Antinociceptive effects induced by desipramine and fluoxetine are dissociated from their antidepressant or anxiolytic action in mice

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Abstract
This study aimed to evaluate the relationship between some aspects of experimental depression, anxiety and the antinociceptive effects of fluoxetine and desipramine in mice. Acute administration of fluoxetine and desipramine (5, 10 and 20 mg/kg, i.p.) showed significant antinociceptive effects in the hot-plate test and against the early and late phase of the mouse formalin test, dissociated from its antidepressant and anxiolytic effects as measured in the forced swimming and in the plus-maze tests, respectively. Neither fluoxetine nor desipramine, at the doses tested, produced significant effects on locomotor activity. Furthermore, both compounds were ineffective in the tail-flick phasic model of nociception. In conclusion, the results suggest that without the distinction of serotonergic and noradrenergic contributions, the acute antinociceptive effects of fluoxetine and desipramine in mice are independent of their sedative, antidepressant and anti-anxiety properties.

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Introduction
There is now substantial evidence that different antidepressants, especially the tricyclics, produce antinociception both in patients with pain of varying aetiology (Blumer and Heilbronn, 1982; Feinmann, 1985; France, 1987; Von Knorring et al., 1983; Walsh, 1983) and in animals submitted to different nociceptive stimuli (Spiegel et al., 1983; Tura and Tura, 1990). However, these results are conflicting and the mechanisms underlying this action remain unclear (Lund et al., 1989, 1990, 1992). It is known that the major mechanism of the action of an antidepressant is mainly related to interference with the reuptake of noradrenaline or serotonin at the neuronal membrane (Rosenblatt et al., 1984; Van Praag, 1983). Furthermore, serotonergic and noradrenergic systems are involved in the neurochemical modulation of pain transmission (Crisp et al., 1991; Fasmer et al., 1989; Jensen and Yaksh, 1984; Kilpatrick et al., 1990; Ollat et al., 1989; Tura and Tura, 1990). Thus, the available evidence supports the role of serotonin and noradrenaline as important neurotransmitters both in the mechanism of action as an antidepressant and in antinociception (Sierralta et al., 1995).

On the other hand, an increasing body of evidence has suggested a neurochemical link between depression and anxiety, and that antidepressants can be used to treat mental dysfunctions other than depression (Bourin et al., 1996; Tollefson, 1995). Although the antidepressant activity may be responsible for a part of the analgesic response, there is evidence indicating that these two effects are independent. For instance, it has been reported that an analgesic response in non-depressed patients is present (Leijon and Boivie, 1989; Sharav et al., 1987), and that analgesic effects occur sooner and with lower drug doses than the antidepressant effects (Davis et al., 1977).

Concerning animal models, Casas et al. (1995) have demonstrated that the antinociceptive properties of amitriptyline were independent of its antidepressant action evaluated in the forced swimming test. Indeed, as pointed out by these authors, current knowledge on the mechanism of the antinociceptive action of antidepressants is largely derived from animal experiments, and most of these studies do not provide information on the emotional aspects of pain.

Since the literature providing information on the emotional aspects of antidepressant-induced analgesia is scanty, and considering that animal models used to
predict antinociceptive activity are unsuitable for predicting other behavioural measures, the present study was undertaken to evaluate the relationship between the potential antidepressant, anxiolytic and antinociceptive effects of two classes of antidepressants in mice. Although a number of tests can be proposed for this aim, the present study is intentionally limited to simple and reliable animal models, such as the forced swimming test and the plus-maze test of anxiety. Also, the influence of the treatment on locomotor activity was investigated. Further, considering that antinociceptive evaluation may vary considerably between different models, different tests of nociception such as tail-flick, hot plate and formalin tests, including both thermal and chemical stimuli, respectively, were used. As noradrenaline and serotonin have been implicated previously in descending modulation of nociception, the noradrenergic and the serotoninergic reuptake inhibitors, desipramine and fluoxetine, respectively, were selected.

**Material and methods**

**Animals**

Male Swiss adult albino mice weighing 30–40 g from our own colony were used. All animals were kept in cages, in groups of 15–20, with free access to laboratory food and water. They were maintained in a temperature-controlled room (23 ± 1 °C) under a 12 h light cycle (lights on 07:00 hours). Each animal was used for one experiment only. All procedures used in the present study complied with the ‘Guide for the Care and Use of Laboratory Animals of The Brazilian Society of Neuroscience and Behaviour’, which operates under accepted guidelines such as ‘Guiding Principles in the Care and Use of Animals’ (DHEW Publication, NIH).

