Electrophysiological Characterization of the Effect of Long-Term Duloxetine Administration on the Rat Serotonergic and Noradrenergic Systems

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ABSTRACT

Duloxetine is a dual serotonin (5-HT)/norepinephrine (NE) reuptake blocker with antidepressant potential. In the present in vivo electrophysiological study, the changes in the function of the rat 5-HT and NE systems after 2- and 21-day administration of duloxetine (20 mg/kg/day) were assessed in the dorsal hippocampus and the dorsal raphe nucleus (DRN). The firing rate of DRN neurons was decreased after 2 days of duloxetine, but returned to the control level after 21-day administration. This recovery of firing rate was presumably due to the desensitization of the DRN somatodendritic 5-HT1A autoreceptors found after long-term duloxetine administration. Overall serotonergic tone was assessed by examining the ability of the 5-HT1A antagonist WAY 100635 to alter hippocampal firing. WAY 100635 increased hippocampal firing rates in 21-day treated rats to a greater extent than in 2-day treated or control rats, suggesting that long-term administration induced an increase in endogenous levels of 5-HT in postsynaptic regions. This increase in 5-HT levels was accompanied by selective changes in the 5-HT and NE systems induced by long-term duloxetine administration, i.e., the desensitization of the alpha-2 adrenergic heteroreceptor on 5-HT terminals and the continued blockade of the 5-HT transporters. In contrast, the sensitivity of the alpha-2 adrenergic and terminal 5-HT1A autoreceptors, as well as that of the postsynaptic 5-HT1A receptor after 21-day treatment was unchanged. Therefore, this study demonstrates that duloxetine increases serotonergic tone in a limbic forebrain structure and may therefore be effective in the treatment of depression.

Both the serotonergic and noradrenergic systems are thought to play an important role in the antidepressant response in humans. For instance, both selective 5-HT reuptake inhibitors (SSRI), such as paroxetine and fluoxetine, and selective NE reuptake inhibitors, such as desipramine and (+)-oxaprotiline (Katz et al., 1993), have been shown to be effective antidepressants. However, all antidepressant drugs to date have a therapeutic lag of a few weeks. Therefore, it was of great interest when the dual 5-HT/NE reuptake blocker venlafaxine as well as the combination therapy of fluoxetine and heterocyclic antidepressants were reported to more effectively and, perhaps, more rapidly attenuate depressive symptoms (Gueli et al., 1995; Nelson et al., 1991; Seth et al., 1992; Weilburg et al., 1989; Zajecka et al., 1995). This suggests a possible interaction or synergy between the serotonergic and noradrenergic systems in the treatment of depression.

Previous studies from our laboratory have shown that long-term administration of SSRIs results in the desensitization of the somatodendritic 5-HT1A autoreceptor and 5-HT1B/1D autoreceptor on 5-HT terminals (Blier and de Montigny, 1994; Chaput et al., 1986b, 1991; Blier and Bouchard, 1994). With autoreceptor regulation decreased, there can be a return to normal firing activity and a greater release of 5-HT per impulse, thus leading to an increased serotonergic transmission. In contrast, long-term administration of NE reuptake inhibitors desensitizes the alpha-2 adrenergic heteroreceptor on 5-HT terminals (Mongeau et al., 1994). Due to this desensitization, there is a decreased level of NE-mediated inhibitory input onto 5-HT terminals, and, presumably, an increase in 5-HT transmission. Thus, via differing mechanisms, long-term antidepressant treatment increases serotonergic neurotransmission, and it has been hypothesized that this increase in serotonergic tone underlies the therapeutic effect of most antidepressant treatments (Blier and de Montigny, 1994). It is therefore of interest to consider that if changes in serotonergic tone were induced more effectively, possibly by stimulating changes in different mechanisms simul-

ABBRIVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); NE, norepinephrine; SSRI, selective serotonin reuptake inhibitor; LSD, lysergic acid diethylamide; DRN, dorsal raphe nucleus; MAOI, monoamine oxidase inhibitor; SIL, silence; ANOVA, analysis of variance.
taneously, there could be a more robust attenuation of depressive symptoms.

Duloxetine [LY 248686; (±)-N-methyl-3-(1-naphthalenyl)-2-thiophenepropanamine] blocks the reuptake of both 5-HT and NE (Wong et al., 1993; Fuller et al., 1994; Engleman et al., 1995; Kihara and Ikeda, 1995; Kasamo et al., 1996). Acute duloxetine administration increases extracellular levels of 5-HT and NE in the rat frontal cortex and hypothalamus (Kihara and Ikeda, 1995; Engleman et al., 1995). Interestingly, long-term administration, while having no effect on basal levels, augments the increase in extracellular 5-HT and NE induced by a challenge dose of duloxetine (Kihara and Ikeda, 1995). Thus, the ability of duloxetine to increase extracellular levels of 5-HT and NE via reuptake blockade is potentiated after long-term administration.

