Antinociceptive Properties of Fenfluramine, a Serotonin Reuptake Inhibitor, in a Rat Model of Neuropathy

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ABSTRACT
Fenfluramine is an indirect agonist of 5-hydroxytryptamine (5-HT) receptors that acts by evoking 5-HT release and blocking 5-HT reuptake in neuronal cells. The current study compared the antinociceptive properties of fenfluramine with those of the tricyclic antidepressants amitriptyline and desipramine in rat models of acute, persistent, and neuropathic pain. In a rat model of neuropathic pain produced by tight ligation of the L5/L6 spinal nerves, i.v. bolus injection of fenfluramine resulted in a dose-dependent and long-lasting (>4 h) blockade of mechanical allodynia (ED50 = 3.5 mg/kg; 95% confidence interval, 2.2–5.4 mg/kg) and cold allodynia (ED50 = 2.4 mg/kg; 95% confidence range, 1.2–4.6 mg/kg). Fenfluramine also prevented tonic pain evoked by the s.c. injection of dilute (5%) formaldehyde solution in a dose-dependent and long-lasting (>4 h) blockade of mechanical allodynia in rats with spinal nerve ligation-induced painful neuropathy or tonic pain in the formalin test. Fenfluramine had differential effects on acute behavioral responses to noxious thermal (heat), chemical (5% formaldehyde solution), and mechanical stimuli; it completely inhibited nociceptive behavior in the acute phase of the formaldehyde solution test and partially inhibited licking and jumping responses in the hot-plate test but did not alter nociceptive thresholds in either the paw pressure test or the tail immersion test. Intracerebroventricular bolus injection of 240 µg of fenfluramine significantly increased mechanical allodynia thresholds; however, the same dose administered spinally by intrathecal bolus injection was ineffective. The inhibitory effects of fenfluramine on mechanical allodynia (and tonic pain behavior in the formaldehyde solution test) were prevented by pretreatment with 10 mg/kg metergoline, a selective antagonist of 5-HT receptors, but not with the µ-opioid receptor antagonist naloxone. These results suggest that fenfluramine produces analgesia in the formaldehyde solution test and the spinal nerve ligation model of neuropathic pain by potentiating, at least in part, supraspinal 5-HT mediated processes.

Neuropathic pain resulting from peripheral nerve injury often leads to chronic and disabling conditions and frequently presents as ongoing pain, allodynia, and hyperalgesia. Because neuropathic pain is often refractory to treatment with conventional analgesics such as opiates and nonsteroidal anti-inflammatory drugs (Arner and Meyerson, 1988; Max et al., 1988; Tanelian and Brose, 1991), considerable research effort has gone into the development of new therapeutic approaches to its treatment.

Antidepressant drugs have been shown to alleviate neuropathic pain in humans (McQuay et al., 1996), but their mechanisms of action are poorly understood. Antidepressants are relatively selective inhibitors of monoamine [e.g., norepinephrine and 5-hydroxytryptamine (5-HT)] reuptake and thereby act to potentiate monoaminergic neurotransmission. Accordingly, their analgesic actions have been attributed to the activation of central norepinephrine and 5-HT systems. The relative contributions of norepinephrine and 5-HT to the analgesic properties of antidepressants have not been clearly elucidated. For example, amitriptyline (a nonselective norepinephrine and 5-HT reuptake inhibitor), desipramine (a relatively selective norepinephrine reuptake inhibitor), and venlafaxine (a relatively selective 5-HT reuptake inhibitor) have been reported to produce antinociception in animal models of painful peripheral neuropathy (Ardid and Guilbaud, 1992; Courteix et al., 1994; Lang et al., 1996; Jett et al., 1997). Other reports, however, have indicated that compounds of these kinds are ineffective in the treatment of neuropathic pain (Coombs et al., 1995; Jett et al., 1997).

