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Interactions of acute morphine with chronic imipramine and fluvoxamine treatment on the antinociceptive effect in arthritic rats

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Abstract

This study was undertaken to investigate the effects induced by chronic systemic administration of two different antidepressants: imipramine (IMI), a dual serotonin-noradrenaline reuptake inhibitor, and fluvoxamine (FVX), a selective serotonin reuptake inhibitor, on the antinociceptive effect of morphine (MOR) in a paw pressure test in adjuvant-induced arthritic rats. For 30 days rats were administered with IMI, FVX or saline (SAL). On days 15 and 30, animals were tested in the paw pressure test 20 min after MOR or SAL administration. MOR induced a significant antinociceptive effect in IMI, FVX and SAL treated rats. But, at 30 days, this increase in pain threshold was significatively higher in IMI than SAL rats. This increase was not seen in FVX rats. These results suggest that a combination of opioid and mixed monoaminergic activities is effective in enhancing the antinociceptive effect of MOR in arthritic rats while only opioid and serotonergic activities have no enhancer effect.

Keywords: Antidepressant; Imipramine; Fluvoxamine; Systemic arthritis; Morphine; Rat

A body of evidence supports the use of opioids in chronic non-cancer pain, including the treatment of arthritic pain [18]. However, the use of opioids to treat these patients is controversial because of concerns about efficacy and safety, and the possibility of addiction or abuse [16]. In these situations, addition of an adjuvant can improve pain relief and minimize the risks of apparition of adverse effects of opioids [14]. In this sense, antidepressant drugs have been used increasingly as adjuvant in the treatment of patients with chronic pain [14]. However, an association of arthritic complaints and treatment with serotonergic antidepressants has been suggested in a case report [8].

In laboratory animals, many antidepressants have been shown to increase morphine (MOR) antinociception. However, Godefroy et al. [7] failed to demonstrate a potentiation of antinociceptive effects of MOR by amitriptyline or imipramine (IMI) given either acutely or chronically using a test of acute nociception (vocalization threshold to graded foot pressure).

From an experimental point of view, the rat with adjuvant-induced arthritis is a model of chronic pain [4]. In this model, rats show hyperalgesia to a mechanical acute noxious stimulus applied on inflamed tissues. However, chronic treatment with tricyclic antidepressants has not shown an analgesic effect on this hyperalgesia measured by a paw pressure test in adjuvant-induced arthritic rats [3]. In spite of this, this chronic pain model is particularly sensitive to MOR [10].

The purpose of the present study was to investigate the effect of chronic administration of IMI, a tricyclic antidepressant, and fluvoxamine (FVX), a selective serotonin reuptake inhibitor (SSRI), on the modification of the pain response to the paw pressure test [17] produced by a single dose of MOR in rats with adjuvant-induced arthritis.

All the experimental procedures were performed according to the ethical guidelines for investigations of experimental pain in conscious animals [20]. The experimental protocol was approved by the Local Committee for Animal Experimentation of the Faculty of Medicine of the University of Cádiz (License no. 079604). Male Wistar adjuvant-induced arthritic rats (150–250 g) supplied by IFFACREDO (France) were used in this study. They were housed in groups of five, and maintained in a controlled

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environment with water and food made available ad libitum. Animals were allowed to adapt to the animal room for at least 1 week prior to use. The antinociceptive responses were determined using an analgesy-meter for the rat paw LETICA LI-7306. A cut-off of 1000 units was set in order to avoid excessive suffering of the animals. For each rat, three determinations were carried out and the average of them was held as the animal response. A control of body weight and tibio-tarsal diameter was carried out at the same time-points used for nociceptive testing. Tibio-tarsal diameter was measured as the mean of circumference diameters of both tibio-tarsal joints. All the animals were evaluated by an observer who did not know the animal treatment. Fifteen days after arthritis induction and after a basal determination of pain threshold, a chronic IMI (10 mg/kg i.p., twice a day), FVX (10 mg/kg i.p., twice a day) or SAL treatment was administered to arthritic rats. At 15 and 30 days, the effect of acute administration of MOR (5 mg/kg s.c.) or SAL was tested 20 min after injection (Fig. 1). At the same time-points weight and tibio-tarsal diameter were determined. Lower limbs inflammation was maintained after completing the experimental protocol.

The results obtained are expressed as the mean ± SEM of the response in the paw pressure test, the rat weight in grams and the tibio-tarsal diameter in millimeters. For statistical analysis, individual group comparisons were made using a two-way ANOVA. Individual treatment effects (differences between groups) were analyzed using a Duncan test following significant main effects of treatment by one-way ANOVA. In weight and tibio-tarsal diameter studies, individual treatment effects were analyzed using Student's *t*-test. The level of significance was $P < 0.05$.

