Efficacy of Duloxetine, a Potent and Balanced Serotonin-Norepinephrine Reuptake Inhibitor in Persistent Pain Models in Rats

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ABSTRACT

5-Hydroxytryptamine (serotonin) (5-HT) and norepinephrine (NE) are implicated in modulating descending inhibitory pain pathways in the central nervous system. Duloxetine is a selective and potent dual 5-HT and NE reuptake inhibitor (SNRI). The ability of duloxetine to antagonize 5-HT depletion in para-chloroamphetamine-treated rats was comparable with that of paroxetine, a selective serotonin reuptake inhibitor (SSRI), whereas its ability to antagonize NE depletion in /H9251-methyl-L-tyrosine-treated rats was similar to norepinephrine reuptake inhibitors (NRIs), thionisoxetine or desipramine. In this paradigm, duloxetine was also more potent than other SNRIs, including venlafaxine or milnacipran and amitriptyline. Low doses of the SSRI paroxetine or the NRI thionisoxetine alone did not have an effect on late phase paw-licking pain behavior in the formalin model of persistent pain; however, when combined, significantly attenuated this pain behavior. Duloxetine (3–15 mg/kg intraperitoneal) significantly attenuated late phase paw-licking behavior in a dose-dependent manner in the formalin model and was more potent than venlafaxine, milnacipran, and amitriptyline. These effects of duloxetine were evident at doses that did not cause neurologic deficits in the rotord test. Duloxetine (5–30 mg/kg oral) was also more potent and efficacious than venlafaxine and milnacipran in reversing mechanical allodynia behavior in the L5/L6 spinal nerve ligation model of neuropathic pain. Duloxetine (3–30 mg/kg oral) was minimally efficacious in the tail-flick model of acute nociceptive pain. These data suggest that inhibition of both 5-HT and NE uptake may account for attenuation of persistent pain mechanisms. Thus, duloxetine may have utility in treatment of human persistent and neuropathic pain states.

Persistent pain results from changes in sensitivity within both ascending and descending pain pathways in the brain and the spinal cord (Wall, 1999; Hunt and Mantyh, 2001). Although a number of neurotransmitters likely modulate the ascending and descending pain pathways, 5-hydroxytryptamine (serotonin) (5-HT) and norepinephrine (NE) have been implicated as mediators of endogenous analgesic mechanisms in the descending pain pathways (Yaksh, 1985; Fields et al., 1991; Jones, 1991; Clark and Proudfit, 1993; Willis and Westlund, 1997; Fields and Basbaum, 1999). Descending input from the cortex, hypothalamus, and amygdala and pretectal nucleus is provided to the midbrain periaqueductal gray, the rostroventral medulla, and the dorsolateral pontomesencephalic tegmentum. Both rostroventral medulla and dorsolateral pontomesencephalic tegmentum project to the spinal dorsal horn. The descending pain pathways form an endogenous pain-modulating circuit consisting of both a descending inhibitory and facilitatory component (Zhuo and Gebhart, 1997; Ren et al., 2000; Millan, 2002; Ren and Dubner, 2002).

Neuropathic pain is a type of persistent pain that arises from functional changes occurring in the pain sensory system after peripheral nerve injury. Sustained or prolonged stimulation of nociceptive afferents (afferent barrage) due to tissue damage or peripheral nerve injury has been implicated in the initiation and maintenance of central neuroplastic changes (Woolf and Mannion, 1999; Ren and Dubner, 2002) culminating in central neuronal hyperexcitability, possibly due to reduced inhibition of nociceptive neurons by neurotransmitters, such as 5-HT and NE in both spinal and supraspinal structures (Ren et al., 2000; Millan, 2002; Ren and Dubner, 2002). The resultant state of central sensitization can produce an ongoing condition of spontaneous, persistent pain as

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine or serotonin; NE, norepinephrine; TCA, tricyclic antidepressant; SNRI, serotonin norepinephrine reuptake inhibitor; /H9251-MMT, /H9251-methyl-L-tyrosine; p-CA, para-chloroamphetamine; MPE, maximum possible effect; NRI, norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor.
well as an increased sensitivity to painful stimuli (hyperalgesia) or to normally nonpainful mechanical or thermal stimuli (allodynia) (Woolf and Mannion, 1999). Although the precise mechanisms involved in the pathogenesis of persistent pain states are not fully understood, there is a growing recognition that disinhibition and imbalance of 5-HT and NE in endogenous pain inhibitory pathways could contribute to persistent pain mechanisms (Ren et al., 2000).