To clarify the role of central serotonergic systems in nociceptive processing, we evaluated the antinociceptive properties of fenfluramine in rat models of acute, persistent, and neuropathic pain. Fenfluramine, a selective and indirect agonist of 5-HT receptors, increases synaptic concentrations of 5-HT by evoking direct release of 5-HT from presynaptic terminals and by inhibiting 5-HT reuptake (Garattini et al., 1975; Orosco et al., 1984; Gobbi et al., 1992). It has behavioral and biochemical effects consistent with its ability to

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; SNL, spinal nerve ligation; MPE, maximum possible effect; i.t., intrathecal; AUC, area under the time-effect curve.
increase 5-HT neurotransmission (see reviews by Garattini et al., 1979; Oroscio et al., 1984). We also examined the ability of the 5-HT receptor antagonist metergoline (Fuxe et al., 1975) to block the analgesic effects of fenfluramine. Our results demonstrate that the systemic administration of fenfluramine blocks persistent pain in the hindpaw formaldehyde solution test and produces a metergoline-reversible blockade of allodynia in neuropathic rats. These findings suggest that potentiation of 5-HT neurotransmission leads to analgesia.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing between 90 and 110 g (Harlan Sprague-Dawley Co., Indianapolis, IN, for the Kim and Chung model) or between 220 and 280 g (Simonson Laboratories, Gilroy, CA) were used. Animals were acclimated to the laboratory environment for 5 to 7 days before entering the study. While in the home cage environment, the animals were allowed free access to water and were maintained on a commercial rat diet under standard laboratory conditions. Room temperature was maintained at 20–23°C and room illumination was on a 12-h light/dark cycle (7 AM/7 PM). All experiments were carried out with the approval of the Institutional Animal Care and Use Committee of Elan Pharmaceuticals.

Drugs

(±)-Fenfluramine hydrochloride, desipramine hydrochloride, amitriptyline hydrochloride, naloxone hydrochloride, clonidine hydrochloride, and morphine sulfate pentahydrate were obtained from Sigma Chemical Co. (St. Louis, MO) and were dissolved in saline (0.9% NaCl solution) for i.v. injection. Metergoline was obtained from Research Biochemicals Inc. (Natick, MA) and was dissolved in a 1% ascorbic acid solution for i.v. injection.

Surgical Procedures

Spinal Catheterization. Following procedures described by Yaksh and Rudy (1976), each animal was placed under halothane anesthesia and implanted with a spinal [intrathecal (i.t.)] polyethylene catheter (PE-10) filled with heparinized (25 IU/ml) saline. The catheter measured 8.5 cm in length and terminated at the lumbar enlargement. The external end of the catheter was sutured to the muscle tissue overlying the cisterna magna. The rats were used for testing 5 to 7 days after surgery.

i.c.v. Cannulation. Using halothane anesthesia, a 23-gauge cannula (6 mm in length) containing a 30-gauge needle (4 mm in length) was implanted into the right lateral ventricle (stereotaxic coordinates: A, −1; L, 2; H, 4 mm). A small stainless steel was implanted into the skull adjacent to the cannula, and the assembly was affixed to the calvaria with dental acrylic. Wound margins were sutured closed, and the animal was allowed to recover for 3 to 5 days before the initiation of further experimental procedures.

Spinal Nerve Ligation (SNL). A painful peripheral neuropathy was produced according to procedures described by Kim and Chung (1992). Using halothane anesthesia, the left L5/L6 spinal nerves were exposed then tightly ligated with 6-0 silk sutures at sites distal to the dorsal root ganglia and proximal to the sciatic nerve.

Pain Testing

Noxious response testing was carried out between 9 AM and 5 PM. The observers were blind to the experimental conditions. Tests measuring responses to acute noxious stimuli (i.e., the paw pressure test, tail immersion test, and hot-plate test) were applied sequentially.

Paw-Pressure Test. The animal was placed in a cone restrainer. With an Ugo Basile analgesymeter (Biological Research Apparatus, Varese, Italy), mechanical pressure was applied to right dorsal hindpaw until a flexion response was elicited. The cut-off pressure was 750 g.

Tail Immersion Test. The animal was placed in a cone restrainer, and the end of the tail (5 cm) was immersed in a 50°C water bath (49.5–50.5°C). The pain threshold was measured as the time required to elicit a flick of the tail. The cut-off time was 30 s.

Hot-Plate Test. The animal was placed on a hot-plate (Hot Plate Analgesia Meter model 39D; IITC, Inc., Woodland, CA) maintained at 52.5°C (52.2–52.8°C). The thermal nociceptive threshold was defined as the time required to elicit either a hindpaw lick or a jump. The cut-off time was 60 s.

