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Effect of chronic and acute administration of fluoxetine and its additive effect with morphine on the behavioural response in the formalin test in rats

A. R. M. Nayebi, M. Hassanpour and H. Rezazadeh

Abstract

Serotonergic systems are involved in the central regulation of nociceptive sensitivity. Fluoxetine, a selective inhibitor of the reuptake of serotonin (5-hydroxytryptamine, 5-HT), was administered orally (0.16, 0.32, 0.8 mg kg^{-1} daily for 7 days), intraperitoneally (0.04, 0.08, 0.16 mg kg^{-1} day^{-1} for 7 days and a single dose of 0.32 mg kg^{-1}) and intracerebroventricularly(10 μg/rat) to rats and nociceptive sensitivity was evaluated using the formalin test (50 μL of 2.5 % formalin injected subcutaneously). The effect of fluoxetine was also studied in the presence of 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT) and after co-administration with morphine. Oral (0.8 mg kg^{-1}), intraperitoneal (0.16 and 0.32 mg kg^{-1}) and intracerebroventricular (10 μg/rat) fluoxetine induced antinociception in the late phase of the formalin test. Furthermore, intrathecal administration of 5-HT (100 μg/rat) induced an analgesic effect. The analgesic effect of fluoxetine (0.16 and 0.32 mg kg^{-1}, i.p.) and 5-HT (100 μg/rat, i.t.) was abolished by pre-treatment with 5,7-DHT (100 μg/rat, i.t.). In addition, the analgesic effect of 5-HT (100 μg/rat, i.t.) was decreased by pre-treatment with naloxone (2 mg kg^{-1}, i.p.). Morphine (5 mg kg^{-1}, i.p.) induced analgesia that was increased by fluoxetine (0.32 mg kg^{-1}, i.p.). These results suggest that fluoxetine has an antinociceptive effect in tonic inflammatory pain through functional alteration of the serotonergic system and also potentiates the analgesic effect of morphine.

Introduction

The formalin test was introduced in 1977 (Dubuisson & Dennis 1977), and has continued to gain popularity as a model of tonic inflammatory pain. The pain produced in the formalin test differs from that of acute nociceptive tests. Specifically, formalin creates a tonic pain secondary to tissue injury, inflammation and central sensitization. Pain behaviour in the formalin test has been postulated to better reflect the pain commonly experienced by humans.

There is much evidence indicating that the serotonergic systems are involved in the central regulation of nociceptive sensitivity (Tonyl et al 1979; Hammond et al 1985; Abhold & Bowker 1990; Giordano 1991; Oyama et al 1996). Increased neurotransmission in ascending or descending serotonin (5-HT)-containing pathways is associated with antinociceptive effects (Lin et al 1980; Berge 1982), while reduced activity in the descending pathways increases the sensitivity to noxious stimulation (Berge 1982). Activation of descending bulbo-spinal neurons by electrical stimulation of the nucleus raphe magnus promotes the release of 5-HT and increases its turnover in the dorsal horn of the spinal cord (Rivot et al 1982; Hammond et al 1985). Serotonin 5-HT_3 and 5-HT_{1A} receptors have been identified

Department of Pharmacology, Faculty of Pharmacy, Tabrize University of Medical Sciences, Tabriz 51664, Iran

R. M. Nayebi, M. Hassanpour, H. Rezazadeh
Correspondence: A. R. M. Nayebi, Department of Pharmacology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz 51664, Iran.
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in the dorsal horn of the spinal cord subserving nociception and analgesia and this, together with the documented involvement of serotonin in these processes, suggests a role for serotonin in pain modulation (Tonyl et al 1979; Oyama et al 1996). Recently we have reported that decreasing testosterone levels (i.e. castration) or blocking of testosterone receptors by flutamide, as a testosterone antagonist, induces analgesia in the late phase of the formalin test that correlates with increase of 5-HT levels in the dorsal horn of the spinal cord (Nayebi & Ahmadiani 1999).