The present in vivo study was designed to examine the effects of the long-term administration of duloxetine on the function of the 5-HT and NE transporters, the somatodendritic 5-HT1A autoreceptor, the terminal 5-HT1B autoreceptor, the alpha-2 adrenergic auto- and heteroreceptors on NE and 5-HT terminals, respectively, and on the degree of tonic activation of postsynaptic 5-HT1A receptors on hippocampus pyramidal neurons. The functioning of the receptors and transporters listed above was determined by using established electrophysiological paradigms designed to measure the effects of these components on recorded neurons. The in vivo electrophysiological techniques of single-unit recording and microiontophoresis as well as the electrical stimulation of the 5-HT pathway were used to determine whether the long-term administration of a dual 5-HT/NE reuptake blocker would induce a greater number of biological changes than those seen after the administration of either a selective 5-HT or NE reuptake blocker.

Methods and Materials

Animal preparation and drug administration. Under halothane anaesthesia, male Sprague-Dawley rats were implanted s.c. with an osmotic minipump (Alza, Palo Alto, CA) that delivered 20 mg/kg/day of duloxetine HCl dissolved in a 50% aqueous ethanol solution. The concentrations were determined based on the mean body weight of the animals during the 2- or 21-day treatment. Control rats were implanted with osmotic minipumps delivering the vehicle. All experiments were performed with the minipumps in place. After implantation, rats were group housed under standard housing and lighting conditions with water and food available ad libitum. At all times, principles of laboratory care, established by the Canadian Committee of Animal Care, were followed.

In preparation for the electrophysiological experiments, rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic frame with the nose bar set -3 mm from the ear bars. Anaesthesia was supplemented in 100 mg/kg doses to maintain a level of anaesthesia at which there were no nociceptive reactions to a paw or tail pinch.

Recording of DRN 5-HT neurons. Extracellular recordings of 5-HT neurons in the DRN were performed with single-barrel microelectrodes pulled in the conventional manner to achieve an impedance of 2 to 7 MΩ. A burr hole was drilled on the midline, 0.9 to 1.2 mm anterior to interaural zero (Paxinos and Watson, 1982). Dorsal raphe 5-HT neurons were encountered just below the Sylvius aqueduct, 5.0 to 6.5 mm ventral to dura and were identified based on well established characteristics (Aghajanian, 1975). For the sampling of the firing rate of 5-HT neurons in rats treated for either 2 or 21 days with duloxetine, five electrode descents were made in each rat in a star shape pattern with each descent being 100 μm from the others. To test the functioning of the somatodendritic 5-HT1A autoreceptor after 21-day treatment, the nonspecific 5-HT agonist, LSD, was injected through a cannula inserted in a lateral tail vein, and the degree to which the agonist inhibited DRN 5-HT neuronal firing was determined for control and duloxetine-treated rats. Despite its lack of specificity, LSD was chosen because, when administered systemically, more specific 5-HT1A agonists such as 5-OH-DPAT have been shown to inhibit the firing of 5-HT DRN neurons via a feedback loop from the frontal cortex and not via the somatodendritic 5-HT1A autoreceptors, whereas LSD acts via the somatodendritic autoreceptors (Ceci et al., 1994; Blier and de Montigny, 1987). The ability of the selective 5-HT1A antagonist, WAY 100635, to reverse the inhibition induced by the i.v. administration of LSD further supports the idea that LSD is inhibiting 5-HT neuronal firing through 5-HT1A receptors (Fig. 2).