Formaldehyde Solution Test. Dilute (5%) formaldehyde solution (50 μl) was injected s.c. into the right dorsal hindpaw. The cut-off time was 60 s. Pain behavior was quantified by counting the incidence of paw flexions. Measurements were taken during 1-min observation periods at 10-min intervals, beginning immediately after formaldehyde solution injection and ending 90 min later.

Mechanical and Cold Allodynia Testing. Mechanical and cold allodynia were assessed in rats with a painful peripheral neuropathy produced by SNL as described above. Mechanical allodynia thresholds were determined according to the methods described by Chaplan et al. (1994). SNL rats were placed in an elevated clear-plastic, wire mesh-bottomed cage, divided into individual compartments of 24 × 10 × 15 cm. After a 15- to 20-min acclimation period, eight von Frey filaments (Stoelting, Wood Dale, IL) with bending forces ranging from 0.4 to 15 g were used to determine the 50% mechanical threshold for paw withdrawal using the up-and-down method. Testing was initiated with the 2.0 g filament. If the initial stimulus failed to evoke a paw withdrawal response, a stronger stimulus was presented; in the event of a paw withdrawal, the next weaker stimulus was chosen. In cases in which response thresholds fell outside the range of detection (i.e., continuous positive or negative responses were observed to the limit of the available stimuli), values of 0.25 and 15 g were assigned, respectively. Otherwise, thresholds were calculated by noting the stimulus level at which the first paw withdrawal response occurred and then collecting four additional responses to the continued presentation of stimuli in the above up-and-down manner. The resulting pattern of positive and negative responses were tabulated, and the 50% response threshold was calculated using the formula:

\[
50\% \text{ threshold (g)} = \left( \frac{X_0 + k \delta}{k} \right) / 10,000
\]

where \( X \) is the value (in log units) of the final von Frey hair used, \( k \) is the value (from table) for the pattern of positive/negative responses, and \( \delta \) is the mean difference (in log units) between stimuli.

Cold allodynia was assessed according to the methods described by Choi et al. (1994). A drop (approximately 15 μl) of acetone was applied to the plantar surface of the left paw. Brisk foot withdrawal (typically 2–5 s after application) was recorded as a positive response. The number of positive responses in five trials was expressed as a percentage of the total number of trials. The interval between each application of acetone was approximately 3 to 5 min.

Gross Activity Monitoring

Gross locomotor activity was quantified using a closed-field (31 × 19 × 18 cm) motion detector (Opto-Varimex Mini; Columbus Instruments International Corporation, Columbus, OH). Each rat was allowed to acclimate to the recording environment for 30 min and then administered an i.v. injection of saline or fenfluramine. Gross activity was continuously monitored for approximately 5 h (9 AM to 6 PM), beginning during the acclimation period. The activity count in successive 10-min intervals was determined.

Data Calculations and Analysis

Nociceptive thresholds were converted to percent maximum possible effect (% MPE) according to the formula % MPE = (post-
treatment value – pretreatment value)/(cut-off value – pretreatment value) × 100, using the assigned cut-off values. To calculate ED_{50} values, dose-response data were fitted to a four-parameter logistic function:

$$\text{Effect} = \frac{E_0 + (E_i - E_0)}{1 + (ED_{50}/D)^m}$$

where $E_0$ is the effect at 0 dose of fenfluramine, $E_i$ is the effect at infinite concentration of fenfluramine, and $m$ is Hill coefficient.

Experimental results are presented as mean ± S.E. Statistical significance was determined by a repeated measures ANOVA followed by post hoc two-tailed t tests with the commercial statistical analysis software (StatView, Abacus Concepts, Berkeley, CA). The statistical significance criterion $P$ value was .05.