**Drugs**

Fluoxetine HCl and desipramine HCl (Research Biochemicals International, USA) were dissolved in distilled water and injected i.p. in a volume of 0.1 ml/10 g body weight. All doses are expressed as weight of salt. The control solutions consisted of an equivalent volume of distilled water.

**Apparatus and procedure**

**Activity counts**

The locomotor activity of each animal was measured in a cage of 40 × 12 × 20 cm with three photocells. Mice received either fluoxetine (5, 10, 20 or 40 mg/kg), desipramine (5, 10, 20 or 40 mg/kg) or control solution. Immediately after injection, each mouse was introduced into the cage and light-beam interruptions were recorded for 60 min.

**Forced swimming test**

This test, validated as a neurobiological model of depression, was performed according to a procedure adapted from Porsolt et al. (1977). Mice were placed individually into a transparent cylinder (25 cm high, 10 cm diam.) containing 10 cm of water maintained at 23 ± 1 °C. The duration of immobility was timed during the last 4 min of a 6-min total test time. A mouse was considered immobile when it only made small movements to keep its head above the water. The effects of drug treatment were assessed 30 min after the injection.

**Elevated plus-maze test**

This model was used to evaluate the anxiolytic or anxiogenic properties of drug treatment. The plus-maze apparatus was made of wood and consisted of two opposite open arms, 30 × 5 cm, and two enclosed arms, 30 × 5 × 15 cm, and was elevated 38.5 cm from the floor. Mice were injected with control solution or antidepressants 30 min prior to the test. Each mouse was placed in the centre of the maze as described in our previous study (Gevaerd and Takahashi, 1996), and the number of entries and the time spent in the open and closed arms was recorded over a 5-min period.

**Nociceptive testing**

**Tail-flick test**

For measurement of the latency of the tail-flick response, a procedure similar to that reported in the literature and adapted in this laboratory was used (Rigon and Takahashi, 1996). Mice were gently restrained in plastic tubes with the tail positioned in the apparatus (Albarch, RS, Brazil) for radiant heat stimulation (90 W). The baseline latencies were determined with three consecutive readings, each 1 min apart, just before drug administration. Following the i.p. administration of antidepressants, the tail-flick responses of each mouse were assessed at 15-min intervals over 45 min. The cut-off time was set at 30 s.

**Hot-plate test**

The hot-plate apparatus (Ugo Basile, Varese, Italy) consisted of a thick aluminium plate floor surrounded by a Perspex cylinder. It was maintained at a temperature of...
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60 ± 0.5 °C (Casas et al., 1995). Mice were individually placed in the centre of the hot plate and the latency for the licking of either forepaw was recorded. Basal latencies were recorded before treatment. Effects of administration of different doses of antidepressants were evaluated at 15-min intervals over 45 min. The cut-off time was 30 s.

**Formalin test**

The formalin test was carried out in an open glass cylinder, 17 cm in diameter, with a mirror placed under the floor to allow unobstructed view of the paws.

Antidepressant or control solution was injected i.p. 15 min before the formalin injection. As described in a previous work (Bittencourt and Takahashi, 1997), each animal was injected with 20 µl of formalin 2.5% into the intraplantar region of the right hindpaw. Mice were then observed for 30 min after formalin injection and the amount of time spent licking the injected paw was timed with a stopwatch. This test has two different phases: the early phase of the nociceptive response normally peaks between 0 and 5 min after formalin injection, and the late phase peaks between 15 and 30 min after formalin injection, possibly reflecting different types of pain (Hunskaar and Hole, 1987).

**Statistical analysis**

A one-way ANOVA test was conducted to analyse the influence of drug treatment upon locomotor activity, forced swimming, the plus-maze test of anxiety, and each phase of the formalin test. A two-way ANOVA for repeated measures was used in which the two factors were phase of the formalin test. A two-way ANOVA for forced swimming, the plus-maze test of anxiety, and each influence of drug treatment upon locomotor activity, a one-way ANOVA test was conducted to analyse the statistical analysis (Hunskaar and Hole, 1987).

The results of the comparisons between antidepressants and control groups on locomotor activity, forced-swimming and plus-maze tests in mice are illustrated in Table 1. Separate one-way ANOVAs revealed that the i.p. administration of desipramine and fluoxetine (5, 10 or 20 mg/kg) did not produce significant changes in the activity counts [F(6,63) = 1.66, p = 0.14], the immobility time in the forced swimming test [F(6,63) = 0.94, p = 0.47] or in the anxiety parameters measured in the plus-maze: percentage number of open-arms entries [F(6,63) = 0.96, p = 0.46], percent time spent on open arms [F(6,63) = 1.03, p = 0.41].

