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Antidepressant-like effect of venlafaxine is abolished in μ -opioid receptor knockout mice

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Abstract

Although the opioid system is known to modulate depression-like behaviors, its role in the effects of antidepressants is not yet clear. We investigated the role of μ -opioid receptors (MOPs) in the effects of venlafaxine, a serotonin and norepinephrine reuptake inhibitor, in the forced swim test using MOP-knockout (KO) mice. Venlafaxine reduced immobility time in wildtype mice (C57BL/6J), but not in MOP-KO mice, although no significant effects were observed on locomotor activity. These results suggest that MOPs play an important role in the antidepressant-like effects of venlafaxine.

Keywords

μ -opioid receptor; knockout mouse; antidepressant

The opioid system has been implicated in mood disorders. Pretreatment with naloxone, a nonselective opioid receptor antagonist, decreased immobility time in mice in the forced swim test (1). Chronic morphine treatment facilitated immobility in the forced swim test (2). These reports indicate that the opioid system impacts behavioral responses to depression-like behavior. Opioid receptors have been classified into at least three subtypes: μ , δ , and κ (MOP, DOP, and KOP, respectively). Several reports have shown that selective ligands for each subtype modulate depression-like behavior in the forced swim test (3–5). Although these three receptor subtypes may be involved in depression-like behavior, even the most selective ligands for a specific opioid receptor subtype possess certain affinities for other subtypes (6), which may contribute to the discrepant findings regarding the role of opioid receptor subtypes. Therefore, the role of each subtype in the modulation of depression-like behavior has not yet been clearly delineated by traditional pharmacological studies that use only selective ligands.

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The development of knockout (KO) mice with MOP gene deletion has revealed a central role of MOPs, rather than other opioid receptor subtypes, in the various effects of opioids, including analgesia, reward, and tolerance (7, 8). Although several compensatory changes might occur in KO animals, these animals have potential utility in investigating the *in vivo* roles of specific proteins. Our previous report showed that MOPs also modulate responses to stress, including depression-like behavior (9). The use of MOP-KO mice could provide novel theories on the molecular mechanisms underlying the modulation of depression-like behavior. Although the opioid system has been implicated in the mechanism of action of antidepressants (10), the precise mechanisms are not yet clear. The present study investigated the role of MOPs in the effects of venlafaxine, a serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine reuptake inhibitor, in the forced swim test using MOP-KO mice.

The present study used wildtype and homozygous MOP-KO mouse littermates on a C57BL/6J genetic background as previously described (7). The experimental procedures and housing conditions were approved by the Institutional Animal Care and Use Committee, and all animal care and treatment were in accordance with our institutional animal experimentation guidelines. Naive adult (>10 weeks old) male mice were group-housed in an animal facility maintained at $22 \pm 2^\circ\text{C}$ and $55 \pm 5\%$ relative humidity under a 12 h/12 h light/dark cycle with lights on at 8:00 am and off at 8:00 pm. Food and water were available *ad libitum*. All behavioral tests were conducted between 1:00 pm and 6:00 pm.

Venlafaxine hydrochloride (LKT Laboratories, St. Paul, MN) was dissolved in saline and injected in volumes of 10 ml/kg.

For the forced swim test, animals were forced to swim in a cylindrical Plexiglas tank (30 cm height $\times$ 30 cm diameter) containing 20 cm deep water for 6 min per day for 5 consecutive days. The water temperature was maintained at approximately 25°C . Immobility time was recorded with an animal activity monitoring apparatus equipped with an infrared detector (SUPERMEX, CompACT FSS, Muromachi Kikai Co., Tokyo, Japan). After each session, the mice were immediately removed from the cylinder, dried with a towel, and kept under a heating lamp until completely dry, before being returned to their home cages. Venlafaxine and saline were administered subcutaneously 20 min before each daily test.

Locomotor activity was assessed with an animal activity monitoring apparatus equipped with an infrared detector (SUPERMEX, CompACT FSS, Muromachi Kikai Co.). Mice were placed individually in $30 \times 45 \times 30$ cm plastic cages, to which they had not been previously exposed, under dim light and sound-attenuated conditions. Locomotor activity was monitored for 30 min. Venlafaxine and saline were administered subcutaneously 20 min before the test.

Data were analyzed with analysis of variance (ANOVA) followed by the Student-Newman-Keuls *post hoc* test. Values of $p < 0.05$ were considered statistically significant.