**Results**

**Effects of i.v. Injection of Fenfluramine, Amitriptyline, and Desipramine on Mechanical and Cold Alloodynia in SNL Rats.** SNL led to mechanical and cold alldynia. Rats with mechanical alldynia thresholds of ≤4 g for 2 to 3 consecutive days were entered into the study. Four groups of SNL rats ($n = 6–14$ in each group) were administered i.v. bolus injections of saline (1 ml/kg) or fenfluramine (1.2, 4, or 12 mg/kg) via the tail vein. Mechanical alldynia thresholds were measured before and at 0.5, 1, 2, and 4 h after treatment. The pretreatment 50% response thresholds were $3.1 ± 0.4$, $2.6 ± 0.2$, $2.0 ± 0.1$, and $3.0 ± 0.2$ g for the above four groups, respectively. As shown in Fig. 1A, fenfluramine, but not saline, blocked mechanical alldynia. At the highest dose tested (12 mg/kg), fenfluramine nearly completely blocked mechanical alldynia ($91 ± 9\%$ MPE) within 0.5 h after i.v. injection. The effect started to decline 1 h postinjection, and at 4 h postinjection was 31% of the peak effect. The antinociceptive effect of fenfluramine was dose-dependent; Fig. 1B shows areas under the time-effect curves (AUCs) for saline and fenfluramine. The calculated ED_{50} was 3.5 mg/kg, with 95% confidence range of 2.2 to 5.4 mg/kg.

As shown in Fig. 2, fenfluramine treatment also suppressed cold alldynia in a dose-dependent manner (ED_{50} = 2.4 mg/kg; 95% confidence range, 1.2–4.6 mg/kg). Cold alldynia was completely blocked within 0.5 h after a single i.v. dose of 12 mg/kg fenfluramine. This effect persisted at nearly the same level during the entire observation period; its effect at 4 h post-treatment was 87% of the peak effect.

Three groups of SNL rats were administered i.v. bolus injections of saline (1 ml/kg, $n = 3$), amitriptyline (4.7 mg/kg, equivalent to one third of 12 mg/kg fenfluramine on a mole basis, $n = 6$), or desipramine (13.5 mg/kg, equivalent to 12 mg/kg fenfluramine on a mole basis, $n = 5$). The doses chosen were maximally tolerable doses; higher doses sacrificed animals. Mechanical and cold alldynia thresholds were measured before and 0.5, 1, 2, and 4 h after treatment. In contrast to fenfluramine, neither amitriptyline nor desipramine blocked mechanical (Fig. 3A) or cold (Fig. 3B) alldynia.

**Effects of i.v. Injection of Fenfluramine on Paw Pressure Test, Tail Immersion Test, and Hot-Plate Test.** Two groups of rats ($n = 8$ in each group) were administered i.v. bolus injections of saline (1 ml/kg) or fenfluramine (12 mg/kg). Nociceptive thresholds in the paw pressure, tail immersion, and hot-plate tests were measured sequentially before and at 0.5, 1, 2, and 4 h post-treatment.

Pretreatment paw withdrawal thresholds in the paw pressure test were $121 ± 21$ and $110 ± 11$ g. Neither saline nor fenfluramine significantly changed nociceptive thresholds during the 4-h observation period (Fig. 4A).

Pretreatment tail-flick thresholds to noxious heat stimuli were $5.2 ± 0.5$ and $5.1 ± 0.4$ s. Neither saline nor fenfluramine significantly changed nociceptive thresholds during the 4-h observation period. Fenfluramine moderately raised the nociceptive threshold; the antinociceptive effect of fenfluramine peaked at $47 ± 11\%$ MPE within 0.5 h after injection and returned to pretreatment baseline levels within 2 h after injection (Fig. 4B).

**Effects of i.v. Injection of Fenfluramine, Amitriptyline, and Desipramine on Formaldehyde Solution Test.** Two groups of rats ($n = 8$ in each group) were administered i.v. bolus injections of saline (1 ml/kg) or fenfluramine (12 mg/kg). One hour later, rats were administered a s.c. injection of 50 µl of 5% formaldehyde solution. In rats treated with saline, formaldehyde solution injection evoked a characteristic biphasic flinch response consisting of an initial, rapidly
decaying acute phase (within 10 min after formaldehyde solution injection) followed by a slowly rising and long-lived (within 90 min after formaldehyde solution injection) tonic phase. Fenfluramine completely blocked both acute and tonic formaldehyde solution-induced flinch responses (Fig. 5).

