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Simultaneous Increases of Extracellular Monoamines in Microdialysates from Hypothalamus of Conscious Rats by Duloxetine, a Dual Serotonin and Norepinephrine Uptake Inhibitor

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Duloxetine (LY248686, [+]-N-methyl-3-(1-napthalenyloxy)-2-thiophene-propanamine) is a potent dual inhibitor of serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE) uptake in hypothalamus and cerebral cortex of rat brain (Wong et al. 1993; Fuller et al. 1994). Consistent with the dual mechanisms of inhibiting 5-HT and NE uptake, duloxetine at 15 mg/kg IP produced large increases in extracellular levels of 5-HT (250%) and NE (1,100%) 30 minutes after systemic administration. Levels of 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) and 5-hydroxyindoleacetic acid (5-HIAA), metabolites of NE and 5-HT, respectively, were reduced, whereas those of dopamine (DA) and its metabolite 3, 4-dihydroxyphenylacetic acid (DOPAC) were not significantly altered. Duloxetine at 7 mg/kg produced less pronounced increases while no consistent effects were observed at 4 mg/kg. In this dose range, duloxetine inhibited 5-HT uptake in platelets ex vivo without inhibiting striatal dopamine (DA) uptake. In the present study we also found that the primary amine (a racemate) of duloxetine is about one-fourth as active as duloxetine to inhibit 5-HT and NE uptake. The potential primary amine metabolite of duloxetine might contribute, in part, to the inhibition of 5-HT and NE uptake in vivo. Thus the ability to produce robust increases of extracellular 5-HT and NE levels suggests that duloxetine may potentially be a highly effective antidepressant agent. ***[Neuropsychopharmacology 12:287–295, 1995]***

KEY WORDS: *Microdialysis; Hypothalamus; Rats; Monoamines; Uptake inhibitors; High-performance liquid chromatography*

Inhibition of presynaptic neurotransmitter uptake should lead to a greater synaptic availability of the targeted neurotransmitter (Fuller and Wong 1990; Geyer et al. 1978; Guan and McBride 1988; Marsden et al. 1979). Applications of in vivo techniques (including microdialysis) have demonstrated that extracellular serotonin in most rat brain areas increases several fold over basal levels upon systemic administration of serotonin uptake inhibitors, including fluoxetine (Auerbach et al. 1989; Dailey et al. 1992; Guan and McBride 1988; Perry and Fuller 1992, 1993a; Rutter and Auerbach 1993), indalpine (Kalen et al. 1988), citalopram (Invernizzi et al. 1992), sertraline (Invernizzi et al. 1991), and clomipramine (Adell and Artigas 1991; Carboni and Di Chiara 1989). Extracellular norepinephrine (NE) concentrations are also elevated upon local or peripheral administra-

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tion of the NE uptake inhibitor desipramine (Dennis et al. 1987; Itoh et al. 1990; Kalen et al. 1988; L'Heureux et al. 1986; Thomas and Holman 1991).

Duloxetine (LY248686, [+]-N-methyl-3-(1-napthalenyloxy)-2-thiophene-propanamine) is a potent inhibitor of serotonin (5-HT) and NE uptake with inhibitor constants (K_i) of 4.6 ± 1.1 and 15.6 ± 2.9 nM, respectively, while it is a much weaker inhibitor of dopamine (DA) uptake with a K_i of 369 ± 38.1 nM (Wong et al. 1993). It weakly interacts at two to three orders of magnitude higher concentrations with neuronal receptors including 5-HT (1A, 1B, 1C, 1D, 2, and 3), NE (α_1, α_2), DA (D_2), muscarinic-acetylcholine, histamine H_1, and opiate (Wong et al. 1993).

In the present study we have examined the effects of duloxetine on extracellular monoamines in microdialysates from hypothalamus of conscious rats. Duloxetine dose-dependently elevated concentrations of 5-HT and NE simultaneously in hypothalamus, suggesting greater availability of both monoaminergic neurotransmitters upon concerted inhibition of 5-HT and NE uptake. The present study also shows that the primary amine of duloxetine, a potential metabolite, inhibits 5-HT and NE uptake in vitro and ex vivo with comparable potency to that of duloxetine. Moreover, duloxetine significantly inhibited 5-HT uptake in platelets ex vivo at doses that had no effect on striatal DA uptake ex vivo.