Recording of dorsal hippocampus CA3 pyramidal neurons. Extracellular recordings of CA3 pyramidal neurons were performed with 5-barrel micropipettes pulled in the conventional manner in order to achieve a tip diameter of 10 to 15 μm. The central barrel was filled with a 2 M NaCl solution and served as the recording barrel. The side barrels were filled with the following solutions: 5-HT creatinine sulfate (20 mM in 200 mM NaCl, pH 3.5-4); NE bitartrate salt (20 mM in 200 mM NaCl, pH 3.5-4); and quisqualic acid (0.75 mM in 200 mM NaCl; pH 8). The last barrel was filled with 2 M NaCl and served as an automatic current balance. The micropipettes were lowered into the dorsal CA3 region of the hippocampus, 4 mm lateral and 4 mm anterior to lambda (Paxinos and Watson, 1982). Neurons were found between 3.5 to 4.2 mm below dura. 5-HT and NE solutions were retained at a current of -9 nA between ejections. Because hippocampal neurons normally do not discharge spontaneously in chloral hydrate anaesthetized rats, a leak or a small ejection current of quisqualate (0 to -4 nA) was used to activate the pyramidal neurons to within their physiological range (8-12 Hz; Ranck, 1975). CA3 hippocampus pyramidal neurons were identified based on several well-established characteristics, namely their large amplitude (0.5-1.2 mV), long duration (0.8-1.2 msec), and alternating of single and complex spike discharge patterns (Kandel and Spencer, 1961). Microiontophoretic applications of 5-HT and NE lasted for 50 sec, and components of the resultant inhibition of the hippocampal neurons were analyzed on-line by computer. These are: the IT50 value, or the time in seconds from the initiation of ejection until the firing rate has decreased by 50% multiplied by the current in nA of ejection, and the RT50 value, or the time in seconds from the termination of ejection until the neuron has recovered 50% of its original firing rate. The IT50 has been shown to be a reliable indicator of the sensitivity of postsynaptic receptors (Brunel and de Montigny, 1988). The RT50 has been shown to serve as a reliable measure of the in vivo activity of 5-HT and NE reuptake processes (de Montigny et al., 1980; Piñeyro et al., 1994). One duloxetine treated and one control rat served as an experimental pair. In order to reduce variation of data between the controls and the treated rats, each pair was studied using the same micropipette.

Determination of serotonergic tone with the 5-HT1A antagonist WAY 100635. CA3 pyramidal neurons in 2- and 21-day duloxetine-treated and control rats were isolated using an activating current of quisqualate (0 to -5 nA) to obtain a baseline firing rate that was within the physiological range. Then, the quisqualate activation was decreased so that neurons were firing at 25 to 30% of their original rate. WAY 100635 was administered in incremental doses of 25 μg/kg through a catheter inserted into a tail vein to establish the effect of the antagonist. It has been shown that WAY 100635, in the anesthetized rat, restores 5-HT neuronal firing if it was attenuated by 5-HT1A autoreceptor activation but does not significantly modify firing in controls (Fletcher et al., 1996). Therefore, in duloxetine-treated animals, where there would be increased extracellular levels of 5-HT in the raphe region, WAY 100635 would restore 5-HT neuronal firing activity. However, because WAY 100635 was given sys-
temically, it would be simultaneously blocking the effects of 5-HT on postsynaptic neurons, thereby canceling out the effect of WAY 100635 on the somatodendritic autoreceptors. Indeed, if the action of the antagonist at the somatodendritic 5-HT1A autoreceptors was influencing the activity of the hippocampal neurons, it would serve to further inhibit their firing rate due to an increased release of 5-HT into the target area. Therefore, it was assumed that any increases in firing activity seen during the administration of WAY 100635 would be a reflection of the action of the antagonist at postsynaptic 5-HT1A receptors. Thus, given that WAY 100635 antagonizes the binding of exogenous 5-HT at postsynaptic 5-HT1A receptors on the CA3 pyramidal neurons, the degree to which the antagonist disinhibits the firing activity would be a direct measure of the tonic level of activation of these receptors by extracellular 5-HT.

**Stimulation of the 5-HT pathway.** The ascending serotonergic pathway was electrically simulated using a bipolar electrode (NE-100, David Kopf, Tujunga, CA) that was implanted into the ventromedial tegmentum (on the midline, AP: +1.0, DV: -8.3 in reference to lambda; electrode was placed at a backward angle of 10°). Two hundred square pulses of 0.5 msec in duration were delivered by a stimulator (S8800, Grass, Quincy, MA) at an intensity of 300 μA and at a frequency of either 1 or 5 Hz. The results of the stimulations upon firing activity were analyzed online by a computer using a Tekmar interface. Peristimulus time histograms were generated to determine the probability of firing, after each stimulation impulse measured as an absolute suppression of firing in msec (SIL). This measure was obtained by dividing the total number of events suppressed by the stimulation by the mean frequency of firing of the neuron (Chaput et al., 1986a). The effects of serotonergic pathway stimulation were determined for the same neuron at 1 and 5 Hz. The degree to which different frequencies of stimulation of the 5-HT pathway inhibit hippocampus pyramidal neurons has been determined to be a measure of the functioning of the terminal 5-HT1B autoreceptor. The rationale for this assumption is, briefly, the greater the frequency of stimulation, the greater release of 5-HT to the initial stimulation, which in turn exerts a greater negative feedback on the 5-HT neurons via the 5-HT1B autoreceptors. Because the release of 5-HT is inhibited more quickly during 5 Hz stimulation, there is an overall smaller release of 5-HT into extracellular space for each action potential reaching 5-HT terminals (Blair et al., 1989) and therefore, an overall smaller inhibition of the hippocampal neurons.

