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Non-selective opioid receptor antagonism of the antidepressant-like effect of venlafaxine in the forced swimming test in mice

Esther Berrocoso, María Olga Rojas-Corrales, Juan Antonio Micó*

Pharmacology and Neuroscience Research Group (PAI-510), Department of Neuroscience (Pharmacology and Psychiatry), Faculty of Medicine, University of Cádiz, Plaza Falla, 9, 11003 Cádiz, Spain

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Abstract

The opioid system has been implicated in mood disorders as well as in the mechanism of action of antidepressants. Since the opioid component in venlafaxine (VLX) is still a matter of discussion, we investigated the role of opioid receptors in the antidepressant-like effect of VLX in the forced swimming test in mice. The non-selective opiate antagonist naloxone at high dose (2 mg/kg, s.c.) antagonized the effect of VLX. In contrast, β-funaltrexamine (40 mg/kg, s.c.), which preferentially blocks μ_1/μ_2 opioid receptors, naloxonazine (35 mg/kg, s.c.), a selective μ_1 opioid antagonist, naltrindole (10 mg/kg, s.c.), a selective δ opioid antagonist, and Nor-binaltorphimine (10 mg/kg, s.c.), which selectively blocks κ-opioid receptors, were all ineffective. Thus, although apparently mediated by the opioid system, the behavioural effect of VLX does not involve specific opioid receptors.

Keywords: Venlafaxine; Antidepressants; Opioid receptors; Naloxone; Forced swimming test; Mice

Venlafaxine hydrochloride (VLX) is a non-tricyclic antidepressant that selectively inhibits serotonin (5-HT) reuptake at low doses, whereas it inhibits both 5-HT and noradrenaline (NA) reuptake at high doses. With respect to opioid receptors, it has been shown to be inactive as a ligand in vitro [7]. Like other antidepressants, VLX is able to relieve several pain conditions, including neuropathic pain. This efficacy of VLX has been demonstrated in animal models [5,16] as well as in clinic pain [8]. It has been shown that the mechanism of the analgesic effects of antidepressants involves monoamines and, in some cases, opioids [20]. Nevertheless, controversy exists regarding the participation of the opioid system in the analgesic action of VLX. Indeed, Schreiber et al. [16] found that in the hot-plate test, the analgesic effect of VLX was influenced by different opioid receptor subtypes. They concluded that this opioid profile may be one of the explanations for its efficacy in severe depression, unlike other antidepressants which lack opioid activity. However, very recently, Marchand et al. [5] using naloxone (NLX) as an opioid receptor antagonist clearly showed that the antihyperalgesic effect of VLX in diabetic rats does not involve an opioid component. In both studies, the possible involvement of the opioid system in the mechanism of action of VLX was explored by means of two different models of pain induction. However, whether or not opioids are involved in the antidepressant-like effect of VLX remains to be elucidated. Other antidepressants, such as tricyclic and non-tricyclic antidepressants, have a clear relationship with the opioid system [3,19]. With regard to the involvement of different opioid receptors in emotional behaviours, a recent study carried out in opioid receptor knockout mice demonstrated a relevant effect of δ opioid receptor improving mood states in mice [4]. Thus, the aim of the present study was to investigate the possible involvement of the different opioid receptors in the main action of VLX, i.e. its antidepressant effect.

Male CD1 mice weighing 25–30 g from the 'Servicio de Experimentación y Producción Animal' (SEPA) of the University of Cádiz were used. Animals were maintained under standard conditions: 12:12 h light/dark schedule (lights on at 08:00 h) with ad libitum food and water and constant temperature (21 ± 1 °C). VLX was a gift from Whyeth-Ayerst Research, USA, and opioid antagonists were purchased from TOCRIS, Spain. VLX, NLX, naltrindole (NALT) and Nor-binaltorphimine (Nor-BNI)

* Corresponding author. Tel.: +34-956-015247; fax: +34-956-015225.
E-mail address: juanantonio.mico@uca.es (J.A. Micó).