It has also been claimed that disturbances in central serotonergic function may be important in the pathogenesis of depression (Coppen 1967; Van Pragg et al 1973; Heinz et al 1999; Lopez et al 1999) and one of the mechanisms underlying the therapeutic effect of antidepressant drugs may involve changes in neurotransmission (Fuxe et al 1983; Fasmer et al 1989). There is a well known association between chronic pain and depression, thus antidepressant drugs have been employed in the treatment of chronic pain (Walsh 1983; Fasmer et al 1989). Antidepressants with different effects on the reuptake of serotonin and noradrenaline have been used clinically (Feinmann 1985; Finestone & Ober 1990; Max et al 1992). In animal studies, some tricyclic antidepressants may elicit antinociceptive effects when given alone, or may potentiate the analgesic effect of opiates (Ossipov et al 1982; Spiegel et al 1983). This study aims to investigate the effect of chronic and acute administration of fluoxetine, a selective inhibitor of the reuptake of serotonin, and its co-administration with morphine on the formalin test.

Materials and Methods

Chemicals

All chemicals were obtained from Sigma Chemical Co. (USA). Solutions were prepared freshly on the days of experimentation. Fluoxetine and the neurotoxin, 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT) were dissolved in ethanol–water (1:10, v/v) and 0.9% saline containing 0.2 mg mL^{-1} ascorbic acid, respectively. 5-HT creatinine sulphate, morphine hydrochloride and desipramine hydrochloride were dissolved in 0.9% saline. The 5,7-DHT (100 μg/rat) was administered intrathecally 5 days before the experiment. Desipramine (10 mg kg^{-1}) was administered intraperitoneally 30 min before 5,7-DHT to prevent uptake of 5,7-DHT into catecholaminergic neurons and subsequent damage. Drugs were administered in a volume of 10 μL by the intrathecal and intracerebroventricular routes 5 min before formalin injection. For intraperitoneal administration the drugs were injected 30 min before the experiments. Drugs were also administered simply by the oral route.

Subjects

The experiments were carried out on male Wistar rats, 275–300 g, housed in standard polypropylene cages, six per cage, under a 12-h light–dark schedule at an ambient temperature of 23 ± 2°C, and allowed food and water. Following surgical implantation of intrathecal and intracerebroventricular cannulas, rats were housed one per cage to avoid possible displacement or disruption of the cannulas. Experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publ. No 85-23, revised 1985).

Surgical procedures

The rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.). For intrathecal administration, rats were cannulated intrathecally with a PE-10 catheter inserted caudally 8.5 cm from the Atlanto-occipital membrane (Yaksh et al 1976). An indwelling intracerebroventricular cannula was implanted with stereotaxic coordinates, AP: −0.8 mm, L: 1.4 mm, V: 3.3 mm, according to Paxinos & Watson (Myers 1977; Paxinos & Watson 1982).

Formalin test

The rats were placed in a quiet room during the light phase of the light–dark cycle. Before injection of formalin, the rats were placed individually in a transparent plastic cage ($30 \times 30 \times 30$ cm) and were left there at least for 30 min. After the rats had adapted to the cage, 50 μL of diluted formalin 2.5% was subcutaneously injected into the plantar region of the hind paw for noxious stimulation. The pain rating was recorded as follows: 0 – weight is borne evenly on both rear paws; 1 – limps during locomotion or rests with injected paw favoured; 2 – injected paw is elevated with, at most, the nail touching the floor; 3 – injected paw is groomed or bitten (Dubuisson & Dennis 1977). Rats were observed for 60 min by an observer blind to treatment. The results of the formalin test are shown as mean of pain scores during the first 5 min (early phase) and 20–60 min (late phase) after formalin injection.

Histology

All rats with intracerebroventricular cannulas were killed at the end of the procedure and the brain dissected to confirm the exact implantation of the cannula.