Three groups of rats (n = 7–8 in each group) were administered i.v. bolus injections of saline (1 ml/kg) and fenfluramine were i.v. bolus injected via the tail vein. B, dose-dependent curve for fenfluramine to block acetone-evoked cold allodynia in neuropathic rats. Anti-cold allodynic effect is expressed as AUC values during the 3-h observation. ED_{50} was calculated according to the formula described in Materials and Methods.

Effects of i.v. and i.t. Injections of Fenfluramine on Mechanical Allodynia in SNL Rats. Three groups of rats (n = 6–8 in each group) were administered i.v. bolus injections of saline (1 ml/kg), amitriptyline (4.7 mg/kg), or desipramine (13.5 mg/kg). Ten minutes later, rats were administered a s.c. injection of formaldehyde solution. Same as in the above experiment, formaldehyde solution produced acute and tonic flinch responses. As shown in Fig. 6, to simplify results, the tonic phase flinches were expressed as the AUC_{10–90 min}. Compared with the saline control group, amitriptyline did not alter acute flinch responses but significantly potentiated tonic flinch responses. In contrast, desipramine blocked acute but not tonic flinch responses (Fig. 6).

Effects of i.e.v. and i.t. Injections of Fenfluramine on Mechanical Allodynia in SNL Rats. Three groups of rats (n = 6–8 in each group) were administered i.e.v. bolus injections of saline (10 µl followed by 10-µl saline flush), fenfluramine (240 µg/rat in 10 µl followed by 10-µl saline flush), or the α2-adrenoceptor agonist clonidine (20 µg/rat in 10 µl followed by 10-µl saline flush). Mechanical allodynia thresholds were measured before and at 0.5, 1, 1.5, 2, and 3 h after treatment. Pretreatment 50% response thresholds were 2.4 ± 0.2, 2.2 ± 0.2, and 2.3 ± 0.2 g, respectively, for these three groups. The i.e.v. injection of saline did not significantly change mechanical allodynia thresholds during the 3-h observation period. The i.e.v. injection of fenfluramine at a dose approximating one-tenth of the fully effective i.v. dose (i.e., 12 mg/kg) significantly raised mechanical allodynia thresholds; however, the antinociceptive effect (MPE, 49 ± 20%) was approximately 50% of that produced by i.v. fenfluramine injection. The i.e.v. injection of morphine completely blocked mechanical allodynia (MPE, 100 ± 0%) for at least 3 h (Fig. 7A).

Fig. 2. A, time-effect curves for fenfluramine (1.2–12 mg/kg) to block acetone-induced cold allodynia in the Kim and Chung rat model of peripheral neuropathy (n = 6 in each group). The anti-cold allodynic effect (mean ± S.E.) is expressed as percentage of acetone response. Saline (1 ml/kg) and fenfluramine were i.v. bolus injected via the tail vein. B, dose-dependent curve for fenfluramine to block acetone-evoked cold allodynia in neuropathic rats. Anti-cold allodynic effect is expressed as AUC values during the 3-h observation. ED_{50} was calculated according to the formula described in Materials and Methods.

Fig. 3. Effects of amitriptyline (4.7 mg/kg) and desipramine (13.5 mg/kg) on von Frey filament-induced mechanical allodynia (A) or acetone-induced cold allodynia (B) in neuropathic rats (n = 3–6 in each group). The antimechanical allodynic effect (mean ± S.E.) is expressed as % MPE, whereas the anti-cold allodynic effect (mean ± S.E.) is expressed as percentage of acetone response as described in Materials and Methods. Saline (1 ml/kg) and fenfluramine were i.v. bolus injected via the tail vein.
Effects of Metergoline and Naloxone on Antimechanical Allodynic Effect of Fenfluramine in SNL Rats.