To evaluate the antidepressant-like effects of venlafaxine, immobility time during the 6 min, 5-consecutive-day forced swim test was analyzed in wildtype and MOP-KO mice (Fig. 1). Two-way, repeated-measures ANOVA of total immobility time during the 6 min test on each of the 5 days with drug dose revealed that immobility time significantly decreased after venlafaxine treatment in wildtype mice ($F_{2,100} = 13.2$, $p < 0.001$), but not in MOP-KO mice (Fig. 1A, B). *Post hoc* comparisons revealed that venlafaxine treatment (10 mg/kg) reduced immobility time in wildtype mice ($p < 0.05$) from Day 1 to Day 4. Venlafaxine treatment (30 mg/kg) also reduced immobility time in wildtype mice ($p < 0.05$) on Day 3 and Day 4. In the saline-treated group, two-way, repeated-measures ANOVA of immobility time with genotypes of mice revealed that immobility time was significantly shorter in MOP-KO mice

than wildtype mice ($F_{1,18} = 9.4, p < 0.01$), similar to our previous report (9). Immobility time in MOP-KO mice was significantly shorter than in wildtype mice on Day 2 ($F_{1,18} = 8.2, p < 0.05$) and Day 3 ($F_{1,18} = 14.7, p < 0.005$). Two-way, repeated-measures ANOVA of immobility time with genotypes of mice also revealed no significant differences between genotypes in either the 10 mg/kg or 30 mg/kg venlafaxine-treated groups.

To test the possible influences of motor dysfunction on the antidepressant-like effects of venlafaxine, locomotor activity in both wildtype and MOP-KO mice was analyzed (Fig. 2). Venlafaxine showed no significant effects on locomotor activity in either wildtype or MOP-KO mice.

In the present study, venlafaxine reduced immobility time in wildtype mice in the forced swim test, an effect that was abolished in MOP-KO mice. These results suggest that MOPs play an important role in the antidepressant-like effects of venlafaxine. This is consistent with previous reports showing that the antidepressant-like effects of venlafaxine in the forced swim test in mice were antagonized by naloxone, a nonselective opioid antagonist (10), although selective antagonists for each opioid subtype were ineffective. Venlafaxine is a non-tricyclic antidepressant that inhibits both 5-HT and norepinephrine reuptake and has no binding affinity for opioid receptors (11). Venlafaxine blocks 5-HT uptake at low doses and norepinephrine uptake at higher doses, and the doses of venlafaxine used in the present study (10 and 30 mg/kg) may act on both 5-HT and norepinephrine transporters (12). Thus, the indirect modulation of 5-HT and norepinephrine neurotransmission by endogenous opioid neurotransmission via MOPs may be hypothesized to be involved in the antidepressant-like effects of venlafaxine.

The locus coeruleus is hypothesized to be one of the most important brain regions implicated in stress, depression, and the mechanisms of action of antidepressants. Stress and depression activate the hypothalamic-pituitary-adrenal axis and increase norepinephrine release in the locus coeruleus. Norepinephrine release in the locus coeruleus is partially regulated by both opioid and noradrenergic mechanisms (13). The locus coeruleus also receives dense 5-HT projections from the dorsal raphe and pericoerulear region (14). Moreover, acute administration of venlafaxine exerted an inhibitory effect on the spontaneous activity of locus coeruleus neurons (15). Thus, a possible mechanism of action of the antidepressant-like effects of venlafaxine may be inhibition of locus coeruleus activity, which may be regulated by endogenous norepinephrine, 5-HT, and opioid systems. Deletion of MOPs might suppress the molecular control of locus coeruleus neurotransmission and depression-like responses in MOP-KO mice.

In the present study, MOP-KO mice showed reduced immobility in the forced swim test, which is consistent with our previous report showing decreased immobility in both the tail-suspension and forced swim tests and reduced stress-induced plasma corticosterone concentrations in MOP-KO mice compared with wildtype mice (9). Thus, MOP-KO mice may be resistant to the stress stimulus and be in an antidepressant-like state. Further studies may reveal the changes in neurotransmission that suppress depression-like responses and abolish the antidepressant-like effect of venlafaxine in MOP-KO mice.

In conclusion, the antidepressant-like effects of venlafaxine were abolished in MOP-KO mice, with no effect on locomotor activity. These results suggest that MOPs play an important role in the modulation of the antidepressant-like effect of venlafaxine.

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A) Wildtype **B) MOP-KO** **Figure 1.**

Effect of venlafaxine on immobility time in the forced swim test in wildtype and MOP-KO mice. Animals were subjected to daily 6 min tests for 5 consecutive days. The figure shows the cumulative immobility time during the 6 min tests over 5 days in (A) wildtype mice that received saline ($n = 12$) or venlafaxine (10 mg/kg, $n = 9$; 30 mg/kg, $n = 7$) and (B) MOP-KO mice that received saline ($n = 8$) or venlafaxine (10 mg/kg, $n = 6$; 30 mg/kg, $n = 6$). # $p < 0.05$, significant difference from corresponding value in saline-treated group. Data are expressed as mean $\pm$ SEM.

Figure 2.

Effect of venlafaxine on locomotor activity in wildtype and MOP-KO mice. The figure shows locomotor activity during a 30 min period of habituation to a novel environment in wildtype mice ($n = 4-6$) and MOP-KO mice ($n = 4$) that received venlafaxine pretreatment 20 min before the test. Each column represents the cumulative activity counts during the 30 min session. [#] $p < 0.05$, significant difference from corresponding value in saline-treated group. Data are expressed as mean $\pm$ SEM.