Statistical analysis

Descriptive statistics and comparisons of differences between means of data sets were calculated by use of InStat software. The data were expressed as mean ± s.e.m. The significant differences between treatment conditions in each phase were first analysed by Kruskal-Wallis nonparametric analysis of variance test. Statistical significance was accepted at the level of $P < 0.05$. In the case of significant variation ($P < 0.05$), the values were compared by Dunn's multiple comparisons test.

Results

Effect of chronic administration of fluoxetine on formalin test

In rats receiving fluoxetine orally (0.16, 0.32 or 0.8 mg kg^{-1} day^{-1} for 7 days), Kruskal-Wallis nonparametric analysis of variance revealed significant interaction between groups in the late phase of the formalin test (Kruskal-Wallis, $P = 0.0038$). Further analysis showed that pain sensitivity was different ($P < 0.05$) between fluoxetine at a dose of 0.8 mg kg^{-1} and vehicle-treated rats (Figure 1).

Intraperitoneal fluoxetine (0.04, 0.08 or 0.16 mg kg^{-1} day^{-1} for 7 days) markedly affected pain sensitivity in the late phase of the formalin test (Kruskal-Wallis, $P = 0.0145$), producing significant ($P < 0.05$) analgesia at a dose of 0.16 mg kg^{-1} (Figure 2).

Effect of acute administration of fluoxetine and 5-HT on formalin test

There was significant difference between groups in the early phase of formalin-induced pain (Kruskal-Wallis, $P = 0.0002$) and between groups in the late phase (Kruskal-Wallis, $P = 0.0001$). Fluoxetine administered intraperitoneally (0.32 mg kg^{-1}) and intracerebroventricularly (10 μg/rat) produced significant analgesia ($P < 0.05$) in the late phase. Furthermore, 5-HT administered intrathecally (100 μg/rat) induced analgesia ($P < 0.05$) in both early and late phases (Figure 3).

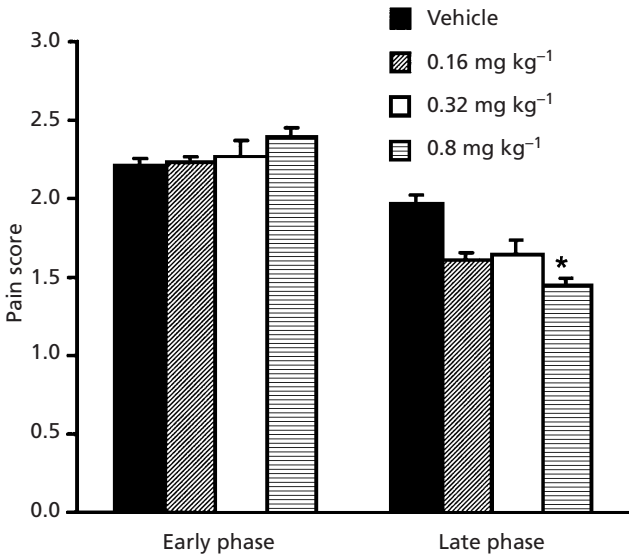

Figure 1 The effect of chronic oral administration of fluoxetine (0.16, 0.32 or 0.8 mg kg^{-1} daily for 7 days) on formalin-induced pain in rats. Each bar represents the mean ± s.e.m. of pain score during the first 5 min (early phase) and 20–60 min (late phase) after formalin injection, n = 6 for each group. Kruskal-Wallis nonparametric analysis of variance followed by Dunn's multiple comparisons test; $*P < 0.05$ when compared with vehicle-treated rats.

