

The Roles of the Opioidergic System and Nitric Oxide in the Analgesic Effect of Venlafaxine

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The noradrenalin and serotonin re-uptake inhibitor venlafaxine has an analgesic effect that is independent of its antidepressant activity; however, the mechanism of this effect remains to be elucidated. This study was performed to investigate the possible roles of the opioidergic system and nitric oxide (NO) pathway in the analgesic effect of venlafaxine. Eighty Wistar rats of both sexes were allocated to 10 groups. The hot plate test was used to assess the antinociceptive/analgesic effect. The temperature of the hot plate was adjusted to $52.5 \pm 1^\circ\text{C}$, the cut-off period was set to be 50 sec; licking of the hind paw was used as a sign of pain perception. Venlafaxine alone (25 mg/kg) showed marked analgesic activity ($p < 0.05$). *N*- ω -nitro-L-arginine (L-NOARG) alone (20 mg/kg) and naloxone alone (2 mg/kg and 4 mg/kg) showed no analgesic activity ($p > 0.05$). Coadministration of low-dose naloxone (2 mg/kg) and both doses of L-NOARG (20 and 40 mg/kg) with venlafaxine (25 mg/kg) did not modify the analgesic effect but high-dose naloxone (4 mg/kg) decreased it significantly ($p < 0.05$). In conclusion, these results suggest that the opioidergic system but not the NO pathway has a role in the analgesic effect of venlafaxine.

Key words—analgesia; venlafaxine; naloxone; L-NOARG

INTRODUCTION

The second-generation antidepressant drug venlafaxine has an intrinsic analgesic effect that is independent of its antidepressant activity. This analgesic effect is of acute onset and occurs at doses lower than those used in the treatment of depression. Venlafaxine is used in the treatment of various types of pain including neuropathic pain for its marked analgesic activity and low frequency of side effects.^{1–4} Still, the mechanism of the analgesic effect remains to be elucidated, as is the case for many antidepressants. In studies on the analgesic effect of venlafaxine, possible interaction with the opioidergic system has been investigated but the results are controversial. Schreiber *et al.* argued that opioid receptors have a role^{5,6} but Marchand *et al.*^{7,8} and Berrocoso⁹ observed no effect.

On the other hand, the L-arginine-NO pathway is ubiquitous in the central nervous system (CNS); it has been shown to participate in thermal inflammatory hyperalgesia and the nociceptive transmission of neuropathic pain.¹⁰ In a study on rats, the nociception and edema induced by glutamate have been attributed largely to the activation of the L-arginine-NO pathway.¹¹ In addition, it has been reported that L-

NOARG decreased the allodynia induced by sciatic nerve ligation in a dose-dependent manner; consequently, it was inferred that the thermal hyperalgesia in this model occurred *via* the NO-cGMP pathway.¹² We are aware of no studies on the interaction between venlafaxine and the NO pathway. This study was performed to investigate the possible roles of the opioidergic system and NO pathway in the analgesic effect of venlafaxine.

MATERIALS AND METHOD

Eighty Wistar rats (275 ± 5 g) of both sexes were allocated to 10 groups of 4 males and 4 females each: the control group (0.2 ml of saline), venlafaxine alone (25 mg/kg), L-NOARG alone (20 mg/kg), L-NOARG alone (40 mg/kg), naloxone alone (2 mg/kg), naloxone alone (4 mg/kg), venlafaxine (25 mg/kg) + L-NOARG (20 mg/kg), venlafaxine (25 mg/kg) + L-NOARG (40 mg/kg), venlafaxine (25 mg/kg) + naloxone (2 mg/kg), venlafaxine (25 mg/kg) + naloxone (4 mg/kg).

The rats were maintained under standard care conditions with a 12h light: 12h dark cycle and provided with laboratory chow and water *ad libitum*. The medical ethics committee approved this study.

Venlafaxine was a gift from Wyeth, Istanbul, Turkey, naloxone and L-NOARG were purchased from Sigma (St. Louis, USA). All drugs were prepared

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fresh with saline just before use.

The rats were allowed 30 minutes to adjust to the laboratory conditions before the experiments. The temperature of the hot plate was adjusted to 52.5 ± 1 °C, and licking of the hind paw was used as a sign of pain perception; the cut-off period (the point at which the study was stopped in order to avoid injury if the animal did not withdraw its foot) was set to be 50 sec.

