The antinociceptive effect of venlafaxine in mice is mediated through opioid and adrenergic mechanisms

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Abstract

The antinociceptive effects of the novel phentylethylamine antidepressant drug venlafaxine and its interaction with various opioid, noradrenaline and serotonin receptor subtypes were evaluated. When mice were tested with a hotplate analgesia meter, venlafaxine induced a dose-dependent antinociceptive effect following i.p. administration with an ED₅₀ of 46.7 mg/kg (20.5; 146.5; 95% CL). Opioid, adrenergic and serotoninergic receptor antagonists were tested for their ability to block venlafaxine antinociception. Venlafaxine-induced antinociception was significantly inhibited by naloxone, nor-BNI and naltrindole but not by β-FNA or naloxonazine, implying involvement of κ₁- and δ-opioid mechanisms. When adrenergic and serotoninergic antagonists were used, yohimbine (P < 0.005) but not phentolamine or metergoline, decreased antinociception elicited by venlafaxine, implying a clear α₂- and a minor α₁-adrenergic mechanism of antinociception. When venlafaxine was administered together with various agonists of the opioid and α₂- receptor subtypes, it significantly potentiated antinociception mediated by κ₁-κ₃- and δ-opioid receptor subtypes. The α₂-adrenergic agonist clonidine significantly potentiated venlafaxine-mediated antinociception. Summing up these results, we conclude that the antinociceptive effect of venlafaxine is mainly influenced by the κ- and δ-opioid receptor subtypes combined with the α₂-adrenergic receptor. These results suggest a potential use of venlafaxine in the management of some pain syndromes. However, further research is needed in order to establish both the exact clinical indications and the effective doses of venlafaxine when prescribed for pain. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Antidepressants; Antinociception; Dopamine; Hotplate; Noradrenaline; Opioid receptor subtypes; Pain; Serotonin; Venlafaxine

Tricyclic antidepressants have been used for decades in the treatment of severe pain in non-depressed patients [19,21]. Nevertheless, their analgesic mechanism of action is still unclear. It apparently involves a direct, central potentiation of the endogenous opioid system [2] or activation of a mixed analgesic effect mediated by serotonergic and/or noradrenergic pathways [1,14,21] or combinations of these mechanisms. Sacerdote et al. [14], concluded that the analgesic effect of clomipramine and amitriptyline and their potentiation of morphine induced antinociception is related to an activation of the endogenous opioid system mediated by serotonin.

Venlafaxine is a structurally novel phentylethylamine antidepressant drug. In vitro, venlafaxine blocks the synaptosomal uptake of noradrenaline and serotonin and, to a lesser degree, of dopamine [6]. The concentrations of venlafaxine necessary to block the reuptake of these monoamines by 50% (IC₅₀) are 0.21 μmol/l for serotonin, 0.64 μmol/l for noradrenaline and 2.8 μmol/l for dopamine [9,10]. Venlafaxine has not been shown to inhibit MAO [10] and its monoamine-inhibitory properties differ from those of the serotonin selective reuptake inhibitors, which show high selectivity for serotonin reuptake only [7]. Moreover, venlafaxine is inactive as a ligand in vitro to α₁-, α₂-adrenoreceptors, muscarinic and histaminergic receptors [3,9,10,13] and no data has been published regarding its binding to opioid receptors. Venlafaxine’s unique pharmacological profile suggests a combined serotonin-noradrenaline mediated antinociceptive effect. In this study we examined this potential antinociception using the mouse hotplate assay.

Male ICR mice weight 25–35 g from Tel-Aviv University colony (Tel-Aviv, Israel), were used. The mice were maintained on a 12:12 h light/dark cycle with Purina rodent chow.
and water available ad libitum. Animals were housed five per cage in a room maintained at 22 \(^\circ\)C and were used only once. Intrathecal injections in mice were made under light halothane anesthesia, using a Hamilton 10 \(\mu\)l syringe fitted to a 30 gauge needle with V1 tubing. The intrathecal (i.t.) injections were introduced by lumbar puncture [5].

