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Research Report

Role of dopamine D3 receptors in basal nociception regulation and in morphine-induced tolerance and withdrawal

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ABSTRACT

Repeated administration of opioids such as morphine leads to the development of tolerance to their pain-relieving effects as well as to physical dependence. Although the association between the dopamine system and the molecular mechanisms of morphine-induced antinociceptive tolerance has been studied, the possible interaction between morphine-induced tolerance and D3 receptors has not been investigated. In the present study, male mice lacking the dopamine D3 receptor gene were used to investigate the function of D3 receptors in the development of morphine-induced tolerance and withdrawal. Compared with wild-type (WT) mice, the dopamine D3 receptor knockout (D3R KO) mice showed pronounced hypoalgesia. The D3R KO mice clearly developed lower morphine-induced tolerance and showed attenuated withdrawal signs compared with the WT mice. These results suggest that D3 receptors regulate basal nociception and are involved in the development of morphine-induced tolerance and withdrawal.

1. Introduction

Opioids are the cornerstone medications in treating moderate to severe pain. Unfortunately, long-term opioid administration leads to morphine-induced analgesic tolerance (Ballantyne and Mao, 2003; Inturrisi, 2002). Tolerance to the analgesic effects of opiates results from the complex changes in various molecular and biochemical pathways (Labuz et al., 2002; Przewlocka et al., 2002; Starowicz et al., 2005). From a molecular perspective, opioid tolerance is associated with the upregulation of the cyclic adenosine monophosphate (cAMP) pathway and G-protein phosphorylation (Law et al., 2004), as well as opioid receptor downregulation, phosphorylation, internalization, and desensitization (Waldhoer et al., 2004). Other studies have proposed the role of dopamine (Cook et al., 2000; Schmidt et al., 2002; Zarrindast et al., 2002), acetylcholine (Bhargava and Way, 1976), glutamate (Xu et al., 2007), and nitric oxide (Kolesnikov et al., 1993) in the development of morphine tolerance.

The mesolimbic dopamine (DA) circuit formed by midbrain DA neurons in the ventral tegmental area projecting to the accumbens nucleus and frontal association cortex plays a key role in reward and motivation (De Boer et al., 1997; Huo et al., 2008; Zhu et al., 2007) as well as in the mediation of

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tonic pain suppression (Manning et al., 1994; Morgan and Franklin, 1990). The mesolimbic DA circuit is a major target of drugs of abuse. However, the conditions required for the development of human opioid tolerance remain unclear. Opioid tolerance is particularly robust in experimental models of acute nociception (Trang et al., 2005). A growing body of evidence strongly suggests that the D3 receptor (D3R) is significantly involved in the mechanisms of drug dependence and abuse (Andreoli et al., 2003; Di Ciano et al., 2003; Heidbreder et al., 2004, 2005; Zeitz et al., 2001). A recent rat-tail-flick test has shown that D2-like (D2/D3) DA receptors are involved in ventrolateral orbital cortex-evoked descending antinociception (Sheng et al., 2009). However, little is known on D3R involvement in morphine-induced analgesic tolerance and withdrawal. In the present study, the involvement of the D3R gene, specifically in morphine-induced tolerance and withdrawal responses, was assessed.

2. Results

2.1. Basal nociceptive thresholds

Basal response latencies were assessed using the tail-flick test, and the difference between genotypes became readily apparent. The D3R KO mice experienced a significantly greater delay in tail withdrawal than their WT counterparts (independent sample *t*-tests: $t=-4.763$, $p=0.001$) (Fig. 1). This result indicates that DA D3Rs regulate basal nociception.