All rats were subjected to the hot-plate test before any treatment to measure the time to withdrawal of the hind leg. Then, the vehicle or the stated agents were given intraperitoneally in a total volume of 0.2 ml. In the venlafaxine+L-NOARG and venlafaxine + naloxone groups, the drugs were injected to different sites. Maximum attention was paid in order not to disturb the animals during the injections. Then the animals were returned to their cages. The hot plate test was performed at 30 and 60 minutes. Intra-group comparisons were made with the paired Wilcoxon test. Different groups were compared with the Mann-Whitney U test. Probability values less than 0.05 were considered significant.

RESULTS

The results of the venlafaxine group (25 mg/kg) were significantly different from the results of the control group ($p < 0.05$) (Fig. 1).

There were no significant differences between the hot-plate test results of the control group and those of the naloxone only and L-NOARG only groups ($p > 0.05$) (Fig. 2).

There were no significant differences between the

results of the venlafaxine group and those of the venlafaxine+low-dose L-NOARG (20 mg/kg) and venlafaxine+high-dose L-NOARG (40 mg/kg) groups ($p > 0.05$) (Fig. 3).

There were no significant differences between the results of the venlafaxine group (25 mg/kg) and the venlafaxine+low-dose naloxone (2 mg/kg) group ($p > 0.05$) (Fig. 4).

Comparison of the results of the venlafaxine group (25 mg/kg) and the venlafaxine + high-dose naloxone (4 mg/kg) group showed that the analgesic activity of venlafaxine was significantly modified ($p < 0.05$) (Fig. 4).

DISCUSSION

In this study, the analgesic/antinociceptive effect of venlafaxine was partially inhibited by naloxone (4 mg/kg) but unmodified by the NOS inhibitor L-NOARG. Venlafaxine is used increasingly in the treatment of neuropathic pain for its acute intrinsic analgesic effect, which is independent of its antidepressant activity.^{13,14} In the present study, a single dose of venlafaxine (25 mg/kg) showed marked an acute analgesic/antinociceptive effect was so strong that in some groups, cut off had to be invoked.

Although the antinociceptive/analgesic activity of venlafaxine is well recognized, its mechanism remains to be elucidated. The possible role of opioid receptors has been investigated in various studies, which yielded controversial results. For example, Schreiber *et al.* reported that the antinociceptive effect of venlafaxine was significantly inhibited by naloxone (10 mg/kg)

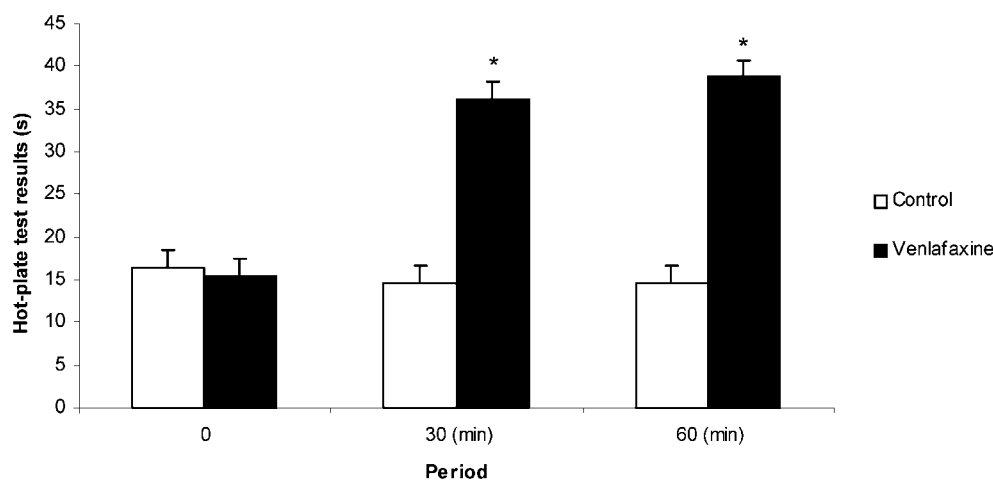


Fig. 1. The Hot-plate Results of the Control and Venlafaxine (25 mg/kg) Groups
* $p < 0.05$