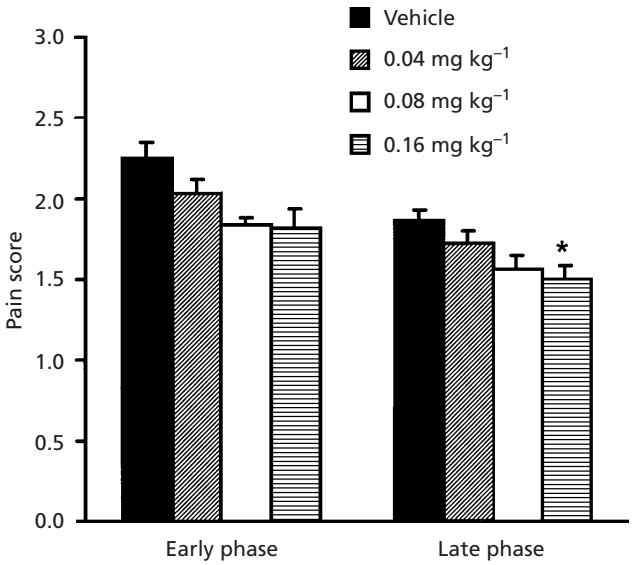

Figure 2 The effect of chronic intraperitoneal administration of fluoxetine (0.04, 0.08 or 0.16 mg kg^{-1} daily for 7 days) on formalin-induced pain in rats. Each bar represents the mean ± s.e.m. of pain score during the first 5 min (early phase) and 20–60 min (late phase) after formalin injection, n = 6 for each group. Kruskal-Wallis nonparametric analysis of variance followed by Dunn's multiple test; $*P < 0.05$ when compared with vehicle-treated rats.

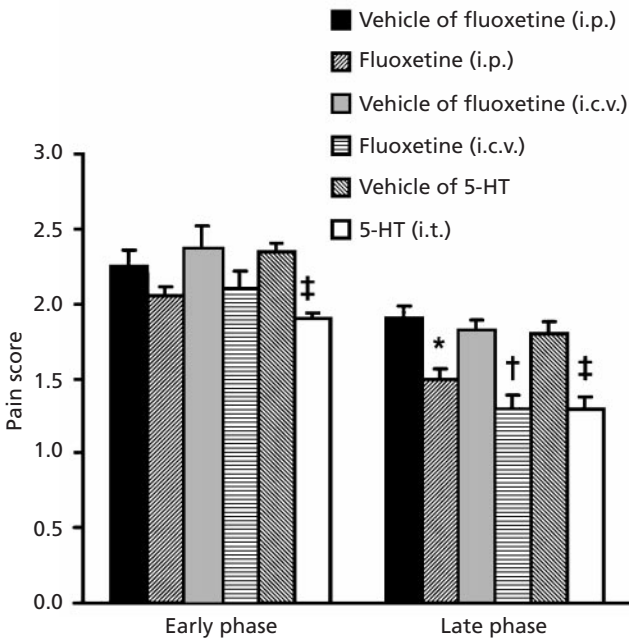

Figure 3 The effect of acute intraperitoneal (0.32 mg kg^{-1}) and intracerebroventricular (10 μg/rat) administration of fluoxetine and intrathecal (100 μg/rat) administration of 5-HT on formalin-induced pain in rats. Each bar represents the mean ± s.e.m. of pain score during the first 5 min (early phase) and 20–60 min (late phase) after formalin injection, n = 6 for each group. Kruskal-Wallis non-parametric analysis of variance followed by Dunn's multiple comparisons test; *$P < 0.05$ when compared with intraperitoneal vehicle-treated rats, †$P < 0.05$ when compared with intracerebroventricular vehicle-treated rats, ‡$P < 0.05$ when compared with 5-HT-vehicle-treated rats.

Effect of 5,7-DHT and naloxone on fluoxetine or 5-HT induced analgesia

Pain sensitivity was significantly different (Kruskal-Wallis, $P = 0.0002$) between groups in the late phase. In groups of rats which were treated by fluoxetine chronically (0.16 mg kg^{-1} daily for 7 days, i.p.) and acutely (0.32 mg kg^{-1}, i.p.), analgesia was reversed significantly ($P < 0.05$) by pre-treatment with 5,7-DHT (100 μg/rat, i.t.). In addition, the analgesic effect of intrathecally administered 5-HT (100 μg/rat) was also reversed significantly ($P < 0.05$) by administration of 5,7-DHT (100 μg/rat, i.t.). Pre-treatment of rats with naloxone (2 mg kg^{-1}, i.p.) significantly decreased ($P < 0.05$) the antinociceptive effect of 5-HT (100 μg/rat, i.t.) (Figure 4).