MATERIALS AND METHOD

Preparation of Synaptosomes for Monoamine Uptake In Vitro

Male Sprague-Dawley rats (Harlan Industries, Cumberland, IN), weighing 130 to 160 g, were killed by decapitation, the brains removed, and the brain areas immediately dissected on ice. Isolated brain areas were homogenized and suspended in 10 volumes of a medium containing 0.32 M sucrose then centrifuged at 1,000 *g* and 4°C for 10 minutes. The supernatant was decanted and centrifuged at 17,000 *g* for 20 minutes. The resulting synaptosomal pellet (P_2) was suspended in fresh medium and kept on ice and used later the same day.

Synaptosomal Uptake of ^{3}H-5-HT and ^{3}H-NE

Synaptosomal preparations (equivalent to 0.5 to 1.0 mg protein) were incubated at 37°C for 5 minutes in 1.0 ml of Krebs bicarbonate medium containing 10 mM glucose, 0.1 mM iproniazid, 1.0 mM ascorbic acid, 0.17 mM ethylenediamine tetraacetic acid (EDTA), 120 nM ^{3}H-NE or 50 nM ^{3}H-5-HT, and compounds at five or more concentrations. The reaction mixture was immediately diluted with 2.0 ml of 0.9% saline and filtered using Whatman GF/B filters under vacuum with a cell harvester (Brandel, Gaithersburg, MD). Filters were rinsed twice with approximately 5 ml of ice-chilled 0.9% saline and were transferred to a counting vial containing 10 ml scintillation fluid (PCS, Amersham, Arlington Heights, IL). Radioactivity was measured using a liquid scintillation spectrophotometer. Accumulation of radioactivity at 4°C represented background and was subtracted from all samples.

Radiolabeled Ligand Binding

The P_2 pellet was resuspended in 50 volumes of ice-cold buffer containing 50 mM Tris-HCl (pH 7.4) and centrifuged at 50,000 *g* for 10 minutes. The resulting pellet was again suspended in 50 volumes of ice-cold buffer and incubated at 37°C for 10 minutes then centrifuged for 10 minutes at 50,000 *g*. The pellet was resuspended in 50 volumes of cold buffer and centrifuged at 50,000 *g* for 10 minutes. The supernatant was discarded, and the remaining pellet was stored at −70°C until use.

Binding of ^{3}H-paroxetine and ^{3}H-tomoxetine to 5-HT and NE uptake sites, respectively, was carried out in 2 ml of reaction medium containing appropriate drug concentration, 0.1 nM ^{3}H-paroxetine or 0.2 nM ^{3}H-tomoxetine, and membrane suspension totaling 50 µg protein/tube as previously described (Wong et al. 1993). Nonspecific binding was determined by adding 1.0 µM of fluoxetine or desipramine for ^{3}H-paroxetine and ^{3}H-tomoxetine binding, respectively. Tubes were incubated at 37°C for 30 minutes, then filtered through Whatman GF/B filters presoaked for 1 hour with 0.05% polyethylenimine with a Brandel cell harvester. The filters were washed by rinsing the tubes with cold Tris-HCl buffer (pH 7.4). Filters were then placed in scintillation vials, 10 ml of scintillation fluid was added, and radioactivity was measured by liquid scintillation spectrophotometry.

^{3}H-5-HT Uptake in Rat Platelets and ^{3}H-DA Uptake in Striatal Homogenates

After fasting overnight, male Sprague-Dawley rats weighing 115 to 145 g were dosed with duloxetine or deionized water by oral gavage. One hour later, the rats were sedated by breathing CO_2 and blood was collected by cardiac puncture into a syringe containing 1.0 ml of 3.8% sodium citrate in normal saline (pH 7.4). Rats were quickly decapitated, the brains removed, and various brain regions dissected. Each striatum was homogenized in 10 volumes of 0.32 M sucrose for measurement of ^{3}H-DA uptake. Blood samples were centrifuged at 100 *g* for 15 minutes at 23°C. The supernatant containing platelet-rich plasma was recovered, an aliquot taken for platelet concentration determination, and the remainder stored on ice for approximately 1