Two groups of SNL rats ($n = 6$ in each group) were administered s.c. injections of vehicle (1% ascorbic acid, 4 ml/kg) or metergoline (10 mg/kg); 2.5 h later, both groups of rats were administered i.v. bolus injections of 10 mg/kg fenfluramine. Mechanical allodynia thresholds were measured before the first treatment (the vehicle or metergoline), 2 h after the first treatment, and 0.5, 1, 1.5, 2, and 3 h after the second treatment (fenfluramine). Pretreatment 50% response thresholds were 2.5 ± 0.2 and 2.5 ± 0.4 g, respectively, for these two groups. Neither vehicle nor metergoline changed mechanical allodynia thresholds (50% response thresholds, 2.1 ± 0.3 and 2.2 ± 0.2 g, respectively) measured 2.5 h after treatment. Intravenous injection of 10 mg/kg fenfluramine produced an antimechanical alldynic effect (94 ± 6% MPE at 0.5 h after injection) in vehicle-pretreated rats that was compatible to that produced in an earlier experiment by 12 mg/kg fenfluramine (Fig. 1A). Metergoline nearly completely blocked the antimechanical alldynic effect of fenfluramine, with 3.5 ± 2.7% MPE at 0.5 h after injection of 10 mg/kg fenfluramine (Fig. 8A).

Two groups of SNL rats ($n = 6$ in each group) were pre-
treated with a single i.p. injection of saline (1 ml/kg) or naloxone (5 mg/kg) 0.5 h before receiving a single i.v. bolus injection of 10 mg/kg fenfluramine. This treatment with high dose (5 mg/kg) of naloxone was shown to be effective in blocking morphine (10 mg/kg) analgesia in the hot-plate test for at least 3 h in a preliminary study (data not shown).

Mechanical allodynia thresholds were measured before the first treatment (saline or naloxone), 0.5 h after the first treatment, and 0.5, 1, 1.5, 2, and 3 h after the second treatment (fenfluramine). Pretreatment 50% response thresholds were 2.6 ± 0.5 and 2.4 ± 0.4 g, respectively. Neither vehicle nor naloxone changed mechanical allodynia thresholds (50% response thresholds: 2.1 ± 0.3 and 2.2 ± 0.2 g, respectively, for the two groups 0.5 h after saline or naloxone treatment). Intravenous injection of 10 mg/kg fenfluramine produced comparable antiallodynic effects in saline- or naloxone-pre-treated rats throughout the 3-h observation period (Fig. 8B).

**Effects of Metergoline on Fenfluramine-Induced Inhibition of Formaldehyde Solution Flinch Responses in Rats.** Four groups of rats (n = 8 in each group) received two treatments: 1) ascorbic acid plus saline, 2) ascorbic acid plus fenfluramine, 3) metergoline plus saline, and 4) metergoline plus fenfluramine. The first treatment was a single s.c. injection of 1% ascorbic acid (4 ml/kg) or metergoline (10 mg/kg), and the second treatment was a single i.v. bolus injection of saline (1 ml/kg) or fenfluramine (6 mg/kg) 1 h after the first treatment. At 30 min later, rats were administered an s.c. injection of 5% formaldehyde solution. Flinch responses were expressed flinch counts for acute phase responses and AUC10–90 min for tonic phase responses. Compared with vehicle control, fenfluramine, but not metergoline, significantly inhibited formaldehyde solution-induced acute and tonic flinch responses. Metergoline significantly restored fenfluramine-induced inhibition of tonic but not acute flinch responses (Fig. 9).

**Effect of Fenfluramine on Gross Activity in Rats.** Two groups of rats (n = 11–12 per group) were administered i.v. bolus injections of saline (1 ml/kg) or fenfluramine (12 mg/kg). Gross locomotor activity was monitored continuously for 4.5 h from the beginning of a 0.5-h pretreatment acclimation period to 4 h postinjection. As shown in Fig. 10, gross activity counts declined during the acclimation period. Intravenous injection of fenfluramine caused transient (approximately 1–3 min) tremors, which accounted for a sharp and short-lived increase in gross activity counts. After this, activity counts declined but overall were significantly (P < .05) higher than those for saline-treated animals.