Tricyclic antidepressants (TCAs) that have 5-HT and/or NE reuptake inhibitor properties, such as amitriptyline and desipramine, are used in the clinical management of persistent pain conditions (Sindrup and Jensen, 1999; Collins et al., 2000; Lynch, 2001) but are limited by side effects including sedation, hypotension, anticholinergic effects, and cardiovascular abnormalities likely due to the affinity of these drugs for cholinergic, adrenergic, and other receptors. In addition, drugs such as amitriptyline have also been suggested to have Na+ channel blocking properties that could also be contributing to their clinical efficacy.

Duloxetine hydrochloride is a potent and selective 5-HT and NE reuptake inhibitor (SNRI) (Wong et al., 1993) that lacks significant affinity for muscarinic, histamine1, α1-adrenergic, dopamine, 5-HT1A, 5-HT1B, 5-HT1D, 5-HT2A, 5-HT2C, opioid receptors, and ion channels including Na+ channels (Bymaster et al., 2001). Duloxetine has also been shown in several in vivo studies to be a balanced inhibitor of 5-HT and NE reuptake (Wong et al., 1993; Wong and Bymaster, 2002). Recently, duloxetine has been shown in humans to be safe and effective in the treatment of depression (Detke et al., 2002). Recently, duloxetine has been shown in humans to be safe and effective in the treatment of depression (Detke et al., 2002; Goldstein et al., 2002; Nemeroff et al., 2002). Because of the proposed role of 5-HT and NE as key mediators of descending pain pathways, duloxetine was evaluated in animal models of persistent and neuropathic pain and in a model of acute nociceptive pain.

The formalin model of persistent pain involves moderate, continuous pain generated by injured tissue after subcutaneous injection of formalin into the hindpaw of the rat, consisting of an early and late phase of paw-licking behavior where the late phase is considered to be an index of persistent pain mechanisms (Shibata et al., 1989; Wheeler-Aceto et al., 1990; Tjolsen et al., 1992). Tight ligation of lumbar L5/L6 spinal nerves (Kim and Chung, 1992) results in signs of neuropathic pain behavior, including mechanical allodynia behavior, and is a well accepted model of neuropathic pain mechanisms (Kim and Chung, 1992). The tail-flick model, in contrast, measures an acute nociceptive response to radiant heat and does not involve a tissue or nerve insult.

Duloxetine was evaluated in the formalin model of persistent pain and the L5/L6 spinal nerve ligation model of neuropathic pain at doses of drug that caused reuptake blockade of 5-HT and NE in vivo and compared with other reuptake inhibitors, including venlafaxine and milnacipran. In addition, the effects of duloxetine, venlafaxine, and milnacipran on α-MMT-induced depletion of NE and p-chlorophenylalanine (p-CA)-induced depletion of 5-HT, in vivo, were also compared with those of paroxetine, thionisoxetine, amitriptyline, and desipramine.

**Materials and Methods**

**Animals.** Rats were maintained at constant temperature and light (12-h light/dark) for 4 to 7 days before the studies. All testing was conducted in the light cycle and the testing room temperature was maintained at 21 to 23°C. Animals had free access to food and water at all times before the day of the experiment. All experiment protocols were approved by the Eli Lilly & Co. Institutional Animal Care and Use Committee.

**Drugs and Injections.** Duloxetine hydrochloride, (+)-N-methyl-3-(1-naphthalenyl)oxy-3-(2-thienyl) propanamine, paroxetine (Paxil), and thionisoxetine were synthesized at Eli Lilly & Co. (Indianapolis, IN) for research purposes. Amitriptyline hydrochloride and desipramine hydrochloride were purchased from Sigma/RBI (Natick, MA). Gabapentin (Neurontin) and Venlafaxine (Effexor) (Lang et al., 1996) were purchased from Bergen Brunswig Drug Co. (Louisville, KY) for research purposes. Drugs or vehicle was administered by i.p. injection or by oral gavage (p.o.) in a volume of 1 to 5 ml/kg. Vehicle for all test compounds was double-distilled water. A pretreatment time of 30 min was chosen for i.p. administration and 60 min for oral administration.