In addition, the effects of serotonergic pathway stimulation were determined for 1 Hz stimulation prior to and following the administration of 10 and 400 μg/kg clonidine. The effects of a low and a high dose of clonidine, an alpha-2 adrenergic receptor agonist, have been determined to be a measure of the sensitivity of the alpha-2 adrenergic auto- and heteroreceptors, respectively. These measures are based on the following rationale. A low dose of clonidine (10 μg/kg) potentiates the effect of ascending 5-HT pathway stimulation, presumably by preferentially stimulating the alpha-2 adrenergic autoreceptors thereby inhibiting the firing of NE neurons and the release of NE, consequently disinhibiting 5-HT terminals. In contrast, a high dose of clonidine (400 μg/kg) inhibits the effect of stimulation, presumably by stimulating alpha-2 adrenergic heteroreceptors on the 5-HT neurons thereby directly inhibiting 5-HT release. Therefore, the same 1 Hz stimulation of the serotonergic pathway results in greater 5-HT release and a greater inhibition of the hippocampal cells after a low dose of clonidine and results in a smaller 5-HT release and lesser inhibition of the hippocampal cells after a high dose. In support of these assumptions, it has been shown that lesioning NE neurons abolishes the effect of the low dose but not of the high dose of clonidine (Mongeau et al., 1993). Two SIL trials were performed for each experimental parameter, i.e., stimulation frequency and clonidine dose, and the average of each trial pair was used for further analyses.

**Drugs.** The following drugs were used: duloxetine HCl (Eli Lilly, Indianapolis, IN), 5-HT creatinine sulfate and NE bitartrate salt (Sigma Chemical Co, St. Louis, MO), quisqualic acid (Toiris Neuramin, Bristol, UK), WAY 100635 (Wyeth-Ayerst, Princeton, NJ) and LSD.

**Statistical analyses.** A two-way analysis of variance was performed on the firing rates of DRN 5-HT neurons in duloxetine-treated and control rats after either 2- or 21-day treatment. A Student’s t test was performed on the effect of 10 μg/kg LSD on DRN 5-HT neuronal firing in treated and control rats. A two-way repeated measures ANOVA was used to determine the effect of treatment and of dose of WAY 100635 on the firing rate of CA3 neurons. Two-way ANOVAs were used to determine the effect of duloxetine treatment and of ejection value on the Iα1E0 values and on the RT0 values. A two-way repeated measures ANOVA was used to determine the effect of treatment and of stimulation frequency on the ability of 5-HT pathway stimulation to inhibit CA3 neuron firing. One-way and two-way repeated measures ANOVAs were performed to test the effect of differing doses of clonidine on the 5-HT pathway stimulation for the treated and control animals. All post hoc analyses used the Student Newman-Keuls.

**Results**

**Effects of 2- and 21-day duloxetine treatment on the firing rate of 5-HT neurons.** The firing rate of DRN 5-HT neurons was significantly decreased after 2 days of duloxetine treatment. However, after 21 days of treatment, firing rates of 5-HT neurons returned to control levels (fig. 1; Flength of treatment 1,206=19.1, P < .0001; Ftreatment 1,206=26.4, P < .0001; Finteraction 1,206=9.3, P = .003).

**Effects of i.v. LSD on the firing of DRN 5-HT neurons after long-term duloxetine treatment.** The 5-HT agonist, LSD, rapidly and completely inhibited the firing of DRN 5-HT neurons in all control rats at a dose of 10 μg/kg. In contrast, this same dose had little or no effect on the firing activity of 5-HT neurons in rats treated for 21 days with duloxetine (t0 = 4.0, P = .004; fig. 2).

**Effect of WAY 100635 on baseline firing rate of CA3 pyramidal neurons after short- and long-term duloxetine administration.** WAY 100635 did not affect the firing activity of CA3 hippocampus pyramidal neurons within a dose range of 25 to 200 μg/kg; i.v. in control rats (figs. 3 and 4). In contrast, WAY 100635 significantly increased the firing rate of hippocampal neurons in rats treated for 21 days with duloxetine (F1,151 = 19.5, P < .0001). In the long-term treated rats, this increase was significant at 25 μg/kg and was dose dependent. The changes induced by a given dose of WAY 100635 was dependent upon the treatment regimen (Finteraction 14,151 = 7.4, P < .0001) with there being an overall

![Fig. 1. Effects of 2- and 21-day treatment with 20 mg/kg/day duloxetine (DUL) or vehicle control (CTL) on the firing rates of DRN 5-HT neurons. The number of neurons recorded is indicated in the boxes at the bottom of each column.](image-url)
significantly greater increase after 21 days of duloxetine administration than either in 2-day treated or control rats (F_{2,21} = 27.0, P < .0001).