Several agents were generously donated as follows: Venlafaxine HCl was a generous gift from Dexon (Hadera, Israel), morphine by Teva (Jerusalem, Israel), naloxonazine by Dr. G.W. Pasternak from Memorial Sloan-Kettering Cancer Center, New York, U50, 488H \{trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolindinyl)]-Cyclohexyl-benzeneacetamide\} by Upjohn Pharmaceuticals (West Sussex, England), \{D-Pen\(^2\),D-Pen\(^5\)\}enkephalin (DPDPE), \(\beta\)-funaltrexamine (\(\beta\)-FNA), naltrindole HCL, nalorphine HCL, naloxone HCL and nor-binaltorphimine (Nor-BNI) were obtained from the Research Technology Branch of NIDA. Ethrane (Enflurane) was purchased from Abbott (Campoverde, Italy). Phentolamine, yohimbine HCl, metergoline, serotonin (5-HT) and clonidine HCl were purchased from Sigma (Israel). All other compounds were purchased from commercial sources. Venlafaxine and Yohimbine HCl were dissolved in distilled water and all the other drugs in physiological saline. The solution of 5-HT contained 0.2 mg/ml ascorbic acid in addition to saline.

Mice were tested with the hotplate analgesia meter as previously described [15,17] using Model 35D, IITC INC. Woodland Hills, CA. USA. The device consisted of a metal plate \((40 \times 35 \text{ cm})\) heated to a constant temperature, on which a plastic cylinder was placed. The analgesimeter was set to a plate temperature of 55.5 \(\pm\) 0.5\(^\circ\)C. The time of latency was recorded between the second the animal was placed on the hotplate surface until it licked its back paw or jerked it strongly or jumped out. Baseline latency was determined before experimental treatment for each mouse as the mean of two trials. Post-treatment latencies were determined after 30 min for venlafaxine administrated intraperitoneal (i.p.) and for opioids which were administrated subcutaneously (s.c.) Post-treatment latencies for DPDPE, which was administered intrathecally, were determine after 15 min. A maximal latency of 30 s was used to minimize tissue damage in the post treatment measurement. Analgesia was defined quantitatively as a doubling or more of baseline values for each mouse. For each dose, at least 10 different mice were checked and their scores were summarized, showing the percentage of animals that became analgesic. Each mouse had been checked once.

Dose-response curves were analyzed using SPSS computer program. This program maximizes the log-likelihood function to fit a parallel set of Gaussian normal sigmoid curves to the dose-response data. Single dose antagonist studies were analyzed using the Fisher’s exact test.

Venlafaxine induced a dose-dependent analgesic effect following i.p. administration (Fig. 1). The ED\(_{50}\) for mice in the hotplate assay for venlafaxine was 46.7 mg/kg \((20.5; 146.5; 95\% \text{ CL})\).

The antinociceptive effect of venlafaxine was antagonized by naloxone from 80 to 20\% \((1 \text{ mg/kg, s.c.; } P < 0.005)\), implying the involvement of opioid mechanisms (Fig. 2).

The involvement of selective antagonists for \(\mu_1\), \(\mu_2\), \(\delta\) and \(\kappa_1\) opioid-receptor subtypes in venlafaxine antinociception of latency was recorded between the second the animal was placed on the hotplate surface until it licked its back paw or jerked it strongly or jumped out. Baseline latency was determined before experimental treatment for each mouse as the mean of two trials. Post-treatment latencies were determined after 30 min for venlafaxine administrated intraperitoneal (i.p.) and for opioids which were administrated subcutaneously (s.c.) Post-treatment latencies for DPDPE, which was administered intrathecally, were determine after 15 min. A maximal latency of 30 s was used to minimize tissue damage in the post treatment measurement. Analgesia was defined quantitatively as a doubling or more of baseline values for each mouse. For each dose, at least 10 different mice were checked and their scores were summarized, showing the percentage of animals that became analgesic. Each mouse had been checked once.