**Blockade of Transporters in Vivo.** Male SD rats (180–230 g; Harlan, Indianapolis, IN) were used for these experiments. Inhibition of the transporters in vivo was determined for 5-HT transporters by blockade of p-CA (10 mg/kg)-induced depletion of serotonin in rat brain and for NE transporters by α-methyltyrosine (α-MMT; 6.25 mg/kg)-induced depletion of norepinephrine in rat cerebral cortex as described previously (Koch et al., 2003). Monoamine concentrations were determined by high-performance liquid chromatography/electrochemical techniques (Koch et al., 2003).

**Formalin Model.** The formalin test was performed in custom made Plexiglas boxes 25 × 25 × 20 cm (length × width × height) in size according to Coderre et al. (1993) based on Shibata et al. (1989) and Wheeler-Aceto et al. (1990). Early (0–5 min) and late (15–40 min) phases of paw-licking behavior were quantitated visually by a blinded observer using an automated behavioral timer, as described previously (Simmons et al., 1998). A mirror placed at the back of the box allowed the unhindered observation of the formalin-injected paw. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, MI) weighing 200 to 230 g were acclimatized individually in the cubicles at least 30 min before the experiment. Formalin (50 μl of a 5% solution in saline) was injected subcutaneously into the dorsal lateral surface of the right hind paw with a 27-gauge needle. Observation started immediately after the formalin injection. Formalin-induced pain was quantified by recording in 5-min intervals the number of seconds each licking event lasted. These recordings were made for 50 min after the formalin injection, using an automated behavioral timer connected to an IBM PC. Scoring of the formalin test was performed according to Codere et al. (1993), Abbott et al. (1995), and Simmons et al. (1998). The sum of time spent licking in seconds from time 0 to 5 min was considered the early phase, whereas the late phase was taken as the sum of seconds spent licking from 15 to 40 min. Drugs were administered intraperitoneally 30 min before formalin.

**L5/L6 Nerve Ligation (Chung Model).** Male Sprague-Dawley rats (Harlan) weighing 150 to 200 g at the time of surgery were used for these experiments. Surgery was performed as described previously (Kim and Chung, 1992). Briefly, neuropathic injury was produced by tightly ligating the left L5 and L6 spinal nerves under gas anesthesia with a mixture of isoflurane (3% for induction and 2% for maintenance) and O2. After surgery, development of neuropathic pain was evaluated daily by measuring mechanical sensitivity of the injured paw to von Frey filaments with incremental bending forces (0.5–15 g) as described by Chaplan et al. (1994). Animals were considered to be neuropathic when they exhibited mechanical allodynia, i.e., paw flinch behavior response to the application of a bending force of less than 2 g for 2 days. Test drug or vehicle was administered p.o., and mechanical threshold for paw flinching was measured at 0.5, 1, 2, 3, 4, and 6 h after dosing. Measurement of the mechanical threshold for paw flinching was also done before surgery (preoperative control). Data are expressed as the threshold force required to elicit a response (grams) and are means ± S.E.M.
Roterdor Test. The ability of the tested compounds to induce ataxia was examined using an automated accelerating rotordor (Omnitech Electronics Inc., Columbus, OH) connected to an IBM PC computer (Simmons et al., 1998). For training and testing purposes, the rotordor was set up to accelerate to 17 rpm in 5 s and maintain that speed for 40 s. Male Sprague-Dawley rats (Charles River Breeding Laboratories) weighing 200 to 230 g were given three training trials to learn to maintain posture on the rotordor before the day of drug testing. The next day, rotordor testing was conducted both at 1 and at 2 h after administration of drug. Rats that maintained posture and did not fall off the rotordor were given a maximum score of 40 s.

Tail-Flick Latency Test. Adult male Sprague-Dawley rats weighing 200 to 230 g (Harlan) were used for these experiments. The tail-flick measurement was made using the Ugo Basile tail-flick unit (Ugo Basile, Comerio, Italy) based on a modification of a method described originally by D’Amour and Smith (1941). The tail-flick unit consisted of an infrared heat source (50-W bulb) of adjustable intensity that was set at 40 units (determined to elicit tail-flick latency of 2–4 s as baseline in naive animals). The infrared source was focused to the base of the tail. The latency time (in seconds) required by the rat to reach the thermal threshold for pain and flick its tail was recorded. Each rat was given one test to determine baseline latency to tail-flick with a cutoff of 10 s set to avoid tissue damage. Animals were then given drug or vehicle and tested at varying time points after administration. Data were calculated as %MPE (maximum possible effect) and expressed as means ± S.E.M. %MPE was calculated using the following formula: %MPE = ([test latency - baseline latency]/cut-off latency (10 s) - baseline latency)) × 100.