Inhibition and recovery times of CA3 hippocampus pyramidal neurons to microiontophoretically applied 5-HT and NE after long-term duloxetine treatment. Twenty-one day treatment with duloxetine (20 mg/kg/day) markedly increased the RT_{50} values to 5-HT (F_{1,152} = 106.9, P < .0001). At 5 nA, RT_{50} values increased by 136% above respective control levels and, at 10 nA, they were increased by 124%. There was a significant difference between the effectiveness of the ejections for both control and duloxetine treated animals (F_{1,152} = 31.3, P < .0001) with there being a longer recovery time after 10 nA than after 5 nA (figs. 5 and 6).

Long-term duloxetine treatment also significantly increased the RT_{50} values to microiontophoretically applied NE (F_{1,140} = 11.0, P = .002). RT_{50} values increased by 118% above control values after 5 nA ejections and by 40% after 10 nA ejections. Again, there was a significant overall effect of ejection value (F_{1,140} = 11.0, P = .002). However, despite no significant interaction (F_{1,140} = 0.18, P = .67), a post hoc examination found only ejection values in the control group to be significantly different from one another (figs. 5 and 6).

Despite the appearance of a trend for an increase in the IT_{50} values for 5-HT following long-term duloxetine administration, the results of a two-way repeated measures ANOVA indicate that duloxetine did not significantly alter the IT_{50} values for microiontophoretically applied 5-HT at 5 or 10 nA (F_{1,107} = 1.77, P = .19). For microiontophoretic application of NE, there was a small but significant increase in IT_{50} values after 21-day treatment with duloxetine (F_{1,107} = 4.67, P = .03). However, post hoc analysis revealed that this was not due to a significant difference in the IT_{50} value for control versus treated animals at 5 nA or at 10 nA, but rather it was significance found only when the data were collapsed (figs. 5 and 6).

Effects of the alpha-2 adrenoceptor agonist clonidine on 5-HT pathway stimulation-induced suppression of firing of CA3 hippocampus pyramidal neurons. In control animals, a low dose of clonidine (10 μg/kg) significantly increased by 40% the suppression of CA3 neuron firing rate induced by ascending serotonergic pathway stimulation (F_{2,11} = 12.5, P = .001). A total of 400 μg/kg of clonidine reversed the increase due to 10 μg/kg clonidine and significantly decreased the SIL value 24% below the control stimulation value (figs. 7 and 8).
A repeated measures ANOVA indicates that there was a significant enhancement (20%) of the stimulation-induced SIL after a low dose of clonidine in rats treated for 21 days with duloxetine ($F_{2,11} = 7.14, P = .01$). Although figure 8 suggests that the increase following 10 μg/kg of clonidine was smaller in duloxetine-treated animals than in controls, the lack of significance of a post hoc pairwise comparison after a two-way repeated measures ANOVA indicates that the duloxetine treatment did not significantly alter the effects of a low dose of clonidine. However, in chronically treated animals, the high dose of clonidine reversed the enhancing effect of the low dose, but there was no significant decrease in SIL values below the control stimulation level (figs. 7 and 8).

Effects of different frequencies of stimulation of the 5-HT pathway on the suppression of firing of CA3 neurons and the effects of long-term duloxetine administration. Increasing the stimulation frequency from 1 to 5 Hz resulted in a significant decrease in the SIL value (absolute suppression of firing of the CA3 neurons in response to pathway stimulation) ($F_{1,55} = 70.5, P < .001$). In control animals, the SIL value induced by 5 Hz stimulations was 28% lower than that induced by 1 Hz stimulations. Similarly, in long-term duloxetine-treated animals, 5 Hz stimulations induced a SIL value 28% lower than that induced by 1 Hz. There was no significant difference between treated and control animals in terms of the abilities of 1 and 5 Hz stimulations to differentially suppress the firing of CA3 hippocampus pyramidal neurons ($F_{1,55} = 0.07, P = .80$; fig. 9).