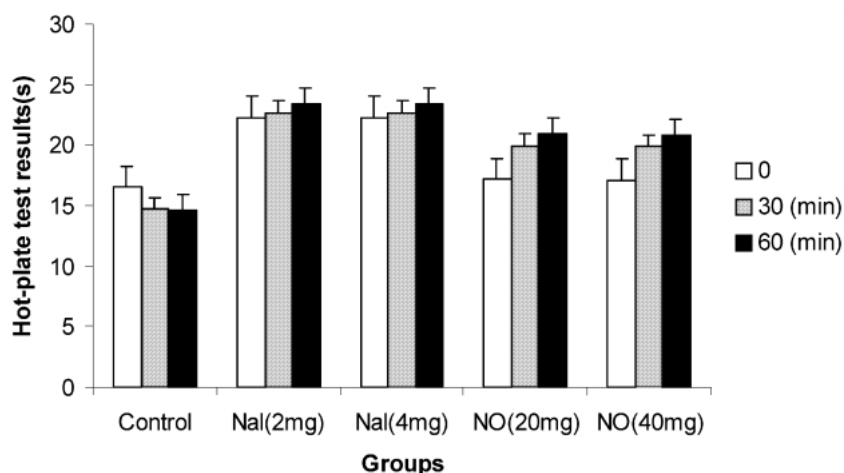


Fig. 2. The Hot-plate Results of the Control Group and Low-dose Naloxone (2 mg/kg), High-dose Naloxone (4 mg/kg), Low-dose L-NOARG (20 mg/kg) and High-dose L-NOARG (40 mg/kg) Groups (Nal: Naloxone, NO: L-NOARG) ($p > 0.05$).

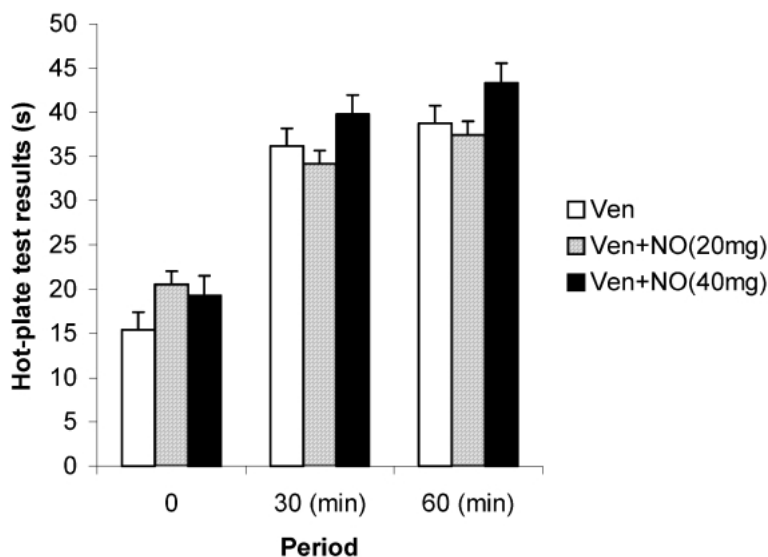


Fig. 3. The Hot-plate Test Results of the Venlafaxine (25 mg/kg), Venlafaxine+Low-dose L-NOARG (20 mg/kg) and Venlafaxine +High-dose L-NOARG (40 mg/kg) Groups (Ven: Venlafaxine, NO: L-NOARG) ($p > 0.05$).

and markedly potentiated by opiates.⁵⁾ This group argued that the κ_1 and κ_3 (OP2) receptors, δ receptors and less importantly μ (OP3) receptors mediate the antinociceptive effect of venlafaxine.⁶⁾

In contrast, in another study, the antinociceptive effect of venlafaxine was decreased by naloxone (1 mg/kg) but the difference did not reach statistical significance; the authors concluded that the opioidergic mechanisms had no role in the analgesic effect of venlafaxine.⁷⁾

In a study by another group, although the μ (OP3)

receptor agonist morphine increased the antinociceptive effect of venlafaxine, the change did not reach statistical significance.⁸⁾

In the present study, 2 mg/kg of naloxone (*i.p.*) did not modify the analgesic effect of venlafaxine whereas 4 mg/kg of naloxone decreased it significantly. Our findings are in accordance with those of Schreiber et al.⁵⁾ As was the case in their protocol, we used rats, the hot plate test and administered the drugs intraperitoneally.