**Discussion**

The essential role of injury-induced sensitization of spinal dorsal horn neurons in the development and maintenance of neuropathic pain has been well documented (Woolf, 1983;Coderre et al., 1993; Woolf et al., 1994). Indeed, in the Kim and Chung model (1992) of neuropathic pain, tight ligation of the L4/L5 spinal nerves produces signs of ongoing pain, allodynia, and hyperalgesia through injured (and intact) fibers or...
the dorsal root ganglion cells, leading to spinal sensitization (Yoon et al., 1996). The Bennett and Xie model (1988) is another commonly used experimental model of mononeuropathy characterized by thermal and mechanical hyperalgesia, is produced by chronic constriction of the sciatic nerve and subsequent spinal sensitization. The present study showed that fenfluramine produced a long-lasting, dose-dependent blockade of mechanical and cold allodynia in the Kim and Chung rat model of neuropathy. Fenfluramine appears to be a specific blocker of neuropathic pain. This conclusion is supported by the following findings. 1) Although sedation may inhibit mechanical and cold allodynia in a nonspecific manner, our cage gross activity study showed that fenfluramine did not sedate the animals. Instead, it slightly increased gross activity. 2) Fenfluramine differentially blocked acute behavioral responses to noxious stimuli. Although fenfluramine completely blocked formaldehyde solution-induced flinch behavior in the acute phase, it partially inhibited licking/jumping responses to heat stimuli, reflected by a smaller inhibitory magnitude and a shorter action duration. Fenfluramine also did not alter nociceptive thresholds in either the paw pressure test or the tail immersion test. These results are consistent with previous reports that fenfluramine neither increases tail-flick responses to heat stimuli in the tail immersion test (Rochat et al., 1982) nor produces analgesia in the radiant heat tail-flick test (Arends et al., 1998). 3) Fenfluramine inhibited formaldehyde solution-induced flinch response in the tonic phase. It is known that the tonic phase of the formaldehyde solution test reflects an injury-induced spinal sensitization of dorsal horn neurons (Dickenson and Sullivan, 1987; Codere et al., 1990), which is fundamental to the development of neuropathic pain (see above). Analgesic effects of antidepressant agents and 5-HT have been shown to involve the opioid system in the central nervous system (Ardid et al., 1991; Ardid and Guilbaud, 1992; Yang et al., 1994; Sierralta et al., 1995). We found that naltrexone at a dose of 5 mg/kg did not block fenfluramine-induced analgesia. In contrast, the selective 5-HT receptor antagonist metergoline (Fuxe et al., 1975) completely blocked fenfluramine-induced inhibition of mechanical allodynia in neuropathic rats and tonic flinch responses in the formaldehyde solution test. Metergoline has been previously reported to block the antinociceptive effects of fenfluramine in the hot-plate test (Rochat et al., 1982). All of these results indicate that the antinociceptive effects of fenfluramine are due to the indirect activation of 5-HT receptors (Garattini et al., 1975; Orosco et al., 1984; Gobbi et al., 1992). It should be pointed out that the role of activation of 5-HT system is controversial in the treatment of neuropathic pain. It has been reported that venlafaxine, a relatively selective 5-HT reuptake inhibitor, relieved thermal hyperalgesia in the Bennett and Xie model of mononeuropathy (Lang et al., 1996). Ardid and Guilbaud (1992) also suggested that antidepressants produce analgesia in the Bennett and Xie model of mononeuropathy mainly by interfering with 5-HT reuptake. On the other hand, it was reported that s.c. administered fluoxetine, a selective 5-HT reuptake inhibitor, at doses up to 30 mg/kg, which was believed to maximally inhibit 5-HT reuptake, did not block either mechanical allodynia or mechanical hyperalgesia in the Kim and Chung model of neuropathy (Jett et al., 1997). It is difficult to explain the inconsistency because the pharmaceutical agents, doses,
administration methods, and animal models that were used varied. Nevertheless, the results of the current investigation strongly support the conclusion that activation of 5-HT systems in the central nervous system blocks neuropathic pain.