Discussion

The present electrophysiological study documented the effects that long-term administration of the dual 5-HT/NE reuptake blocker duloxetine has upon the serotonergic and noradrenergic systems. The administration of duloxetine for 2 days decreased the firing rates of 5-HT DRN neurons, but the activity of these neurons recovered to control levels after 21-day treatment. The recovery of firing was accompanied by a desensitization of the somatodendritic 5-HT1A autoreceptors in the DRN. As shown by the ability of WAY 100635 to disinhibit the firing of hippocampal neurons in 21-day treated duloxetine rats to a greater extent than in 2-day treated or control rats, long-term duloxetine administration produced an increase in the overall serotonergic tone, resulting in a greater tonic stimulation of the postsynaptic 5-HT1A receptors in the hippocampus. This increase in tone was permitted by the desensitization of the alpha-2 adrenergic heteroreceptor on the 5-HT terminals that would allow a greater release of 5-HT per impulse, and the continued ability of duloxetine to block the reuptake of 5-HT that would allow the released 5-HT to remain in the synapse for a greater period of time. Because the functioning of the terminal 5-HT1A autoreceptors, the postsynaptic 5-HT1A receptors and the alpha-2 adrenergic autoreceptors did not change after long-term duloxetine treatment, these components probably did not contribute to the increased serotonergic tone.

Although duloxetine is a dual 5-HT/NE reuptake inhibitor, it shows a greater potency in blocking 5-HT transporters than NE transporters (Wong et al., 1993; Kasamo et al., 1996). Therefore, the dose of duloxetine used in the present study was chosen based on a previous study from our laboratory indicating that 20 mg/kg/day was the smallest dose that could significantly block both 5-HT and NE reuptake (Kasamo et al., 1996). To examine the effects of long-term duloxetine administration on the tonic activation of postsynaptic 5-HT1A receptors in a situation analogous to that of a depressed patient receiving the
drug, experiments were conducted with the minipumps in place delivering duloxetine. To maintain continuity, further experiments designed to examine the functioning of multiple components of the 5-HT and NE systems were also conducted with the minipump in place. Given that duloxetine exhibits weak or no affinity for the receptors examined in this study, the presence of...
duloxetine was probably not a confounding factor (Wong et al., 1993). However, the presence of duloxetine limits the conclusions that could be drawn about the functioning of the 5-HT and NE transporters.

Previous studies have shown that 5-HT neuronal firing is decreased after short-term treatment but recovered to control levels after long-term treatment with SSRI, presumably due to desensitization of the somatodendritic 5-HT\textsubscript{1A} autoreceptors (Blier and de Montigny, 1994). Our study demonstrated that duloxetine has a similar profile of action on both the firing activity of DRN 5-HT neurons and the somatodendritic 5-HT\textsubscript{1A} autoreceptor (figs. 1 and 2).

Similar to chronic administration of antidepressants from different classes such as befoxatone, paroxetine and mirtazapine (Haddjeri et al., 1997), long-term duloxetine treatment induced an increase in the tonic activation of the postsynaptic 5-HT\textsubscript{1A} receptors in the hippocampus. WAY 100635, which had no intrinsic activity in the hippocampus of control animals, induced an increase in hippocampal neuron firing in rats treated with duloxetine (figs. 3 and 4). The interpretation that this denotes an increase in serotonergic tone is based on the following reasoning. Long-term antidepressant treatment acting on 5-HT neurons, i.e., SSRI and monoamine oxidase inhibitors, decrease regulatory feedback allowing a greater effect per electrical impulse reaching 5-HT terminals (see introduction for references). With a greater amount of 5-HT in the extracellular space, there is presumably a greater tonic inhibitory activation of 5-HT\textsubscript{1A} receptors on hippocampal neurons. Therefore, in control animals, WAY 100635, a selective 5-HT\textsubscript{1A} receptor antagonist, did not alter the firing rate of hippocampal neurons suggesting that the tonic activation of 5-HT\textsubscript{1A} receptors is not detectable in such an anaesthetized preparation. The ability of WAY 100635 to slightly but significantly increase firing activity after short-term administration suggests that duloxetine can modestly increase activation of the postsynaptic 5-HT\textsubscript{1A} receptor. It is important to emphasize that upon systemic administration of WAY 100635, the firing activity of 5-HT neurons in rats treated for 2 days was restored to the level seen in controls and 21-day treated rats (fig. 2). Therefore, the small effect detected at 2 days of treatment may be a consequence of dual 5-HT/NE reuptake blockade. The observation that there was a greater increase in WAY 100635-induced hippocampal neuronal firing after 21-day duloxetine treatment than after 2-day treatment suggests this probe is capable of assessing, not only the intrinsic activity of duloxetine, but also alterations in endogenous 5-HT levels resulting from adaptive changes occurring after long-term antidepressant administration. Furthermore, the 21-day-treated animals were actually receiving less than 20 mg/kg/day by the time of testing vs. the 2-day-treated rats receiving the full 20 mg/kg/day due to the osmotic minipumps being filled with a drug concentration determined by the predicted mean body weight over the treatment period. In conclusion, the antagonist disinhhibited the target neurons of rats treated chronically with duloxetine, thus unveiling a markedly enhanced tonic activation of the postsynaptic 5-HT\textsubscript{1A} receptors.