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were administered 30 min before the test and β-funaltrexamine (β-FNA) and naloxonazine (NAZ) 24 h before the test. Control animals received only saline. Drugs were dissolved in saline (NaCl 0.9%) and injected in a volume of 0.1 ml/100 g of body weight. The different treatments were administered under blind conditions and ten mice were used per group. The opioid mediation of the antidepressant-like effect of VLX was investigated in one experimental series for several days. All treatment groups were included on each experimental day to randomize biological variability and experimental conditions.

The forced swimming test in mice (FST) [10] was used to evaluate the antidepressant effect of VLX and to assess the possible involvement of the different opioid receptor subtypes. Mice were placed individually in glass cylinders (height 25 cm, diameter 10 cm) containing water 15 cm deep at 23 °C, and left there for 6 min. The total duration of immobility during the last 4 min was video-recorded and evaluated by a blinded researcher. Reduction of immobility in this test was predictive of antidepressant activity. A mouse was judged to be immobile when it remained floating in the water and made only the movements necessary to keep its head above the surface. The water was changed between testing individual animals to avoid methodological artefacts like the existence of pheromones or other products excreted by mice.

To investigate the role of opioid receptors in the antidepressant-like effect of VLX, it was tested first with progressive doses of the non-selective opioid receptor antagonist NLX (0.5, 1, 2 mg/kg, s.c.), and then with the following selective opiate antagonists: β-FNA (40 mg/kg, s.c.), which preferentially blocks μ_1 and μ_2 opioid receptors [9]; NAZ (35 mg/kg, s.c.), a selective μ_1 opioid antagonist [9]; NALT (10 mg/kg, s.c.), a selective δ opioid antagonist [11]; and Nor-BNI (10 mg/kg, s.c.), which selectively blocks k-opioid receptors [17]. The doses of the selective opioid antagonists were chosen to maximize the antagonistic effect of these compounds while preserving their receptor specificity.

The results are expressed as the mean ± SEM of immobility time in seconds. In the case of the interaction of VLX with opioid antagonists, the mean time of immobility of the combined treatment groups was also expressed as the percentage change from the drug-alone group (see Table 1). Data were analyzed by one-way analysis of variance (ANOVA) and post-hoc comparison of means was carried out with the Student–Newman–Keuls test. A P value of <0.05 was considered to be significant.

VLX (2.5–20 mg/kg, i.p.) induced a dose-dependent antidepressant effect ($F_{(4,45)} = 6.433$, $P < 0.001$) in the FST in mice (Fig. 1). Doses of 2.5 and 5 mg/kg decreased the immobility time compared with saline controls, but not significantly so. The minimal significant effective dose was VLX 10 mg/kg. Therefore, this dose was chosen to study the involvement of the opioid system in the mechanism of action of VLX.

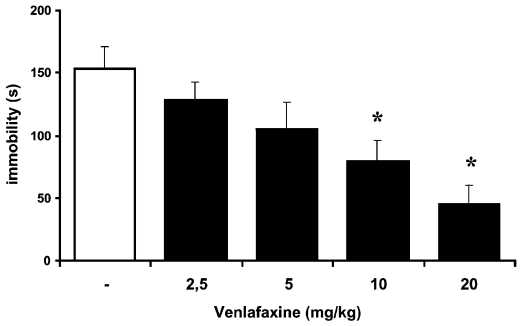

Fig. 1. Effect of VLX (2.5–20 mg/kg, i.p.) on immobility time in the FST in mice. Results are expressed as the mean ± SEM (s). Data were analyzed by one-way ANOVA followed by Student–Newman–Keuls test. *$P < 0.05$ vs. saline-treated mice.

We then studied the involvement of opioid receptors in the behavioural effect of an effective dose of VLX (10 mg/kg, i.p.) (Fig. 2, Table 1). VLX was tested with the non-selective opioid receptor antagonist NLX (0.5, 1 and 2 mg/kg, s.c.). While the results obtained with the co-administration of VLX + NLX (0.5 mg/kg) and VLX + NLX (1 mg/kg) were not significantly different from that obtained with VLX, the highest dose of NLX used, VLX + NLX (2 mg/kg), increased the immobility time with respect to VLX alone (+27.53% compared to VLX).