Data Analysis for All Tests. Data were analyzed by analysis of variance and Dunnett’s t test and Tukey’s test using JMP version 3.2 (SAS Institute, Cary, NC) statistical software. A significance of p < 0.05 was considered to be statistically different from vehicle group. All data are presented as means ± S.E.M.

Results

Antagonism of p-CA-Induced Depletion of Brain 5-HT Concentrations by Uptake Inhibitors (Fig. 1A). Duloxetine was compared with a number of compounds known to be inhibitors of 5-HT reuptake. The potency of compounds to inhibit the p-CA-induced depletion of whole rat brain 5-HT is shown in Fig. 1A. Data are expressed as percentage of control, where each individual experiment was plotted against its own control group (overall mean control 5-HT = 2.76 ± 0.34 nmol/g; mean p-CA-treated 5-HT = 1.48 ± 0.27 nmol/g, n = 6 experiments, 3–5% variability between each experiment). Compounds were administered i.p. 1 h before 10 mg/kg i.p. p-CA, which depleted brain 5-HT between 37 and 56%, 2 h after p-CA administration, paroxetine was the most potent blocker of the p-CA-induced depletion of brain 5-HT, the dose required to block 5-HT depletion by 50% (ED50 dose) was 1.2 mg/kg i.p. followed by duloxetine (ED50 = 2.3 mg/kg i.p.), venlafaxine (ED50 = 5.9 mg/kg i.p.), thioisoxetine (ED50 = 14.1 mg/kg i.p.), amitriptyline (ED50 = 22.7 mg/kg i.p.), desipramine (ED50 = 23.0 mg/kg i.p.), and milnacipran (ED50 = 24.6 mg/kg i.p.). More importantly, the efficacy of duloxetine was comparable with paroxetine in blocking p-CA-induced depletion of brain 5-HT, suggesting that in vivo, duloxetine was a potent and efficacious 5-HT reuptake inhibitor.

Antagonism of p-CA-Induced Depletion of Whole Rat Brain 5-HT Concentrations by Uptake Inhibitors (Fig. 1A). Duloxetine was compared with a number of compounds known to be inhibitors of 5-HT reuptake. The potency of compounds to inhibit the p-CA-induced depletion of whole rat brain 5-HT is shown in Fig. 1A. Data are expressed as percentage of control, where each individual experiment was plotted against its own control group (overall mean control 5-HT = 2.76 ± 0.34 nmol/g; mean p-CA-treated 5-HT = 1.48 ± 0.27 nmol/g, n = 6 experiments, 3–5% variability between each experiment). Compounds were administered i.p. 1 h before 10 mg/kg i.p. p-CA, which depleted brain 5-HT between 37 and 56%, 2 h after p-CA administration, paroxetine was the most potent blocker of the p-CA-induced depletion of brain 5-HT, the dose required to block 5-HT depletion by 50% (ED50 dose) was 1.2 mg/kg i.p. followed by duloxetine (ED50 = 2.3 mg/kg i.p.), venlafaxine (ED50 = 5.9 mg/kg i.p.), thioisoxetine (ED50 = 14.1 mg/kg i.p.), amitriptyline (ED50 = 22.7 mg/kg i.p.), desipramine (ED50 = 23.0 mg/kg i.p.), and milnacipran (ED50 = 24.6 mg/kg i.p.). More importantly, the efficacy of duloxetine was comparable with paroxetine in blocking p-CA-induced depletion of brain 5-HT, suggesting that in vivo, duloxetine was a potent and efficacious 5-HT reuptake inhibitor.

Fig. 1. Effects of uptake inhibitors on 5-HT or NE depletion. A, antagonism of p-CA-induced depletion (10 mg/kg i.p.) of rat brain 5-HT concentrations by uptake inhibitors administered 1 h before p-CA. Tissue samples collected 2 h after p-CA. The data are expressed as percentage of antagonism of p-CA-induced depletion of rat brain 5-HT concentrations. Mean ± S.E., n = 5/group, * p < 0.05 compared with the p-CA group. B, antagonism of α-MMT-induced depletion of rat cerebral cortical concentrations of NE by uptake inhibitors administered 1 h before α-MMT (6.25 mg/kg s.c.). Tissue samples collected 4 h after α-MMT. Data expressed as percentage of control. Mean ± S.E., n = 5/group, * p < 0.05 compared with the α-MMT group. Data for duloxetine, venlafaxine, and milnacipran are reploted from Koch et al. (2002) for comparative purposes.