Basal determination did not show any differences in pain response between experimental groups. At days 15 and 30, MOR displayed antinociceptive effects in rats treated with IMI (Fig. 2), FVX (Fig. 3) or SAL. In contrast, antidepressants did not show antinociceptive effects at this time-point. At day 30, when MOR was administered in rats chronically treated with IMI the increase was significatively

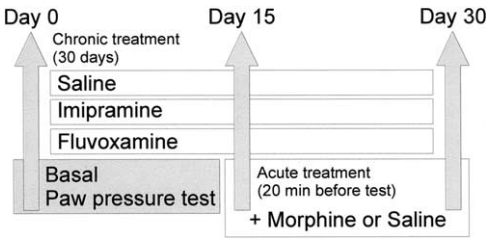

Fig. 1. Experimental protocol. Rats were tested in the paw pressure test (Basal). Then a chronic treatment during 30 days with IMI (10 mg/kg, i.p., twice a day) or FVX (10 mg/kg, i.p., twice a day) or SAL (0.9%) was performed. On days 15 and 30 (12 h after the last drug dose) the animals were tested in the paw pressure test 20 min after the administration of MOR (5 mg/kg, s.c.) or SAL. Thus, the initial three groups (IMI, FVX or saline treated) became six: SAL + SAL ($n = 10$), SAL + MOR ($n = 10$), IMI + SAL ($n = 9$), IMI + MOR ($n = 9$), FVX + SAL ($n = 9$) and FVX + MOR ($n = 9$).

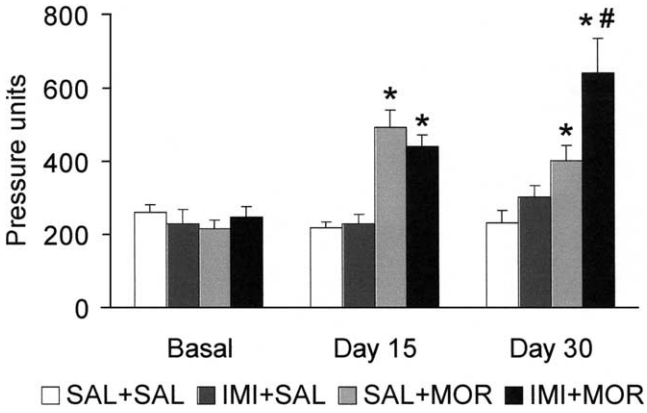

Fig. 2. Effect of acute administration of MOR in arthritic rats chronically treated with IMI on pain response to a paw pressure test. IMI treatment does not modify the antinociceptive effect of MOR at day 15 (*$P < 0.05$ vs. SAL + SAL and SAL + IMI). At day 30, the antinociceptive effect of MOR (*$P < 0.05$ vs. SAL + SAL and SAL + IMI) is greater in IMI treated rats than SAL treated rats ($^{\#}P < 0.05$ vs. SAL + MOR).

higher than that observed in rats chronically treated with SAL (Fig. 2). On the other hand, in FVX treated rats MOR did not display an enhanced effect (Fig. 3). With respect to body weight measures, IMI rats showed a lower weight than FVX and SAL rats at 15 and 30 days (Table 1). In this sense, serotonin and noradrenaline reuptake inhibition is a mechanism responsible for a reduced food intake in rats due to sibutramine, venlafaxine or duloxetine administration [9]. This could be the reason for the effect induced by IMI in body weight. With respect to tibio-tarsal diameter measures, IMI treated rats showed a significant increase in tibio-tarsal diameter compared to SAL rats at the same time-points (Table 2). This increase could be due to a negative effect produced by IMI-induced noradrenaline reuptake inhibition, since noradrenaline exerts a negative influence on the inflammatory process in arthritic joints [13].

The results obtained in this work did not show an analgesic effect of chronic treatment with antidepressants on acute pain response measured by a paw pressure test in

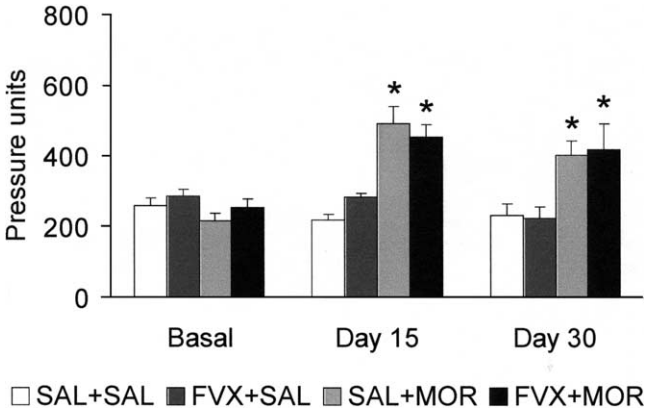

Fig. 3. Effect of acute administration of MOR in arthritic rats chronically treated with FVX on pain response to a paw pressure test. FVX treatment does not modify the antinociceptive effect of MOR during the treatment (*$P < 0.05$ vs. SAL + SAL and SAL + IMI).