In the literature, the effects of amitriptyline and desipramine on neuropathic pain and persistent pain also are not consistent. It was reported that acute i.v. injections of amitriptyline (0.5 mg/kg) or desipramine (2 mg/kg) blocked thermal hyperalgesia in the Bennett and Xie model of mononeuropathy. In contrast, i.c.v. injection of desipramine (0.25–8 mg/kg) blocked mechanical hyperalgesia in the rat model of diabetic neuropathy produced by streptozotocin (Courteix et al., 1994). It was also reported that systemic administrations of amitriptyline (20 mg/kg) and desipramine (3–100 mg/kg) blocked tonic flinch responses in the formaldehyde solution test (Acton et al., 1992; Jett et al., 1997). In contrast, it was reported that acute treatment with amitriptyline or desipramine did not block established mechanical allodynia in either the Bennett and Xie model (Coombs et al., 1995) or the Kim and Chung model (Jett et al., 1997). Our results show that in contrast to fenfluramine, neither amitriptyline (4.7 mg/kg, equivalent to one third of 12 mg/kg fenfluramine at the mole base) nor desipramine (13.5 mg/kg, equivalent to 12 mg/kg fenfluramine at the mole base) administered i.v. at the maximum tolerable doses blocks mechanical or cold allodynia in neuropathic rats. In addition, neither amitriptyline nor desipramine blocks persistent pain in the formaldehyde solution test. In fact, amitriptyline potentiates formaldehyde solution-induced hyperalgesia.

It is interesting to note that fenfluramine inhibits the paw-licking or jumping response in the hot-plate test but does not inhibit the tail-flick response in the tail immersion test despite the fact that both responses are evoked by noxious thermal (heat) stimuli. Similar results were also reported previously (Rochat et al., 1982). These observations suggest that fenfluramine acts at, at least in part, a supraspinal level. It is known that the tail-flick response to noxious stimuli is a simple spinal reflex, whereas the paw-licking response to noxious thermal stimuli is a complex one involving the functional integration of brain and spinal cord processes. For example, the tail-flick response to noxious stimuli is preserved in the chronic spinalized animals (Franklin and Abbott, 1989; Advokat, 1993). The hypothesis that fenfluramine acts, at least in part, at the supraspinal level is strongly supported by the fact that spinal i.t. injection of fenfluramine at a dose (240 μg/rat), approximately equivalent to one tenth of the fully effective systemic dose (12 mg/kg), does not block mechanical allodynia during a 3-h observation period in neuropathic rats. In contrast, i.c.v. injection of fenfluramine at the same dose produces a partial analgesia compared with that produced by systemic administration. Although the laterial ventricular area is a site of action for fenfluramine, other brain regions that mediate fenfluramine-induced analgesia need to be determined precisely through microinjection mapping and other techniques.

Our observation that spinal i.t. injection of fenfluramine is ineffective in producing analgesia in neuropathic rats is rather surprising. In the past, much research interest has focused on the role of descending spinal serotonergic pathway on pain modulation. Although it was reported that i.t. administration of 5-HT increased sensitivity to noxious stimuli (Zemlan et al., 1988; Millan et al., 1991; Bervoets and Millan, 1994), spinal administration of 5-HT is generally shown to reduce sensitivity to noxious stimuli in animals (Wang, 1977; Yaksh and Wilson, 1979; Kuraishi et al., 1985). It was recently reported that lumbar spinal transplantation of serotonergic neurons, which was proved to increase spinal 5-HT synthesis, alleviated mechanical and cold allodynia and thermal hyperalgesia in the Bennett and Xie model of mononeuropathy (Etgen et al., 1997). Our negative results with fenfluramine may be related to its properties of indirect activation of 5-HT receptors or its distribution in the spinal cord.

In summary, i.v. administration of fenfluramine blocks mechanical and cold allodynia in the Kim and Chung rat model of peripheral neuropathy and formaldehyde solution-induced persistent (“chronic”) pain but has differential effects on acute pain induced by noxious thermal, mechanical, or chemical stimuli. In contrast to fenfluramine, amitriptyline or desipramine, at the maximum tolerable doses, is ineffective in blocking either allodynia in neuropathic rats or tonic flinch responses in the formaldehyde solution test. The administration of i.c.v., but not i.t., fenfluramine produces a partial analgesia in neuropathic rats. The antinociception of fenfluramine is nearly entirely prevented by pretreatment with the 5-HT receptor antagonist metergoline but not with the μ-opioid receptor antagonist naloxone. The results of the present study suggest that fenfluramine, at least in part, acting supraspinally in the lateral ventricular area and other areas in the brain, produces a specific blockade of neuropathic pain through indirect activation of 5-HT receptors, presumably through direct release of 5-HT and inhibition of 5-HT reuptake from neuronal synapses.

References


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