Next, we studied the involvement of the μ_1, μ_2, δ and k-opioid receptors. VLX was co-administered with β-FNA (40 mg/kg) and the selective μ_1 antagonist NAZ (35 mg/kg) to differentiate between a possible μ_1 and/or μ_2 mediation. β-FNA increased the immobility effect of VLX but not significantly so (+8.26% compared to VLX). Furthermore, the selective μ_1 antagonist, NAZ, did not reverse this effect,

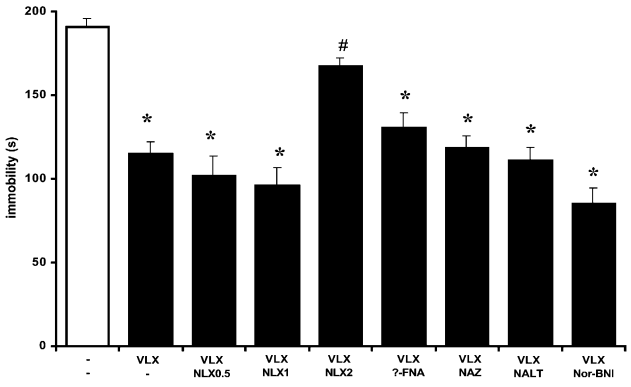

Fig. 2. Effect of different opioid antagonists on the antidepressant effect of VLX (10 mg/kg, i.p.) in the FST in mice. Results are expressed as the mean ± SEM (s). − + −, saline + saline; VLX + −, venlafaxine (10 mg/kg, i.p.) + saline; VLX + NLX, venlafaxine (10 mg/kg, i.p.) + naloxone (0.5–2 mg/kg, s.c.); VLX + β-FNA, venlafaxine (10 mg/kg, i.p.) + β-funaltrexamine (40 mg/kg, s.c.); VLX + NAZ, venlafaxine (10 mg/kg, i.p.) + naloxonazine (35 mg/kg, s.c.); VLX + Nor-BNI, venlafaxine (10 mg/kg, i.p.) + Nor-binaltorphimine (10 mg/kg, s.c.); VLX + NALT, venlafaxine (10 mg/kg, i.p.) + naltrindole (10 mg/kg, s.c.). Data were analyzed by one-way ANOVA, followed by Student–Newman–Keuls test. *$P < 0.05$ vs. saline-treated mice; $^{\#}P < 0.05$ vs. VLX-treated mice.

Table 1
Interaction of different opioid antagonists with VLX (10 mg/kg, i.p.) in the FST in mice

Opioid antagonist	Dose	Antagonist alone	VLX + antagonist	% Change from VLX alone
NLX	0.5	170.70 ± 12.86	101.80 ± 26.89*	−6.76*
	1	184.50 ± 4.71	95.90 ± 24.61*	−9.86*
	2	187.60 ± 10.86	167.20 ± 11.51#	+27.53#
β-FNA	40	161.10 ± 17.38	130.44 ± 20.44*	+8.26*
NAZ	35	157.50 ± 13.51	118.11 ± 17.40*	+1.79*
NALT	10	187.60 ± 11.47	111.10 ± 17.71*	−1.89*
Nor-BNI	10	170.90 ± 12.35	85.00 ± 21.96*	−15.57*

Values are expressed as the mean ± SEM (s). The mean time of the combined treatment group is also expressed as the percentage change from the VLX group. Mean time of VLX group, 114.70 ± 16.63 s; mean time of saline group, 190.70 ± 10.98 s. *$P < 0.05$ vs. saline-treated mice; #$P < 0.05$ vs. VLX-treated mice.

as expected. Similarly, the κ-antagonist, Nor-BNI (10 mg/kg), and the selective δ opioid antagonist, NALT (10 mg/kg), also failed to modify the effect of VLX in any way.