Table 1
Evolution of body weight of animals during chronic antidepressant treatment in arthritic rats

	Basal	Day 15	Day 30
SAL	174.10 ± 2.87	208.35 ± 3.49	240.30 ± 4.92
IMI	171.55 ± 2.13	190.72 ± 2.26*	210.94 ± 3.14*
FVX	175.05 ± 1.61	211.45 ± 2.71	246.80 ± 3.75

*$P < 0.001$ vs. SAL.

arthritic rats. At the same time, acute administration of MOR produced an antinociceptive effect in both IMI or FVX or SAL treated rats. However, there was an augmentation of the antinociceptive effect of MOR in IMI treated rats. Weight gain and paw volume only were modified by chronic treatment with IMI. The former parameter had an increase significantly lower than arthritic control rats while the paw volume had an enhancement significantly greater than control rats.

The lack of an antinociceptive effect of IMI chronic treatment in paw pressure is in accordance with previous reports in adjuvant-induced arthritic animals [3,7]. In our work, we used similar doses and time intervals between induction and measurement of the pain response and we have obtained similar results with IMI in paw pressure. With respect to SSRI there is no evidence of a possible analgesic effect of chronic treatment in the paw pressure test in arthritic animals to compare with FVX results.

On the other hand, the antinociceptive effect produced by acute administration of MOR is in concordance with previous reports. Thus, i.v. MOR increased the vocalization threshold to foot pressure in adjuvant-induced arthritis in a dose-dependent manner [10]. Other opiates such as tramadol also induced an antinociceptive effect in this test in arthritic rats [11]. Moreover, in both reports the antinociceptive effect was increased in arthritic compared to normal rats, revealing an enhanced sensitivity to opiates [10,11].

However, the goal of this work was the evaluation of the interaction between chronic antidepressant treatment and MOR acute administration in arthritic rats. In this sense, Baraldi et al. [1] reported that chronic IMI treatment (20 mg/kg, 20 days) showed a 'per se' potent analgesic effect and enhanced MOR analgesia in a hot-plate test. In contrast, chronic administration of IMI (10 mg/kg) during 2 or 4 weeks did not affect MOR antinociception in a paw pressure test in arthritic rats [7]. In this work, we have used IMI 20 mg/kg per day but we have not obtained an analgesic effect per se in a paw pressure test as shown previously in a hot-plate test by Baraldi et al. [1]. However, this lack of effect in the paw pressure test is comparable to that reported with a dose of 10 mg/kg per day by Godefroy et al. [7]. In relation to potentiation of an antinociceptive MOR effect, IMI produced an enhancement of MOR analgesia as shown previously in a hot-plate test using the same dose [1], whereas Godefroy et al. [7] failed to demonstrate an enhancement with 10 mg/kg per day. Therefore, this effect could be related to the dose used.

Table 2
Evolution of tibio-tarsal diameter (mm) during chronic antidepressant treatment in arthritic rats

	Basal	Day 15	Day 30
SAL	12.61 ± 0.78	11.97 ± 0.76	12.78 ± 1.00
IMI	13.49 ± 0.61	15.73 ± 0.92**	15.99 ± 1.02*
FVX	12.75 ± 0.61	12.75 ± 0.76	12.10 ± 0.67

*$P < 0.05$ and **$P < 0.005$ vs. SAL.

Two mechanisms could contribute to the enhanced antinociceptive effect of MOR in IMI treated rats. First, chronic treatment with antidepressants induces an increase in the density of cells expressing mu-opioid receptors in rat forebrain [5,6], and second, chronic peripheral inflammation induces an increased sensitivity to opiates [10,11]. However, the effect on opiate receptor density has been described for IMI and fluoxetine [5,6], and other SSRIs such as FVX, and it is possible that IMI and FVX could display this action in a similar way. This fact does not explain the observed differences in this work. On the other hand, the increased sensitivity to opiates could contribute to differential IMI and FVX effects because IMI induced a higher inflammation as compared to SAL treated rats. This increased inflammation could be related to the influence of serotonin on the peripheral inflammation level. In this sense, Hood et al. reported a worsening of arthritic complaints after treatment with serotonergic antidepressants [8]. It is reasonable to think that FVX, a SSRI, could potentiate peripheral inflammation to a greater extent than IMI, a mixed monoamine reuptake inhibitor. However, IMI is the drug that shows a proinflammatory effect. On the other hand, IMI and fluoxetine have shown a peripheral anti-inflammatory effect in localized paw inflammation [2,15]. Therefore, other factors participating in the IMI mechanism of action could be related to the observed increase in generalized inflammation. In this sense, desipramine, a noradrenaline reuptake inhibitor, induced an increase of edema in arthritic mice whereas fluoxetine, a SSRI, induced an opposite effect [12]. In addition, at later stages of inflammation, the number of peripheral mu-opioid receptors appears to increase and may enhance opioid efficacy [19]. Thus, IMI could lead to a great number of peripheral mu-opioid receptors suitable for MOR binding that could explain the increased antinociceptive effect displayed by MOR after 30 days of IMI treatment.

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