hour. A 0.1-ml aliquot was mixed with 2.0 ml Krebs bicarbonate buffer (pH 7.4) containing 10 mM glucose, 0.1 mM iproniazid, 1.0 mM ascorbic acid, 0.17 mM EDTA, and 105 nM ^{3}H-5-HT. Tubes were incubated at 37°C for 10 minutes, except the tubes used to represent nonspecific uptake which were kept at 4°C, after which all tubes were chilled to 4°C and 0.1 ml of 20% formaldehyde was quickly added to each tube to terminate the reaction. Platelets were harvested by filtration through Whatman GF/B filters using a cell harvester (Brandel, Gaithersburg, MD). After rinsing two times with cold normal saline, the filters were transferred to 20-ml scintillation vials, 10 ml of Ready Solv HP scintillation fluid was added, and the vials were counted by liquid scintillation spectroscopy for 5 minutes.

Uptake of ^{3}H-DA in rat striatal homogenates was performed as described for platelet uptake with the following exceptions: a 0.1-ml aliquot of striatal homogenate was added to 1.0 ml Krebs bicarbonate buffer with contents listed above except that 110 nM ^{3}H-dopamine was substituted for ^{3}H-5-HT, and incubated for 3 minutes at 37°C. No formaldehyde was added to the tubes.

Microdialysis Assays of Monoamines and Metabolites

Sprague-Dawley rats (Harlan or Charles Rivers) weighing 270 to 300 g were surgically implanted with microdialysis probes under chloral hydrate/pentobarbital anesthesia (170 and 36 mg/kg IP in 30% propylene glycol, 14% ethanol) (Perry and Fuller 1992). A David Kopf stereotaxic instrument was used to implant the probe unilaterally in the hypothalamus at coordinates rostral −1.5 mm, lateral −1.3 mm, and ventral −9.0 mm (Paxinos and Watson 1986). After a 48-hour recovery period, rats were placed in a large plastic bowl with a mounted liquid swivel system (CMA/120 system for freely moving animals; Bioanalytical Systems, West Lafayette, IN). Filtered artificial cerebrospinal fluid (CSF—150 mM NaCl, 3.0 mM KCl, 1.7 mM $CaCl_2$, and 0.9 mM $MgCl_2$) was perfused through the probe at a rate of 1.0 µl/minute. The output dialysate line was fitted to a high performance liquid chromatography (HPLC) valve.

The HPLC method was similar to that previously described (Perry and Fuller 1992) with some modifications. A 10-port HPLC valve (Valco Instruments, Houston, TX) with a 20-µl sample loop was used in a configuration with a small-sample cleanup column (Spherisorb 3µ ODS2, 2 × 10 mm, Keystone Scientific, Bellefonte, PA) that trapped late-eluting peaks in the dialysate samples. When the valve was in the inject position, dialysate from the sample loop was injected on to the sample cleanup column and then onto the analytical column (Spherisorb 3µ ODS2, 2 × 150 mm, Keystone Scientific, Bellefonte, PA). When the valve was in the load position and dialysate sample was being collected in the sample loop, the sample cleanup column was being backflushed with mobile phase. The valve was in the inject position for 5 minutes and in the load position for 25 minutes with a total run time of 30 minutes.

The mobile phase for both the cleanup and analytical columns was the same and consisted of 75 mM potassium acetate, 0.5 mM EDTA, 1.4 mM sodium octanesulfonic acid and 8% methanol (pH 4.79). The flow rate for both columns was 0.25 ml/minute, and the analytical column was maintained at 40°C while the cleanup column was mounted close to the valve at room temperature. An electrochemical detector (EG&G PARC, Princeton, NJ) with a dual glassy carbon electrode was used to detect NE, DA, 5-HT, and their metabolites 3-methoxy-4-hydroxyphenylethyleneglycol (MPHG) 3,4-dehydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA). NE, DA, and 5-HT were assayed at a potential of 350 mV, sensitivity setting of 0.5 nA/V, and DOPAC, 5-HIAA, and HVA at a potential of 750 mV, sensitivity setting of 50 nA/V. The output of both channels was analyzed and quantitated by a chromatography data system (EZChrom, Scientific Software, San Ramon, CA) on a Compaq 486/33 computer. The sensitivity for NE, 5-HT and DA was approximately 0.1 pmol/ml dialysate or 2 fmol injected on to the column. Basal levels were measured for at least 120 minutes prior to drug administration (Table 1).