Since the dose of 40 mg/kg of both compounds induced a significant decrease in motor activity, this sedative dose was excluded from other behavioural measures (data not shown).

**Tail flick-test**

Figure 1 depicts the effects of i.p. treatment with antidepressants on the tail-flick nociceptive responses. A two-way ANOVA revealed no statistically significant differences among the main treatment factors [F(6,63) = 1.35, p = 0.24] or in the interaction between treatment and time [F(18,189) = 1.22, p < 0.23]. However, a significant effect of time was observed [F(6,63) = 4.00, p < 0.008]. Overall, these results suggest that desipramine and fluoxetine at all doses tested failed to significantly alter the tail-flick latencies of mice.

**Table 1.** Effects of acute treatment (i.p.) of fluoxetine, desipramine and saline on the responses of mice in locomotor activity, forced-swimming and elevated plus-maze tests

<table>
<thead>
<tr>
<th>Treatment (mg/kg i.p.)</th>
<th>Locomotor activity counts</th>
<th>Forced swimming time (s)</th>
<th>Plus maze</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% EOA</td>
</tr>
<tr>
<td>Saline</td>
<td>483.1 ± 20.4</td>
<td>140.7 ± 5.2</td>
<td>16.8 ± 2.0</td>
</tr>
<tr>
<td>Fluoxetine (5)</td>
<td>397.5 ± 36.3</td>
<td>137.6 ± 8.7</td>
<td>15.1 ± 2.9</td>
</tr>
<tr>
<td>Fluoxetine (10)</td>
<td>510.9 ± 37.7</td>
<td>110.7 ± 9.2</td>
<td>16.2 ± 2.1</td>
</tr>
<tr>
<td>Fluoxetine (20)</td>
<td>421.2 ± 34.6</td>
<td>125.2 ± 9.6</td>
<td>13.5 ± 2.6</td>
</tr>
<tr>
<td>Desipramine (5)</td>
<td>462.8 ± 40.2</td>
<td>125.9 ± 8.7</td>
<td>12.3 ± 2.7</td>
</tr>
<tr>
<td>Desipramine (10)</td>
<td>387.9 ± 61.2</td>
<td>126.5 ± 6.9</td>
<td>13.9 ± 3.0</td>
</tr>
<tr>
<td>Desipramine (20)</td>
<td>397.1 ± 35.9</td>
<td>120.6 ± 10.1</td>
<td>17.0 ± 3.5</td>
</tr>
</tbody>
</table>

Data are expressed as means ± s.e.m. for 10 animals in each dose.
% EOA, mean percentage number of entries on the open arms.
% TOA, mean percentage of time spent on the open arms.
Figure 1. Effects of acute treatment (5, 10 and 20 mg/kg i.p.) of fluoxetine and desipramine on the nociceptive responses of mice in the tail-flick test. Drugs were administered immediately following baseline latency at time zero. Data are expressed as means ± s.e.m. for 10 animals in each dose.

Figure 2. Antinociceptive effects of acute treatment (5, 10 and 20 mg/kg i.p.) of fluoxetine and desipramine in the hot-plate test. Drugs were administered immediately following baseline latency at time zero. Data are expressed as means ± s.e.m. for 10 animals in each dose. *p < 0.05; **p < 0.01 compared to the saline-treated group, Newman–Keuls test.

Hot-plate test
The results from the hot-plate test are depicted in Figure 2. A two-way ANOVA of these data showed significant treatment effects [F(6,63) = 8.14, p < 0.0001], a significant time factor [F(3,189) = 11.80, p < 0.0001], and a significant interaction between treatment and time [F(18,189) = 2.85, p < 0.0002]. Subsequent comparisons indicated that both antidepressants at doses of 10 and 20 mg/kg, significantly increased the latency time of forepaw licking, as shown in Figure 2 (Newman–Keuls test, p < 0.05). These antinociceptive effects were detected 15 min after i.p. injections of the drugs (Newman–Keuls test).

Formalin test
The antinociceptive effects of desipramine and fluoxetine in both phases of the mouse formalin test are illustrated in Figure 3. Separate one-way ANOVAs showed significant treatment effects for the early phase [0–5 min: F(6,63) = 7.97, p < 0.0001] and late phase [15–30 min: F(6,63) = 13.81, p < 0.0001]. Subsequent post-hoc tests confirmed that desipramine and fluoxetine at all doses (5, 10 and 20 mg/kg, i.p.) significantly attenuated the time of paw licking during both phases of the test (Newman–Keuls test, p < 0.05).