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was then assessed (Fig. 2). Administered 24 h prior to testing, β-FNA (40 mg/kg, s.c.) is a selective μ₁ and μ₂ antagonist and naloxonazine (35 mg/kg, s.c.) is a selective μ₁ antagonist [11]. Similarly, the δ selective antagonist naltrindole (20 mg/kg, s.c.) blocks δ analgesia [11] and norBNI (10 mg/kg, s.c.) is a selective κ₁ antagonist [20]. All these antagonists neither mediate antinociception by themselves nor change the latencies of baselines of pretreated animals.

NorBNI reversed venlafaxine antinociception at the same dose it reversed κ₁ antinociception mediated by U50, 488H and naltrindole reversed δ antinociception at the same dose that reversed DPDPE antinociception (P < 0.005; Fig. 2). β-FNA and naloxonazine reversed only partially venlafaxine antinociception. The activity of each of the antagonists was confirmed with its prototypic agonists (data not shown). This results imply a clear κ₁- and δ-mechanisms of action and only a minor involvement of μ-mechanisms.

When multiple doses of the selective agonist of μ-subtype morphine were coadministered with vehicle or with an inactive (subthreshold) dose of venlafaxine (2.5 mg/kg, i.p; Table 1), a non-significant three-fold shift to the left in the dose-response curve was observed. The ED₅₀ of morphine without venlafaxine was 5.8 mg/kg and with venlafaxine was 1.8 mg/kg.

When various doses of the selective agonist of δ-subtype DPDPE were injected with or without an inactive dose of venlafaxine (2.5 mg/kg, i.p; Table 1), an almost four-fold shift to the left in the dose-response curve was detected (P < 0.005). ED₅₀ of DPDPE without venlafaxine was 320 ng (i.t) and with venlafaxine was 90 ng, i.t.

When the selective agonist of the κ₁-subtype U50, 488H was injected with or without an inactive dose of venlafaxine (2.5 mg/kg, i.p; Table 1), a six-fold shift to the left in the dose-response curve (P < 0.005) was obtained. ED₅₀ of U50, 488H without venlafaxine was 5.7 mg/kg, s.c. and with venlafaxine was 1.0 mg/kg, s.c.

The selective agonist of the κ₃-subtype nalorphine was then administered with or without an inactive dose of venlafaxine (2.5 mg/kg, i.p; Table 1). A shift to the left of more then 70-fold of the dose-response curve was found (P < 0.005). ED₅₀ of nalorphine without venlafaxine was 29.3 mg/kg, s.c. and with venlafaxine was 0.4 mg/kg, s.c.

These results suggest that venlafaxine, when administrated together with opiates, significantly potentiates antinociception mediated by κ₁-, κ₃- and δ-opioid receptor subtypes.

In order to assess the involvement of the serotonergic and adrenergic systems in venlafaxine antinociception, the effect of phenotolamine (α₁-, α₂-adrenergic antagonist), yohimbine (α₂-adrenergic antagonist) and metergoline (a non-selective serotonin receptor antagonist) were examined (Fig. 2). Venlafaxine-induced antinociception was significantly inhibited only by yohimbine (at the same dose needed to reverse clonidine antinociception; P < 0.05), implying an α₂-adrenergic mechanism of action. Multiple doses of the selective agonist of the α₂-adrenergic system were coadministered with vehicle or with an inactive (subthreshold) dose of venlafaxine (2.5 mg/kg, i.p; Table 1). When clonidine, a selective α₂-agonist, was administered alone, a dose-depend antinociception was evident. Concomitant administration of clonidine with inactive dose of venlafaxine (2.5 mg/kg) potentiated clonidine’s antinociception and shifted its dose-response curve almost six-fold (P < 0.05) to the left. ED₅₀ value of clonidine alone was 0.34 mg/kg (0.03; 1.1) and with venlafaxine 0.06 mg/kg (0.02; 0.13).