The antidepressant-like effect of VLX in the FST is in agreement with other studies. Redrobe et al. [12] showed that VLX reduced immobility time from 8 to 16 mg/kg in the FST in mice. Because VLX enhances spontaneous motor activity at 16 mg/kg in mice, we chose the dose of 10 mg/kg of VLX for our studies. At this dose, VLX already shows a clear antidepressant-like effect without affecting motor activity. On the other hand, none of the opiate antagonists used significantly modified the immobility time by themselves, a finding in agreement with other studies with the same test, at least in the case of NLX [3].

In the first step, the behavioural effect of VLX was clearly antagonized by the highest dose of NLX used (2 mg/kg), suggesting an opioid involvement. This antagonism is consistent with previous results for tricyclic antidepressants [3], i.e. NLX at 2 mg/kg antagonized the effect of clomipramine (20 and 30 mg/kg) and imipramine (20 and 30 mg/kg) in the FST.

In line with this observation, the analgesic effect of antidepressants has been frequently related to the opioid system. Ardid and Guilbaud [1] demonstrated that NLX antagonizes the effect of clomipramine in a model of neuropathic pain, and we showed that NLX is also able to antagonize the analgesic effect of several tricyclic antidepressants in different models of acute pain [20]. With respect to VLX, Schreiber et al. [16] reported that the antinociceptive effect of VLX is antagonized by NLX (1 mg/kg) and nor-BNI (10 mg/kg), a κ-antagonist, and is potentiated by various κ_1, κ_3 and δ opioid agonists in the hot-plate test in mice. They concluded that the antinociceptive effect of VLX involves κ and δ opioid receptor subtypes. In contrast, Marchand et al. [5] recently failed to show any opioid involvement in the effect of VLX in a model of neuropathic pain.

NLX, a non-specific opiate antagonist, is able to block all the subtypes of opioid receptors ($\mu > \delta/\kappa$) to differing degrees [2]. Thus, in a second series of experiments examining the effects of various selective antagonists of opiate receptors, none succeeded in reducing the behavioural effect of VLX. Since all these antagonists were used at concentrations that selectively block a different subtype of opioid receptor, it may be that the antidepressant-like effect of VLX does not involve a specific subtype of receptor in the FST in mice.

Several hypotheses could account for these results. First, the antidepressant effect of VLX could result from the combined action of various subtypes of opioid receptors. Indeed, a tendency to a partial antagonism was seen with β-FNA. Second, NLX may have produced its effects via an indirect mechanism involving NE and/or 5-HT [15,18]. If this is the case, a third hypothesis is that different opioid mechanisms associated with multiple individual transmitters are involved, given that the analgesic and antidepressant action of VLX involves activation of both NE and 5-HT as well as other neurotransmitters (see Rossby et al. [14]).

In fact, the antidepressant effect of VLX could be mediated by a monoamine/opiate balance. Redrobe et al. [12] showed that the effect of low dose (8 and 16 mg/kg) VLX in the FST in mice was antagonized by PCPA (DL-*p*-chorophenylalanine), a serotonergic metabolism inhibitor, while the dose of 32 mg/kg remained unaffected. The same effect was observed with DSP-4, a noradrenergic neurotoxin. This compound antagonized the effect of VLX at 16 mg/kg, but the dose of 32 mg/kg remained effective. Interestingly, VLX shows some similarities with tramadol [6], an analgesic with opiate and monoaminergic activity that we have shown to elicit an antidepressant-type effect [13].

In summary, several lines of biochemical and behavioural evidence suggest that the opioid system could be involved in the mechanism of action of tricyclic and non-tricyclic antidepressants. However, in our study, the antidepressant effect of VLX was not clearly related to a specific opioid receptor. Moreover, given that two recent studies [5,16] using different pain models draw opposite conclusions regarding the opioid effect of VLX, additional research will be necessary to establish the mechanism underlying opioid receptor mediation in the antidepressant and analgesic effect of VLX and to extend these studies to other antidepressants with these properties.

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