Drugs

[^{3}H]-5-HT, [^{3}H]-DA, [^{3}H]NE, and [^{3}H]-paroxetine were purchased from New England Nuclear Corp. (Boston, MA) and [^{3}H]-tomoxetine was supplied by Amersham Laboratories (Buckinghamshire, England). Desipramine hydrochloride was obtained from Merrell-National

Table 1. Mean ± SEM Basal Levels of Monoamines and Their Metabolites in Rat Hypothalamus Dialysates

	Compound (*pmol/ml*) (*n* = 6)						
	NE	DA	5-HT	MHPG	DOPAC	HVA	5-HIAA
Mean	0.472	0.528	0.406	7.597	169.12	47.14	713.5
±SEM	0.063	0.052	0.030	0.278	17.48	4.92	33.7

Laboratories (Cincinnati, OH). Duloxetine, fluoxetine, the racemate of duloxetine ([±]-N-methyl-3-(1-napthalenyloxy)-2-thiophene-propanamine), and the primary amine of the duloxetine racemate were synthesized at the Eli Lilly research laboratories (Indianapolis, IN). Drugs used in vivo were prepared in filtered deionized water and administered by IP injection.

Evaluation and Statistical Analyses

Binding and uptake data were converted to percentages of control, and a Student's *t*-test was used to compare the control with the drug-treated groups. Extracellular levels of the amines and their respective metabolites in microdialysates were calculated by comparing peak heights with those of 50 pmole standards. The mean value of the four samples immediately preceding drug administration served as the basal level for each experiment and data were converted to percentages of basal. Paired *t*-tests were used to compare the mean of the basal values from the time point immediately preceding drug administration to those of each time point thereafter.

RESULTS

Inhibition of Uptake In Vitro

Duloxetine, its racemate, and the racemate of its primary amine each inhibited 5-HT and NE uptake in synapotosomes of rat hypothalamus and/or cortex (Table 2). Since the positive isomer of the primary amine of duloxetine was not available, the racemate of duloxetine was included for comparison. In hypothalamus duloxetine was the most potent inhibitor of both 5-HT and NE uptake with K_i values of 2.4 ± 0.7 and 4.1 ± 1.0 nM, respectively. The racemate of duloxetine and of its primary amine inhibited 5-HT uptake in hypothalamus with K_i's of 6.7 ± 2.0 and 8.2 ± 2.4 nM, respectively, and in cortical synaptosomes with K_i's of 5.6 ± 1.2 and 9.1 ± 1.7 nM. The potency to inhibit NE uptake by the racemate and the primary amine was five times higher in hypothalamus preparations (K_i's of 4.9 ± 1.2 and 26.0 ± 6.1 nM) than in cortical preparations (K_i's of 25.7 and 135.2 nM, respectively). Duloxetine, a positive isomer, has been shown to inhibit 5-HT and NE uptake in rat cortical preparations with K_i values of 4.6 and 15.6 nM, respectively (Wong et al. 1993).

Table 2. Inhibition of 5-HT and NE Uptake by Duloxetine, Its Primary Amine, and the Racemate of the Primary Amine In Vitro*

		K_i (*nM*) Inhibition of Uptake	
Compound	**Brain Area**	**5-HT**	**NE**
Duloxetine	Hypothalamus	2.4 ± 0.7	4.1 ± 1.0
Primary amine	Hypothalamus	8.2 ± 2.4	26.0 ± 6.1
	Cortex	9.1 ± 1.7	135.2
Racemate	Hypothalamus	6.7 ± 2.0	4.9 ± 1.2
	Cortex	5.6 ± 1.2	25.7

* Synaptosomal preparations of cerebral cortex or hypothalamus in duplicate samples were incubated at 37°C for 3 minutes in Krebs bicarbonate medium containing seven concentrations (1 to 1,000 mM) of the drug tested.