Fig. 2. Effects on formalin-induced paw-licking behavior with the selective norepinephrine reuptake inhibitor thioisoxetine (0.2, 1, and 3 mg/kg) and selective serotonin reuptake inhibitor paroxetine (1 mg/kg) alone or thioisoxetine (0.3, 1, and 3 mg/kg) in the presence of paroxetine (1 mg/kg) administered intraperitoneally, 30 min before formalin injected into the paw. In combination experiments, paroxetine and thioisoxetine were administered on two different sides, 1 min apart. Whereas thioisoxetine or paroxetine alone did not attenuate paw-licking behavior, when combined, they showed a significant attenuation of formalin-induced paw-licking behavior. Mean ± S.E., n = 5 to 12. Data expressed as inhibition of total time spent licking the injured paw as percentage of control. Late phase, 15 to 40 min after administration of formalin into the paw.
Antagonism of α-MMT-Induced Depletion of Brain NE Concentrations by Uptake Inhibitors (Fig. 1B). Figure 1B compares the potency of duloxetine with the ability of several reuptake inhibitors to inhibit the α-MMT-induced depletion of rat cortical norepinephrine concentrations. Data are expressed as percentage of control, where each individual experiment was plotted against its own control group (overall mean control norepinephrine = 2.17 ± 0.20 nmol/g; mean α-MMT norepinephrine = 1.02 ± 0.16 nmol/g, n = 6 experiments, 3–5% variability between each experiment). Compounds were administered i.p. 1 h before 6.25 mg/kg s.c. α-MMT, which depleted cortical norepinephrine concentrations between 41 and 63%, 4 h after administration average α-MMT norepinephrine = 1.02 ± 0.16 nmol/g, n = 6 experiments). Desipramine was the most potent blocker of the α-MMT-induced depletion of cortical norepinephrine concentrations (ED_{50} = 2.5 mg/kg i.p.), followed by thionisoxetine (ED_{50} = 3.8 mg/kg i.p.), duloxetine (ED_{50} = 14.9 mg/kg i.p.), and milnacipran (ED_{50} = 43.5 mg/kg i.p.). Amitriptyline and paroxetine had ED_{50} values >50 mg/kg i.p. (the highest doses tested), and venlafaxine had an ED_{50} values >100 mg/kg i.p.). The efficacy of duloxetine was comparable with that of selective NRIs, desipramine, and thionisoxetine, in blocking α-MMT-induced depletion of cortical norepinephrine concentrations, confirming that in vivo, duloxetine was also a potent and efficacious NRI.

Effects of a Combination of Thionisoxetine and Paroxetine on Formalin-Induced Late Phase Paw-Licking Pain Behavior (Fig. 2). The selective NRI thionisoxetine alone did not have an effect on late phase paw-licking pain behavior in the formalin model at the low doses of 0.3, 1, and 3 mg/kg, administered intraperitoneally 30 min before formalin.

However, when these doses of thionisoxetine were combined with a 1-mg/kg i.p. dose of paroxetine, a selective SSRI, the 1- and 3-mg/kg dose of thionisoxetine showed a statistically significant attenuation of formalin-induced paw-licking behavior when administered 30 min before formalin. When analyzed by a two-way analysis of variance test, the parallel shift of the thionisoxetine dose response in the presence of paroxetine was shown statistically to be additive. These data suggested that although inhibition of 5-HT or NE reuptake alone had a minimal effect in attenuating formalin-induced late phase paw-licking pain, the combination of both actions produced a significant reduction in pain behavior.

Total paw-licking time in the late phase is expressed as percentage of control. Overall total paw-licking time in the late phase was 377 ± 22 s.

Effects of Duloxetine, Venlafaxine, Milnacipran, Amitriptyline, and Gabapentin on Formalin-Induced Late Phase Paw-Licking Pain Behavior (Fig. 3, A–C). Duloxetine (3, 10, and 15 mg/kg i.p.) significantly attenuated for-
malin-induced late phase paw-licking behavior in a dose-dependent manner. Two other selective dual reuptake inhibitors, venlafaxine (10 and 30 mg/kg i.p.) and milnacipran (10 and 30 mg/kg i.p.), also attenuated late phase paw-licking behavior in this model. However, duloxetine was more potent than either venlafaxine or milnacipran in attenuating pain behavior in this model of persistent pain (Fig. 3A). Amitriptyline was efficacious in attenuating late phase paw-licking behavior at 10 and 30 mg/kg i.p. (Fig. 3B).