Effect of fluoxetine on analgesia produced by morphine

Pain responses were significantly different between groups in the early phase (Kruskal-Wallis, $P = 0.0005$) and between groups in the late phase (Kruskal-Wallis, $P = 0.0001$). Morphine (5 mg kg^{-1}) administered intraperitoneally induced marked ($P < 0.05$) analgesia both in early and late phases of the formalin test. This analgesic effect was potentiated ($P < 0.05$) by co-administration of intraperitoneal fluoxetine (0.32 mg kg^{-1}) (Figure 5).

Discussion

Antidepressant drugs have been widely used in the treatment of chronic pain (Butler 1984; Theesen & Marsh 1989; Finestone & Ober 1990; Schweyen 1990; Max et al 1992; Kharkevich & Churukanov 1999; Reimann et al 1999; Sawynok et al 1999). They may be useful even when there is no evidence of clinical depression (Feinmann 1985). Development of selective inhibitors of the reuptake of 5-HT has raised the hope that these drugs may be more effective in patients with chronic pain (Watson & Evans 1985; Davidoff et al 1987).

The nociception test used in this study differs from other commonly used tests, such as the hot-plate and tail-flick methods, in the fact that moderate, continuous pain of some duration is employed, rather than threshold-level pain. The aversive response elicited by intraplantar injection of formalin is composed of two phases (Dubuisson & Dennis 1977). The late phase, expressed 20 min after formalin injection, is considered to be more useful than the early phase in the evaluation of drugs employed clinically in the treatment of inflammatory pain (Dubuisson & Dennis 1977; Hunskaar et al 1985). Our results show that chronic administration of fluoxetine, a selective 5-HT reuptake inhibitor, by the oral and intraperitoneal route, produces analgesia in the late phase of the formalin test. Oral administration was used here to minimize stresses that are usually induced when other routes are used. These results confirm previous reports showing that 5-HT reuptake inhibitors such as alaproclate, citalopram, clomipramine and zimeldine have an analgesic effect in the formalin test (Fasmer et al 1989; Lund et al 1990). Other animal studies have shown a wide variety of effects on nociception; both reduced and increased responses to nociceptive stimuli have been found (Richenberg et al 1985) which may partly reflect differences between drugs, doses and tests. In our study, intraperitoneal and intracerebroventricular administration of fluoxetine, as a single dose, also induced an analgesic effect in the late phase. Other reports have shown that inhibition of