Discussion
This study demonstrated that the acute i.p. administration of desipramine and fluoxetine, inhibitors of noradrenaline and serotonin reuptake, respectively, induced antinociceptive effects in the hot-plate and formalin tests, dissociated from their antidepressant or anxiolytic effects, in mice. These findings confirm and extend other studies showing that these antidepressants elicit antinociceptive effects in different animal models (Ansuategui et al., 1989; Ardid et al., 1992; Eschalier et al., 1981; Fialip et al., 1992;
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Figure 3. Antinociceptive effects of acute treatment (5, 10 and 20 mg/kg i.p.) of fluoxetine and desipramine in the mouse formalin test. Nociceptive responses in the early phase (0–5 min after the injection of formalin) and the late phase (15–30 min after the injection of formalin) were scored as the amount of time spent licking the hind-paw. Antidepressants were administered 15 min before the injection of formalin. Data are expressed as means ± S.E.M. for 10 animals in each dose. *p < 0.01 compared to the saline-treated group. Newman–Keuls test.

Goldstein et al., 1990; Rigal et al., 1983). A prominent feature of our study is that the antinociceptive action of the drugs was obtained without altering their action in the forced swimming test, a predictor model of antidepressant effect, or in the plus-maze test, a reliable and simple model of anxiety. It is important to realize, however, that in the present study mice were not experimentally selected into depressed or anxious groups prior to antinociceptive evaluation. Nevertheless, these results are also in partial agreement with the findings showing that the antinociceptive effect of amitryptiline was independent of its antidepressant effect (Acton et al., 1992).

It is important to note that in the present study, the effects of desipramine and fluoxetine on motor activity were taken into account in order to select the dosage range for the other behavioural tests. Thus, the failure of antidepressants to alter the immobility time and the anxiety measures in the plus-maze test might be related to the selected low doses of the drugs and/or the schedule of drug administration. Indeed, in reports demonstrating a significant anti-immobility action of antidepressants, higher doses of these compounds were used (File and Tucker, 1986). In addition, it appears that the anxiolytic effect of antidepressants is usually observed following repeated drug administration (Bodnoff et al., 1988).

Although antidepressants elicit an antinociceptive effect in a great variety of nociceptive models, it seems that these drugs are more effective according to the following stimulus ranges: chemical > mechanical > electrical > thermal (Eschalier et al., 1992). Accordingly, it is worth noting that the antinociceptive effects of desipramine and fluoxetine were observed during both the early and late phases of the formalin test. This test constitutes a chemical model of tonic pain involving peripheral and central components, relevant to the study of longer-lasting pain (Sugimoto et al., 1986). Thus, it is known that the early phase may be due to direct effects on nociceptors and that it can be inhibited by centrally acting analgesics. In contrast, the late phase seems to be due to an inflammatory response partly mediated by prostaglandins, and it can be inhibited by non-steroid anti-inflammatory drugs and steroids, as well as the centrally acting drugs (Hunskaar and Hole, 1987). On the other hand, in the hot-plate test in which a strong thermal stimulus was used (60 °C), involving central integration, it is believed both antidepressants caused a modest but statistically significant increase in the latencies of reaction time. Further, desipramine and fluoxetine failed to induce antinociception in the tail-flick model, a spinal reflex type of pain test also using a thermal stimulus. A particular problem with this test is the possible effect of skin temperature, a parameter influenced by local blood flow and ambient temperatures.

The quite similar pattern of responses displayed by desipramine and fluoxetine employed here do not support the contention that the selective inhibitors of the reuptake of serotonin have stronger antinociceptive effects than less selective drugs (Ageel et al., 1986). Certainly, these findings raise questions requiring additional studies. Therefore, the exact mechanism involved in the antinociceptive effects of desipramine and fluoxetine remain unsolved here. It is important to note that the majority of studies carried out on this topic have implicated the participation of opioids (Eschalier et al., 1981; Takahashi and Paz, 1987; Valverde et al., 1994) or monoaminergic mechanisms, mainly serotonin and noradrenaline (Eschalier et al., 1981; Fasmer et al., 1989; Micó et al., 1997). Moreover, it has recently been suggested that desipramine-induced antinociception may involve NMDA mechanisms (Mjellem et al., 1993).

In conclusion, the data presented in this study suggest that desipramine and fluoxetine possess antinociceptive properties at a dosage range devoid of sedative, anti-
depressant or anxiolytic effects in mice. In addition, the similar profile of responses induced by both antidepressants indicates that a predominant noradrenergic or serotonergic involvement is not present.

References


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