Gabapentin (10, 30, and 50 mg/kg i.p.), an antiepileptic drug that is widely used in the treatment of various neuropathic pain states, attenuated formalin-induced late phase paw-licking behavior, but the effects beyond 30 mg/kg showed a plateau effect. Duloxetine was more potent and efficacious in this model (Fig. 3C).

Total paw-licking time in the late phase is expressed as percentage of control. Each compound was plotted against its own vehicle control. Overall total paw-licking time in the late phase across compounds was $359.16 \pm 30.98$ s, and variability across experiments was 5 to 9%.

**Reversal of Mechanical Allodynia Behavior by Duloxetine in L5/L6 Spinal Nerve-Ligated Rats and Comparison with Venlafaxine and Milnacipran (Fig. 4, A–C).** Duloxetine was tested for its ability to reverse mechanical allodynia behavior in L5/L6 spinal nerve-ligated rats. The effects of orally administered duloxetine (10, 20, and 30 mg/kg p.o.) were evaluated over several time points. Duloxetine (Fig. 4a) dose-dependently reversed mechanical allodynia behavior in this model of neuropathic pain. The effects of the highest (30 mg/kg) dose of duloxetine were shown to occur at 1, 2, 3, and 4 h after administration and last up to 6 h, with the peak effects occurring 3 h after oral adminis-

![Graph](image_url)

**Fig. 4.** Effects of duloxetine, venlafaxine, and milnacipran on mechanical allodynia behavior over time in L5/L6 spinal nerve-ligated rats as measured by graded von Frey filaments. A, duloxetine (10, 20, and 30 mg/kg p.o., $n = 6–15$) showed dose-dependent reversal of mechanical allodynia behavior in this model. Effects were evident for 4 h after administration. B, venlafaxine (p.o., $n = 8$) reversed mechanical allodynia at 1 and 2 h at the 100-mg/kg dose and from 2 to 6 h at the 300-mg/kg dose. C, milnacipran (p.o., $n = 10–12$) significantly reversed mechanical allodynia at the 300-mg/kg dose beginning at 1 h after administration. The effects were evident until the 6-h time point was measured. The 200-mg/kg dose showed effects only at the 4-h dose p.o.). Mean ± S.E. +, $p < 0.05$ compared with vehicle; #, $p < 0.1$ compared with vehicle. Pre, presurgery baseline; base, postsurgery baseline. Data expressed as response (grams), gram force applied to ipsilateral hindpaw.
tration, whereas the effects of the 20-mg/kg dose of duloxetine were highest at 3 and 4 h after administration.

Venlafaxine (100 and 300 mg/kg p.o.; Fig. 4b) and milnacipran (200 and 300 mg/kg p.o.; Fig. 4c) also significantly reversed mechanical allodynia behavior in the L5/L6 spinal nerve-ligated rats. The effects of 300 mg/kg venlafaxine were evident from 2 to 6 h after dosing, whereas the effects of 300 mg/kg milnacipran were evident from 1 to 6 h after dosing. However, in comparison with duloxetine, both dual reuptake inhibitors were less potent and less efficacious in this model of neuropathic pain.

Effects of Duloxetine in the Tail-Flick Test of Acute Nociceptive Pain. Duloxetine (3, 10, 20, and 30 mg/kg p.o.) produced minimal effects in attenuating the tail-flick latency in the tail-flick test of acute nociceptive pain, with statistically significant, but modest effects occurring only at the 20- and 30-mg/kg doses at 4 h after administration (Fig. 5).

Effects of Duloxetine, Venlafaxine, Milnacipran, and Amitriptyline in the Rotorod Test of Sedation/Ataxia and Neuromuscular Function (Fig. 6, A–D). Duloxetine (Fig. 6A), venlafaxine (Fig. 6B), and milnacipran (Fig. 6C) did not show performance deficits in the rototod test of sedation/ataxia and neuromuscular function at any of the doses tested after i.p. administration. These doses showed efficacy in the formalin model. Higher doses of milnacipran showed visible signs of ataxic behavior and were not used in pain studies. Whereas amitriptyline did not have significant effects at doses below 50 mg/kg (Fig. 6D), it caused performance deficits at the 50-mg/kg dose in this test.