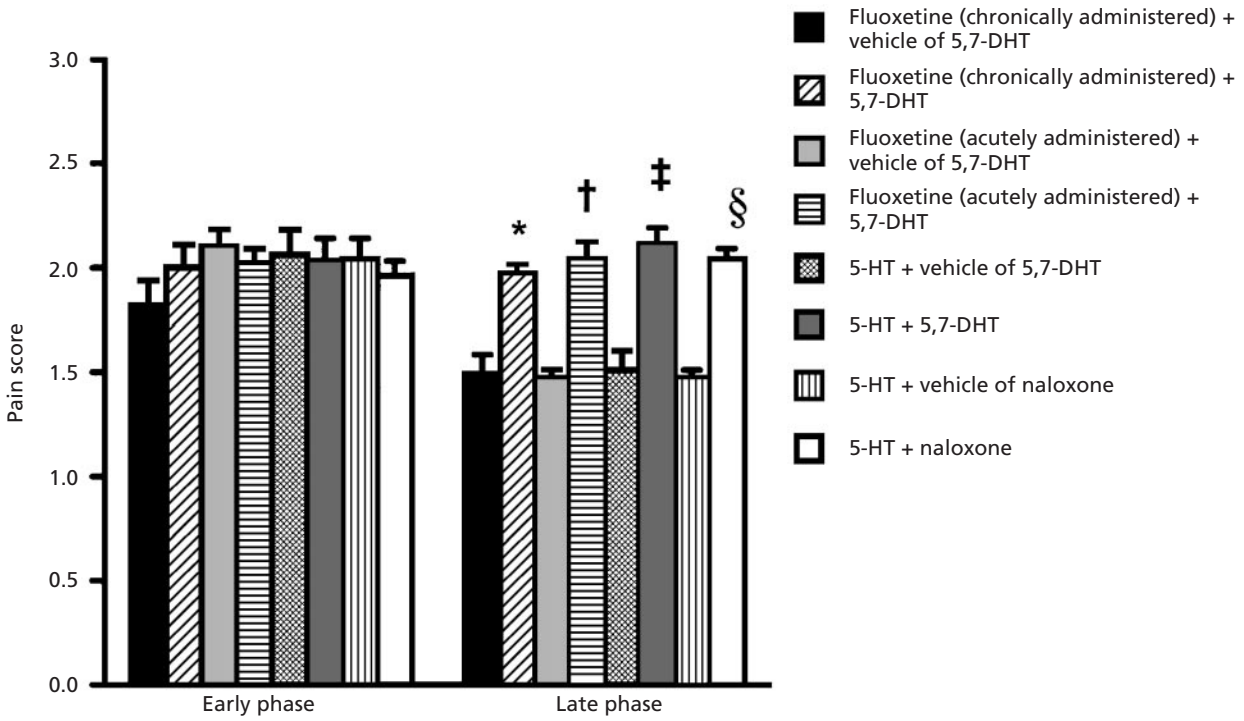

Figure 4 The effect, on formalin-induced pain, of fluoxetine (0.16 mg kg^{-1} daily for 7 days and a single dose of 0.32 mg kg^{-1}, i.p.) and 5-HT (100 μg/rat, i.t.) in 5,7-DHT (100 μg/rat, i.t.) pre-treated rats and 5-HT (100 μg/rat, i.t.) in naloxone (2 mg kg^{-1}, i.p.) pre-treated rats. Each bar represents mean $\pm$ s.e.m. of pain score during the 5 min (early phase) and 20–60 min (late phase) after formalin injection, n = 6 for each group. Kruskal-Wallis nonparametric analysis of variance followed by Dunn's multiple comparison test; *$P < 0.05$ when compared with chronic fluoxetine administration in vehicle-treated rats, †$P < 0.05$ when compared with acute fluoxetine administration in vehicle-treated rats, ‡$P < 0.05$ when compared with 5-HT administration in vehicle-treated rats, §$P < 0.05$ when compared with 5-HT administration in vehicle-treated rats.

5-HT uptake was positively correlated with the plasma concentration of fluoxetine (Lemberger et al 1978). Therefore, we can conclude that analgesic effect of fluoxetine produced by chronic and acute administration are due to an increase in 5-HT level in the CNS. Injection of 5-HT by the intrathecal route also produced analgesia in the late phase of the formalin test. These results are in agreement with different studies that account for the pain-modulating effect of 5-HT (Tonyl et al 1979; Hammond et al 1985; Abhold & Bowker 1990; Giordano 1991; Oyama et al 1996). Recently, we have shown that 5-HT concentration in the dorsal horn of the spinal cord of rats increases in an analgesic state, and this can be followed by decrease of testosterone levels (i.e., castration) or by blocking testosterone receptors (Nayebi & Ahmadiani 1999).

Other studies, using microdialysis in freely moving rats, indicate that fluoxetine increases dopamine concentrations in the pre-frontal cortex (Pozzi et al 1999) and striatum (Malone & Taylor 1999) and also interacts with the GABA(A) receptor complex (Tunnicliff et al 1999). We found that in groups of rats that were pre-treated with 5,7-DHT, as a neurotoxin of the serotonergic system, the analgesic effects of both chronic and acute fluoxetine administration were abolished significantly. This indicates that its antinociceptive effect is exerted through interaction with serotonergic neurons.