We found venlafaxine to induce dose-dependent antinociception in the mouse hotplate assay following i.p. administration. This effect was antagonized both by naloxone (implying involvement of the opioid system) and by yohimbine (implying involvement of α₂-adrenergic mechanisms), but was unaffected by the nonselective serotonin antagonist mianserin. When administered together with various opioid receptor agonists, venlafaxine significantly potentiates antinociception mediated by κ₁-, κ₂- and δ-opioid receptor subtypes. When administered at an inactive dose together with the selective α₂-agonist clonidine, it significantly potentiated α₂-antinociception. These results are surprising, since venlafaxine’s pharmacological profile did not suggest opioid-mediated antinociception.

In a series of previous studies we evaluated the antinociceptive effect and mechanisms of several antidepressants of the newer generations. All of them were found to enhance opioid analgesia when co-administered with opioid receptor agonists. However, all but mianserin were found to induce non-opioid-mediated antinociception. Moclobemide induced a selective α₁-adrenergic-mediated antinociception [18], while nefazodone, fluvoxamine and fluoxetine, induced serotoninergic-mediated antinociception [12,15,17]. Only the tetracyclic antidepressant mianserin, when injected alone i.p., elicited a biphasic dose-dependent antinociceptive effect, abolished by naloxone, indicating an involvement of opioid mechanisms [16].

The findings regarding κ- and δ-opioid receptor involvement in venlafaxine’s antinociception are both novel and difficult to interpretation. It may involve a significant finding in the preclinical studies of venlafaxine, its effect on β-adrenergic receptors. In the rat pinal nerve model of noradrenergic sensitivity, treatment with venlafaxine caused a subsensitivity of the β-adrenergic-linked cyclic adenosine

Table 1
Receptor selective agonists ED₅₀ with and without venlafaxine

<table>
<thead>
<tr>
<th>Receptor subtypes</th>
<th>Without venlafaxine</th>
<th>With venlafaxine</th>
</tr>
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<tbody>
<tr>
<td>Morphine (μ₁)</td>
<td>5.8 (3.5; 14.2)</td>
<td>1.8 (0.9; 3.6)</td>
</tr>
<tr>
<td>DPDPE (δ)</td>
<td>320 (178; 653)</td>
<td>90 (49; 174)*</td>
</tr>
<tr>
<td>U50488H, H (κ₁)</td>
<td>5.7 (2.2; 103.2)</td>
<td>1.0 (0.4; 0.4)*</td>
</tr>
<tr>
<td>Nalorphine (κ₃)</td>
<td>29.3 (16.5; 68.5)</td>
<td>0.4 (0.2; 0.7)*</td>
</tr>
<tr>
<td>Clonidine (α₂)</td>
<td>0.34 (0.03; 1.1)</td>
<td>0.06 (0.02; 0.13)*</td>
</tr>
</tbody>
</table>

*Each test group contained 10 mice. The numbers in parentheses are the 95% confidence limits of the ED₅₀. P ≤ 0.05 for difference from the group without venlafaxine.
monophosphate (c-AMP)-generating system. Thus venlafaxine reduced noradrenergic responsiveness after both acute and chronic treatment [9]. This down regulation of the noradrenergic system, combined with other properties of venlafaxine, may induce an indirect activation of the opioid system supraspinally, even though the κ-opioid system is most active spinally. However, further study is needed to establish the exact location of this interaction.

Another (and more feasible) explanation may be a dopamine-mediated involvement of the opioid system. Venlafaxine exerts a clear dopamine reuptake inhibitory effect [6]. Increase of the dopamine levels at the synaptic cleft, as well as dopamine D2 receptor agonists and mixed dopamine D1/D2 receptor agonists were reported to increase dose-dependingly the nociceptive threshold of mice. This antinociceptive effect was mediated through the opioid system [4,8].

Venlafaxine has a mild profile of side effects and a favorable potential rapid-onset of action. These characteristics, combined with its clear opioid-mediated antinociceptive properties, indicate its possible use for treatment of pain syndromes. Further clinical studies are necessary in order to establish the exact clinical indications and recommended dosage for the use of venlafaxine in the pain clinic.