Given that there was a greater tonic activation of postsynaptic 5-HT\textsubscript{1A} receptors after long-term duloxetine administration, we then sought to determine the mechanisms through which this increase could have occurred. In the present study, clonidine, an alpha\textsubscript{2}-adrenergic heteroreceptor agonist, was used to probe the effects of long-term duloxetine administration on the alpha\textsubscript{2}-adrenergic heteroreceptors (Mongeon, et al., 1994). Results indicate that long-term duloxetine administration desensitized the alpha\textsubscript{2}-adrenergic heteroreceptors (figs. 7 and 8). In control animals, a high dose of clonidine (400 \(\mu\)g/kg) attenuated the ability of 5-HT pathway stimulation to inhibit hippocampus pyramidal cell firing, presumably by directly acting on the heteroreceptors of 5-HT terminals, thus inhibiting 5-HT release. Long-term treatment with duloxetine attenuated this negative feedback system suggesting that the alpha\textsubscript{2}-adrenergic heteroreceptors had desensitized. This finding is in agreement with changes induced by long-term administration of drugs that increase synaptic availability of NE, such as the NE reuptake inhibitor nisoxetine, and the MAOI befoxatone (Blier and Bouchard, 1994; Mongeon et al., 1994), suggesting that the desensitization of the alpha\textsubscript{2}-adrenergic heteroreceptors is
physiologically relevant, and that this effect may stem from the ability of duloxetine to block the reuptake of NE. Therefore, their attenuated function would free 5-HT terminals from the increased NE inhibitory influence being exerted via sustained NE reuptake blockade.

As was the case with SSRIs, the RT50 values of hippocampus CA3 pyramidal neurons after the microiontophoretic application of 5-HT or NE, shown previously to be a measure of the function of the transporter mechanism (Pinéyro et al., 1994), were significantly and current-dependently increased in duloxetine treated animals (figs. 5 and 6). This demonstrates that the dose of duloxetine used here, 20 mg/kg/day, was sufficient to markedly block the reuptake of both 5-HT and NE. Although the results of our study cannot be directly compared to the acute and 2-day administration duloxetine study by Kasamo et al. (1996), it appears that the ability of each current (5 or 10 nA) to inhibit the recovery of neuronal firing in animals chronically treated with duloxetine was not different from the ability of the same currents to inhibit the recovery of firing in acutely or 2-day-treated animals. This suggests that the 5-HT and NE transporter mechanisms are not altered after long-term administration of duloxetine. Recent in vitro studies in our laboratory demonstrating no change in the sensitivity of the 5-HT and NE transporters after 3-week administration of duloxetine support this contention (Rueter et al., in press). A continued effectiveness of duloxetine to block the 5-HT transporter could lead to an enhancement of the extracellular levels of 5-HT. In contrast to the unaltered effectiveness of duloxetine to block the 5-HT and NE transporters after long-term treatment, the long-term administration of the SSRI paroxetine (Pinéyro et al., 1994) or of a NE reuptake inhibitor, such as desipramine (Lacroix et al., 1991) has been shown to desensitize the 5-HT and NE transporters, respectively.

In agreement with previous studies, stimulations of the ascending serotonergic pathway at 1 Hz in control rats inhibited the firing rate of hippocampus pyramidal CA3 neurons to a significantly greater extent than did 5 Hz stimulations (fig. 9). As outlined in "Materials and Methods," this finding has been interpreted as a measure of the functioning of the terminal 5-HT1H autoreceptors (Chaput et al., 1986a). It has been shown that chronic treatment with an SSRI such as paroxetine desensitizes these receptors thereby significantly decreasing the differential effects of 1 vs. 5 Hz pathway stimulation (Chaput et al., 1991). In contrast, long-term duloxetine treatment did not alter the differential effects of 1 vs. 5 Hz stimulation, therefore suggesting that there was no alteration in the sensitivity of the terminal 5-HT1H autoreceptors with long-term duloxetine treatment (fig. 9). Therefore, this component of the 5-HT system, which regulates the release of 5-HT per impulse, presumably does not participate in the enhancement of serotonergic tone induced by long-term duloxetine administration.