According to some reports, activation of serotonergic neurons in the raphe magnus is not necessary for opioid analgesia (Gao et al 1998), but in this study we showed that the antinociceptive effect of intraperitoneally administered 5-HT was decreased by naloxone pre-treatment. This confirms several reports that indicate possible interaction between serotonergic and opioidergic neurons (Abbott & Young 1989; Coda et al 1993). Despite research efforts, morphine remains the standard opioid analgesic for control of post-surgical and cancer-related pain. Although it can substantially decrease even severe pain in most cases at adequately large oral and parenteral doses, the therapeutic potential of morphine

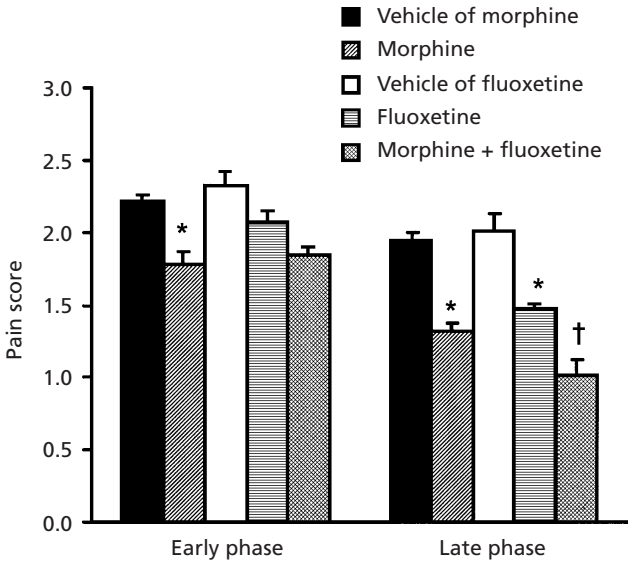

Figure 5 The effect of morphine (5 mg kg^{-1}, i.p.), fluoxetine (0.32 mg kg^{-1}, i.p.) or fluoxetine (0.32 mg kg^{-1}, i.p.) co-administration with morphine (5 mg kg^{-1}, i.p.) on formalin-induced pain in rats. Each bar represents the mean ± s.e.m. of pain score during the 5 min (early phase) and 20–60 min (late phase) after formalin injection, n = 6 for each group. Kruskal-Wallis nonparametric analysis of variance followed by Dunn's multiple comparison test; *$P < 0.05$ when compared with vehicle-treated rats, †$P < 0.05$ when compared with morphine-treated rats.

is often limited by subjective side-effects (nausea, sedation, mood alteration) and, at higher doses, by respiratory depression. Since serotonin is involved in modulation of nociception and also according to several reports about interactions between serotonergic and opioidergic systems (Abbott & Young 1989; Coda et al 1993), fluoxetine, as a specific serotonin reuptake inhibitor, is considered a reasonable candidate enhancer of opioid analgesia. The results of this study show that co-administration of fluoxetine with morphine significantly increases the analgesic effect of morphine in rats. Studies in man have shown that fenfluramine, as a 5-HT releaser, enhances the analgesic potency of morphine without a parallel increase in opioid side-effects (Coda et al 1993). In another study, the antinociceptive potency of morphine was found to be increased by co-injection with fenfluramine (Arends et al 1998). During the initial 2–6 h after fenfluramine administration, fenfluramine releases 5-HT from serotonergic neurons (Orosco et al 1984) and thereafter (e.g. 24 h) serotonergic neurons are partially depleted of neurotransmitter and may be hypofunctional (Shoulson & Chase 1975). Therefore, this drug would not be expected to be a useful drug for pain management. We offer that fluoxetine, which increases synaptic 5-HT without depleting serotonergic neurons, may allow improvement of opioid therapy for persistent pain states, such as cancer pain, by increasing the analgesia-to-side-effect ratios of morphine and similar opioids. However, the exact mechanism of interaction between serotonergic and opioidergic systems is not clear and remains to be elucidated.

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