The group that reported that naloxone did not

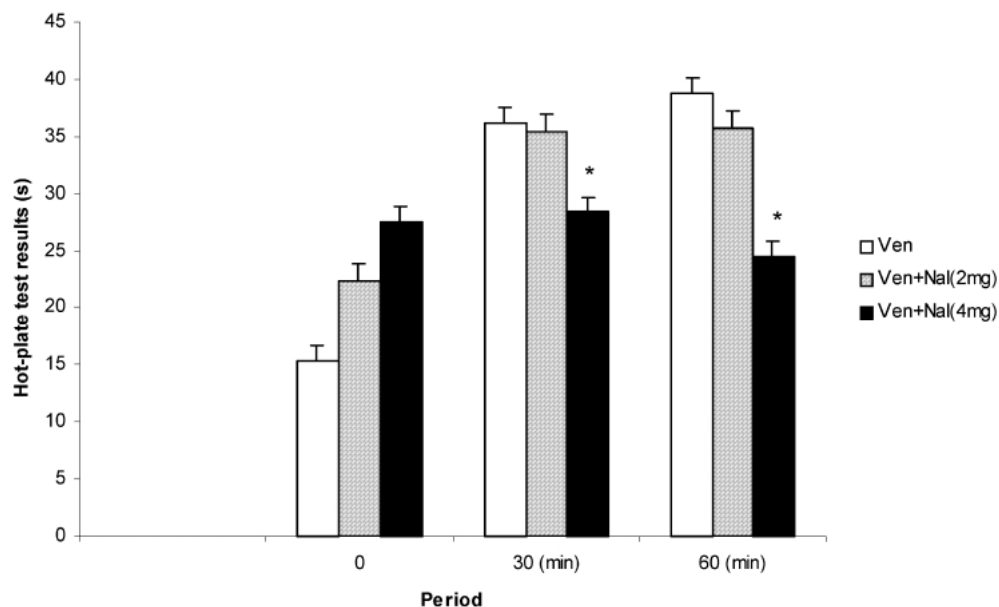


Fig. 4. The Hot-plate Test Results of the Venlafaxine (25 mg/kg), Venlafaxine+Low-dose Naloxone (2 mg/kg) and Venlafaxine+High-dose Naloxone (4 mg/kg) Groups
(Ven: Venlafaxine, Nal: Naloxone) * $p < 0.05$.

modify the analgesic activity of venlafaxine, also used the hot plate test on rats but the dose of naloxone was lower (1 mg/kg). In other studies, naloxone was used at 3 mg/kg, 5 mg/kg and 10 mg/kg.^{15–17} If this group had used other doses, they may have observed statistically significant differences. We found 2 mg/kg of naloxone to be ineffective whereas 4 mg/kg of naloxone decreased the analgesic effect significantly. In accordance with the results of others, we observed no analgesic or hyperalgesic effects of naloxone alone.

On the other hand, there are many studies on the participation of the NO-cGMP pathway in nociception.^{18–20} In a study on rats, the L-arginine-NO pathway has been shown to play a critical role in the glutamate- and NMDA-mediated nociceptive responses. This group showed that *i.p.*, *i.c.v.*, *i.t.* and local administration of the NO-synthase (NOS) inhibitor L-NOARG inhibited glutamate-induced nociception.²¹ In another study, L-NOARG decreased the allodynia caused by sciatic nerve ligation in a dose-dependent manner and it was shown that the thermal hyperalgesia after sciatic nerve ligation is mediated by NO.¹²

Ferreira *et al.* showed that intraperitoneally administered NOS inhibitors L-NOARG and L-NAME showed no effect by themselves and therefore argued that the NO-cGMP pathway has no role either at the spinal or supraspinal level in the induction of thermal

hyperalgesia.¹¹

In the present study, we observed that the L-NOARG did not affect the thermal hyperalgesia induced by the hot plate which is in accordance with the results of Ferreira *et al.* Therefore, it may be concluded that in contrast to the nociception induced by glutamate and allodynia caused by sciatic nerve ligation, the NO-cGMP had no role in the induction of thermal hyperalgesia induced by the hot plate test. This is supported by the fact that L-NOARG did not modify the analgesic/antinociceptive effect of venlafaxine. Had the NO-cGMP pathway played a role, we would expect the analgesic/antinociceptive effect of venlafaxine to be potentiated by L-NOARG. We did not have an opportunity to make any comparisons with the published literature, because to the best of our knowledge, this is the first study on the potential role of NO and NOS inhibitors on the analgesic effect of venlafaxine.

In conclusion, venlafaxine shows a marked intrinsic acute analgesic activity in rats. This effect is partially mediated by the opioidergic system; in the rat, the NO-cGMP pathway has no role in the thermal allodynia induced by the hot plate and the antinociceptive effect of venlafaxine is not modified by the NOS inhibitor L-NOARG. Based on our data, we recommend the combination of opiate analgesics with venlafaxine in the treatment of severe pain such as cancer pain.

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