Clonidine, an alpha-2 adrenergic antagonist, was used to probe the effects of long term duloxetine administration on the alpha-2 adrenergic autoreceptors (Mongeau et al., 1994). Long-term duloxetine did not alter the ability of a low dose of clonidine to augment the effects of 5-HT pathway stimulation, suggesting that there was no alteration in the sensitivity of the alpha-2 adrenergic autoreceptor (fig. 8). This finding is in agreement with a variety of in vitro studies that found no change in the alpha-2 adrenergic autoreceptor after chronic NE reuptake blockade (Schoffelmeer and Mulder, 1982; Moret and Briley, 1994). However, it is in conflict with previous electrophysiological work that found changes in the sensitivity of alpha-2 adrenergic autoreceptor after chronic desipramine treatment (Lacroix et al., 1991). This discrepancy may stem from the different methodologies, drug regimens or modes of drug administration, or may reflect a lack of physiological significance to the small changes in alpha-2 adrenergic autoreceptor sensitivity. Based on these findings from our study, it appears that this component of the NE system does not contribute to the increased activation of postsynaptic receptors.

The inhibitory effect of 5-HT upon the firing rate of neurons in the CA3 region of the hippocampus, in the conditions used in our study, is entirely mediated via the 5-HT1A receptor as has been shown in several electrophysiological studies including the one presented here (for review see de Montigny and Blier, 1992). For example, the selective 5-HT1A receptor antagonist, WAY 100635, blocks the effects of 5-HT and some 5-HT agonists in the hippocampus in a variety of electrophysiological and behavioral models (Fletcher et al., 1996; Rueter et al., 1997; Gariboldi et al., 1996). Furthermore, our study has shown that WAY 100635 can antagonize endogenous 5-HT if the levels of 5-HT have been elevated by long-term 5-HT reuptake blockade. Although this antagonist can also act on alpha-1 adrenoceptors, the antagonism of 5-HT compounds is achieved at doses of WAY 100635 too small to affect these alpha-1 adrenoceptors (Waszczak et al., 1996).

Our data suggest that long-term administration of duloxetine does not significantly alter the sensitivity of postsynaptic 5-HT1A receptors. The I50 values for 5-HT, shown to be a valid measure of receptor sensitivity (Brunel and de Montigny, 1988), were unaltered by chronic duloxetine treatment. A trend toward an increase of I50 values was found with microiontophoretically applied NE, suggesting a decrease in the sensitivity of the postsynaptic alpha-2 adrenergic receptors located on the hippocampal neurons after long-term duloxetine administration. However, given the lack of differences found with a post hoc analysis and an inability to replicate this result in this laboratory (Rueter et al., in press) it is unclear whether this finding is of functional significance (fig. 6).

Long-term NE reuptake blockade has been shown to potentially increase the tonic activation of the postsynaptic alpha-1 adrenoceptors in the hippocampus (Lacroix et al., 1991). Our study reveals the possibility of enhanced tonic levels of NE due to the continuing ability of duloxetine to block the NE transporter and the trend toward a desensitization of the alpha-2 adrenergic autoreceptors on NE terminals (figs. 5, 6 and 8). Thus, similar to the changes in the 5-HT system induced by duloxetine, there could be a greater amount of NE in the synapse due to ongoing NE reuptake blockade. We would have liked to directly assess the tonic activation of the postsynaptic alpha-1 adrenoceptor in a manner similar to 5-HT1A receptor activation, i.e. test the ability of an alpha-1 adrenoceptor antagonist to disinhibit the firing of hippocampal neurons. However, alpha-1 adrenoceptor antagonists are known to directly inhibit raphe firing (Baraban and Aghajanian, 1980). Thus, the results of this probe would be confounded by changes in the 5-HT system, thus rendering the approach inappropriate.

It is interesting to note that long-term administration of a
dual 5-HT/NE uptake inhibitor did not merely result in a summation of the changes induced by a 5-HT reuptake inhibitor given alone plus those induced by a NE reuptake inhibitor given alone. This may be the result of interactions between the two neurotransmitter systems. For instance, it has been shown that the administration of a 5-HT reuptake inhibitor can attenuate the ability of an alpha-2 adrenergic agonist to inhibit 5-HT release (Blier et al., 1990). Similarly, it may be that, in the presence of NE reuptake blockade, 5-HT reuptake inhibition no longer induces the changes it would if it occurred alone.

In summary, the electrophysiological effects observed after long-term administration of duloxetine suggest that this drug does indeed act as other antidepressants, increasing serotonergic tone after long-term administration. This enhancement is presumably due to the altered functioning of components of both the 5-HT and NE systems, i.e., the 5-HT1A autoreceptors, the alpha-2 adrenergic heteroreceptors and the 5-HT and NE transporters. These observations indicate that duloxetine, given in doses high enough to impact both the 5-HT and NE systems, should be useful in the treatment of depression.

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References


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