Discussion

In vivo neurochemical studies on synaptosomal uptake inhibition and microdialysis studies have previously shown that duloxetine is a potent and selective 5-HT and NE reuptake inhibitor in brain (Wong et al., 1993; Bymaster et al., 2001; Wong and Bymaster, 2002; Koch et al., 2003). Koch et al. (2003) showed that although duloxetine, venlafaxine, and milnacipran exhibited dual 5-HT and NE reuptake inhibition properties in vivo, duloxetine was more potent. In the present study, in rats that were depleted of serotonin with p-CA, duloxetine was as efficacious as paroxetine, an SSRI, in blocking p-CA-induced depletion of 5-HT content, whereas in rats depleted of norepinephrine with α-MMT, duloxetine was as efficacious as the selective NRI thionisoxetine or desipramine in increasing NE content. These data reiterate that functionally, duloxetine is a relatively balanced dual reuptake inhibitor of 5-HT and NE in vivo, consistent with other published studies on duloxetine’s ability to change extracellular levels of 5-HT and NE in the brain (Wong and Bymaster, 2002).

The present study has further demonstrated the efficacy of duloxetine in two preclinical models of persistent pain, the formalin model and the L5/L6 spinal nerve ligation model at doses consistent with uptake inhibition in vivo, and a rank order of potency of duloxetine > venlafaxine ≥ milnacipran, similar to that observed in the neurochemical studies (Figs. 1, A and B; Koch et al., 2003). Thus, we postulate that the efficacy of duloxetine in these pain models is mediated via enhanced 5-HT and NE transmission resulting from potent in vivo blockade of 5-HT and NE reuptake sites.

In the formalin model, the selective NRI thionisoxetine or the SSRI paroxetine alone did not have an effect on late phase paw-licking pain behavior in the formalin model when tested at doses that show functional activity as an NRI or SSRI in the neurotransmitter depletion studies described above (Fig. 1, A and B). However, when an inactive dose of paroxetine was combined with inactive doses of thionisoxetine, a statistically significant attenuation of late phase formalin-induced paw-licking behavior was observed. These data suggested that although inhibition of 5-HT or NE reuptake alone had a minimal effect in attenuating formalin-induced late phase paw-licking pain, the combination of both actions produced a significant reduction in pain behavior. Thus, the effects of paroxetine and thionisoxetine together were found to be additive. Thus, increasing both 5-HT and NE via reuptake inhibition may be more beneficial in the attenuation of persistent pain mechanisms. However, the possibility that a pharmacokinetic interaction between paroxetine and thionisoxetine may have led to enhanced activity when combined cannot be ruled out.

Duloxetine was efficacious in reversing late phase paw-licking behavior, an index of persistent pain, in the formalin model in rats after intraperitoneal administration. The effects were dose-dependent. Furthermore, duloxetine displayed higher potency than both venlafaxine and milnacipran in reversing late phase paw-licking behavior in the formalin test. In the effective dose range, duloxetine was comparable with or more potent than amitriptyline and gabapentin in this model.

Importantly, the selective dual reuptake inhibitors duloxetine as well as venlafaxine and milnacipran did not show neurological deficits as measured by the rototod test at doses that attenuated pain behavior in the formalin model, whereas amitriptyline caused significant deficits in performance in the rototod test at the highest dose tested, likely due to being less selective than duloxetine, venlafaxine, and milnacipran.

In the lumbar L5/L6 spinal nerve ligation model of neuro-
Pathic pain, duloxetine (10–30 mg/kg) significantly reversed mechanical allodynia behavior by 3 h after oral administration. In this neuropathic pain model, duloxetine was more potent than venlafaxine and milnacipran.

Of particular interest in these studies is the reduced efficacy of duloxetine in the tail-flick test of acute nociceptive pain at oral doses that showed good efficacy in the L5/L6 spinal nerve ligation model. Several factors may account for this discrepancy: 1) In the tail-flick test, there is no tissue or nerve damage involved, unlike in the formalin or neuropathic pain models; and 2) the tail-flick nociceptive pain response involves mainly a spinal mediated reflex, whereas injury or nerve ligation triggers more complex supraspinal neurotransmitter mediation and modulation. These data would thus suggest that duloxetine is more likely to be effective when there is persistent activation of pain pathways, unlike opiate agents, such as morphine, which are known to be more efficacious in acute nociceptive pain models. The present data support the efficacy of duloxetine in persistent rather than acute pain states.

