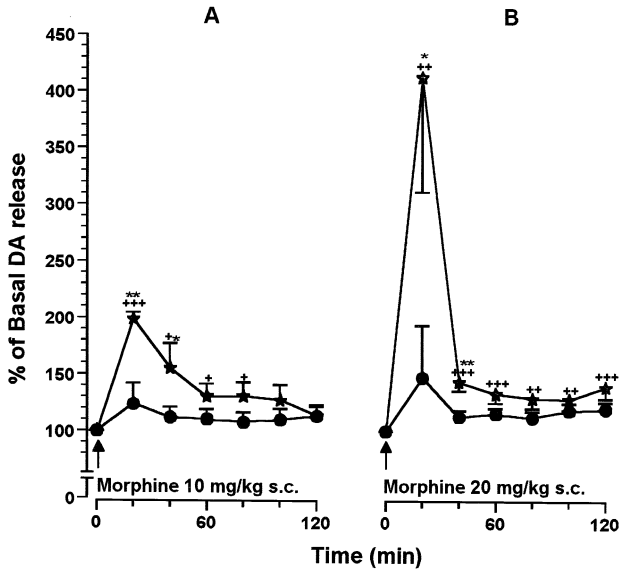

Fig. 1. The effect of morphine (A) 10 and (B) 20 mg/kg, s.c., on dopamine release in the nucleus accumbens of cannabinoid CB_1 KO (●) and wild-type (★) mice is shown. Values are expressed as a mean (±S.E.M.) percent variation of basal values. For 10 mg/kg morphine (A): ANOVA revealed a significant main effect of treatment ($F(1,86) = 6.080$; $P \leq 0.03$); a significant main effect of repeated measures ($F(6,86) = 10.359$; $P \leq 0.001$) and a significant interaction between factors ($F(6,86) = 4.016$; $P \leq 0.002$). At 20 mg/kg morphine (B): ANOVA revealed a significant main effect of treatment ($F(1,145) = 7.056$; $P \leq 0.01$); a significant main effect of repeated measures ($F(6,145) = 5.377$; $P \leq 0.001$) and a significant interaction between factors ($F(6,145) = 3.249$; $P \leq 0.01$). $n = 5$ or more for each group. $^{+}P \leq 0.05$, $^{++}P \leq 0.01$ and $^{+++}P \leq 0.001$ vs. basal values; $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ vs. cannabinoid CB_1 KO mice.

dependently increased dopamine release in WT mice. In WT mice the peak effect of morphine at both doses was observed 20 min after injection. Morphine increased dopamine release by 100% and 300% when administered at 10 and 20 mg/kg, respectively. The dose-related increase in dopamine release by morphine in WT mice is consistent with previous results obtained in rats (Di Chiara and Imperato, 1987). By contrast, morphine produced a similar stimulation of locomotor activity in both KO and WT mice (data not shown).

Our results, taken together with previously published data showing that cannabinoid CB_1 KO mice do not self-administer morphine (Ledent et al., 1999), indicate that activation of limbic dopamine release is essential for the expression of the rewarding properties of morphine.

Moreover, the present data also strengthen previous pharmacological evidence indicating a functional link between cannabinoid and opioid systems (Tanda et al., 1997; Ledent et al., 1999) and suggest that a functional cannabinoid system is required for the expression of the biochemical effects of morphine on mesolimbic dopamine transmission.

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