2.2. Effect of D3R on acute and chronic morphine-induced antinociceptive tolerance

On day 1, treatment with a low dose of morphine (1.0 mg/kg) showed no different antinociceptive effect in D3R KO compare with WT groups (independent sample *t*-tests: $t=-0.065$, $p=0.949$). Both 3.0 and 5.0 mg/kg morphine injections induced higher latencies in the D3R KO mice than in the WT mice on day 1 (independent sample *t*-tests: for 3 mg/kg, $t=-6.548$, $p=0.000$; for 5 mg/kg, $t=-4.997$, $p=0.000$) (Fig. 2), indicating

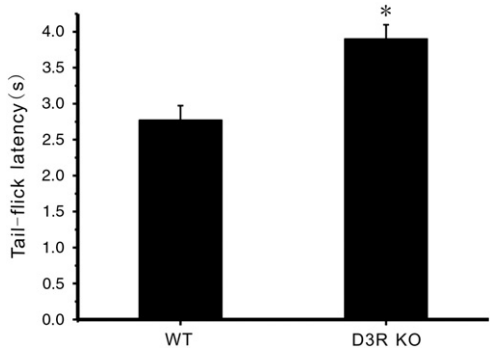

Fig. 1 – Baseline tail-flick latencies of D3 receptor mutant (D3R KO) and wild-type (WT) mice. Values were presented as means ± SEM. The average baseline tail-flick latency of WT mice was significantly lower than that shown by D3R KO mice. *$p<0.05$ compared with WT mice (independent samples *t*-tests. n = 12 per genotype).

that D3R KO mice displayed enhanced morphine-induced analgesia compared with WT mice at middle and high dose morphine in the tail-flick nociception models. To test the effects of D3R on adaptation to repeated morphine treatments, the mice were given repeated morphine injections (1.0 or 3.0 or 5.0 mg/kg for 5 days) and tested for analgesic tolerance 30 min after drug administration. At the 1.0 mg/kg dose, the mice showed no antinociceptive effect in both D3R KO and WT groups on days 1 to 5, and showed no significant development of tolerance to the antinociceptive activity (D3R KO: $t=0.748$, $p=0.470$; WT: $t=1.582$, $p=0.142$) (Fig. 2). At the 3.0 mg/kg dose, significant differences between genotype and time were revealed. The D3R KO mice showed significantly increased tail-flick latencies on days 1 ($t=-6.548$, $p=0.000$) and 5 ($t=-6.714$, $p=0.000$) compared with the WT mice. Both D3R KO ($t=2.499$, $p=0.030$) and WT ($t=4.670$, $p=0.001$) mice showed significantly reduced tail-flick latencies after repeated injection (day 5) compared with those after acute (day 1) injection (Fig. 2). Considerable effects on genotype and time were also revealed at the 5.0 mg/kg dose. The D3R KO mice showed significantly increased tail-flick latencies on days 1 ($t=-6.786$, $p=0.000$) and 5 ($t=-4.693$, $p=0.001$) compared with WT mice. Both D3R KO ($t=9.421$, $p=0.000$) and WT ($t=5.810$, $p=0.000$) mice showed significantly reduced tail-flick latencies after day 5 compared with those after day 1 (Fig. 2). These data indicate that at 3.0 or 5.0 mg/kg morphine concentrations, both D3R KO and WT mice showed high antinociceptive activity and developed tolerance to morphine-induced antinociception. However, after receiving repeated morphine injections, the analgesic effect of the same morphine dose was much more preserved in D3R KO than in WT mice. Moreover, the calculated degrees of tolerance for both 3.0 and 5.0 mg/kg morphine injections in D3R KO mice (11.54% ± 4.84% and 28.68% ± 2.54%, respectively) were significantly lower ($t=2.205$, $p=0.050$ and $t=2.650$, $p=0.023$) than those calculated in WT mice (26.49% ± 5.64% and 46.28% ± 5.37%, respectively). Overall, these results suggest that D3R KO mice exhibited enhanced acute analgesic effects of morphine and clearly developed a lower morphine tolerance.

2.3. Naloxone-induced morphine withdrawal

The degrees of naloxone-precipitated morphine withdrawal in D3R KO mice and in their WT littermates were calculated. The global withdrawal scores for the D3R KO mice were significantly decreased compared with those for the WT mice after the promotion of morphine withdrawal by the naloxone injection ($t=5.158$, $p=0.000$) (Fig. 3).

3. Discussion

The purpose of the present study was to assess the effects of D3R on morphine-induced antinociceptive tolerance. The main findings show that D3R regulates the basal pain threshold, participates in the acute analgesic response to morphine, and is involved in the development of morphine-induced tolerance and withdrawal.

DA is a major neurotransmitter in the mammalian brain. DA receptors can be classified into two families according to