Recent studies suggest that persistent inflammation or tissue or nerve injury results in hyperexcitability of dorsal horn neurons within the spinal cord, also called central sensitization (Dubner and Ruda, 1992; Coderre and Katz, 1997; Mannion and Woolf, 2000). Central sensitization is characterized by altered responsiveness of dorsal horn neurons, expansion of receptive fields and plasticity of neuronal connections within the pain transmitting pathways leading to increased neuronal activity at supraspinal sites and to dysfunction of the endogenous spinal and supraspinal pain inhibitory mechanisms (Coderre and Katz, 1997; Urban and Gebhart, 1999; Mannion and Woolf, 2000; Ren et al., 2000). An imbalance of the excitatory and inhibitory mechanisms within both the ascending and descending pain inhibitory pathways could ultimately lead to persistent pain (Urban and Gebhart, 1999; Woolf and Mannion, 1999; Ren et al.,

![Fig. 6. Effects on performance in the rotorod test of sedation/ataxia. Measurements were made at different time points after intraperitoneal administration in pretrained rats. (A) Duloxetine, n = 8; (B) venlafaxine, n = 9; (C) milnacipran, n = 9; (D) amitriptyline, n = 8; no deficits in performance were noted at doses tested, except at the highest dose tested (50 mg/kg) in amitriptyline-treated rats. *p < 0.05 compared with no-drug group.

Data from: Iyengar et al.
2000; Hunt and Mantyh, 2001; Ren and Dubner, 2002). Thus, restoring this balance, for example with 5-HT and NE re-uptake inhibitors, could be beneficial in persistent pain conditions in humans.

SSRIs have not proven to be as effective against neuropathic pain as anticipated (Sindrup and Jensen, 1999). In a study examining pain reduction among patients with neuropathic pain, fluoxetine was less effective than TCAs amitriptyline and desipramine and fared no better than placebo (Max et al., 1992). Paroxetine (Kennedy et al., 2000) has found some utility in the treatment of chronic, daily headaches (Jackson, 1998). In general, the SSRIs are less effective in the treatment of diabetic neuropathy, compared with TCAs (Sindrup and Jensen, 1999). Venlafaxine may have analgesic effects (Lang et al., 1996) because it inhibits the reuptake of both serotonin and norepinephrine. Its side effect profile is similar to the other SSRIs and can include agitation, insomnia, or somnolence, gastrointestinal distress and inhibition of sexual functioning, consistent with the possibility that at lower doses, venlafaxine seems to be more of an SSRI and requires higher doses to show dual SNRI properties in humans as is evident in one reported study of venlafaxine in diabetic neuropathic pain (Davis and Smith, 1999). Several meta-analyses of randomized double blind controlled studies of reuptake inhibitors, including SSRIs and TCAs, in neuropathic pain conditions (Sindrup and Jensen, 1999; Collins et al., 2000; Lynch, 2001), have further suggested that enhancing both 5-HT and NE transmission as opposed to either one alone could result in a better therapeutic outcome. The TCAs have been used for the treatment and management of neuropathic pain for some 25 years (Max et al., 1992; Sindrup and Jensen, 1999; Collins et al., 2000; Lynch, 2001). The mechanism of action in relieving of neuropathic pain by the TCAs is thought to be due to the inhibition of reuptake of serotonin and norepinephrine or just norepinephrine within the central nervous system; however, other possible mechanisms of action include α-adrenergic blockade, sodium channel effects, and NMDA receptor antagonism. Nevertheless, the side effect profile of the TCAs, including sedation, hypotension, anticholinergic, and cardiovasculard abnormalities, has limited their use in the treatment and management of neuropathic and other persistent pain states. Thus, the more selective dual uptake inhibitors may offer a safer alternative.

In summary, the present results with duloxetine, a selective and potent SNRI that enhances 5-HT and NE neurotransmission in the central nervous system in a relatively balanced way, further supports the suggestion that 5-HT and NE, play a significant role in attenuating persistent pain mechanisms, presumably via descending modulatory pain pathways. The efficacy of duloxetine in models of persistent pain and neuropathic pain, suggests that in addition to its reported antidepressant activity, duloxetine may exhibit efficacy in the treatment of neuropathic pain and other persistent pain conditions in humans. In addition, persistent pain is a frequently cited feature of depression among primary care patients in a wide range of cultural settings that can be refractory to multiple treatment modalities where duloxetine may have utility.

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