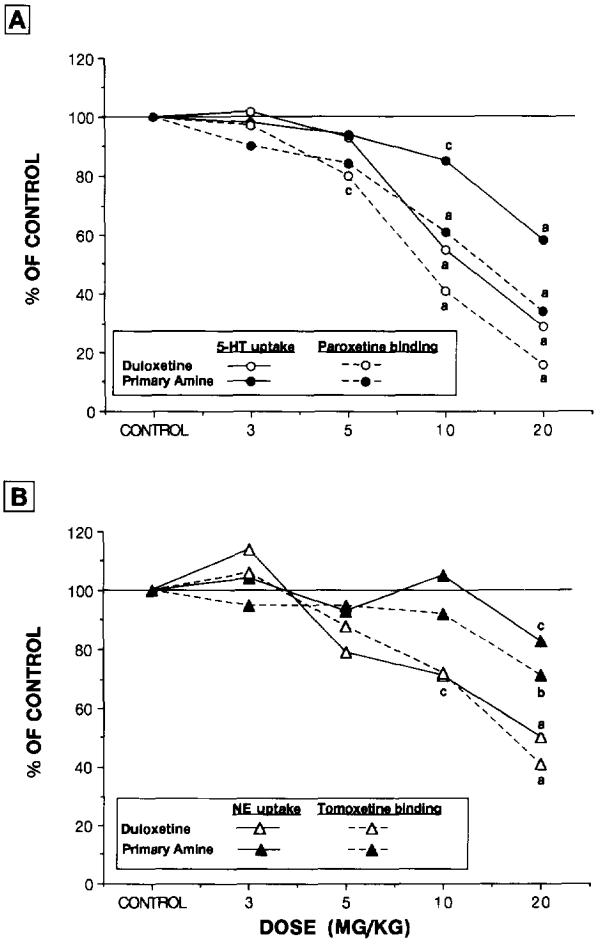

Figure 1. Ex vivo inhibition of uptake and binding in rat hypothalamus: Oral administration of duloxetine or its primary amine dose-dependently inhibited ^{3}H-5-HT uptake and ^{3}H-paroxetine binding (**A**), as well as ^{3}H-NE uptake and ^{3}H-tomoxetine binding (**B**), in rat hypothalamus preparations. Uptake: synaptosomes (equivalent to 1.0 mg protein) were incubated in triplicate at 37°C for 5 minutes in Krebs bicarbonate buffer (pH 7.4) containing 10 mM glucose, 0.1 mM iproniazid, 0.2 mg/ml ascorbic acid, 0.2 mM EDTA, and 0.05 µM ^{3}H-5-HT or ^{3}H-NE. Binding: synaptosomes (equivalent to 0.05 mg protein) were incubated in triplicate at 37°C for 30 minutes in 50 mM Tris-HCl buffer (pH 7.4) containing 150 mM NaCl, 5.0 mM KCl, 0.1 nM ^{3}H-paroxetine, or 0.2 nM ^{3}H-tomoxetine with or without 1.0 µM fluoxetine or desipramine, respectively.

Inhibition of Uptake Ex Vivo

Upon administration at 20 mg/kg PO in vivo the primary amine of duloxetine inhibited 5-HT and NE uptake ex vivo in rat hypothalamus by 42% and 17%, respectively, while duloxetine produced a 71% and 50% inhibition (Figure 1). Duloxetine or its primary amine compound administered in vivo also dose-dependently inhibited the sodium dependent ^{3}H-paroxetine binding to the 5-HT uptake site and ^{3}H-tomoxetine binding to the NE uptake site in rat hypothalamus homogenates (Figure 1). After oral administration of duloxetine up to 20 mg/kg, binding of ^{3}H-paroxetine and ^{3}H-tomoxetine was inhibited by 84% and 59%, respectively. The primary amine compound inhibited the binding of ^{3}H-paroxetine and ^{3}H-tomoxetine by 66% and 29%, respectively (Figure 1).

In a separate study, while there was no significant change in striatal ^{3}H-DA uptake over the entire dose range of duloxetine (1 to 30 mg/kg PO), ^{3}H-5-HT uptake in blood platelets ex vivo was dose-dependently inhibited with an ED_{50} of 7.7 mg/kg (Figure 2), causing a maximum inhibition of 96% at the 30-mg/kg dose.

Monoamines and Metabolites in Microdialysates

Following sampling for four 30-minute intervals to establish baseline values (Table 1), rats were treated with duloxetine (4, 7, or 15 mg/kg IP). After administration with the high dose (15 mg/kg) of duloxetine, extracellular 5-HT and NE levels increased most profoundly by 240 ± 25% and 1,100 ± 380%, respectively, above baseline within the first 30-minute interval (Figures 3A and 4A). The elevated levels of 5-HT were maintained significantly above basal levels for up to 3 hours. The elevated NE levels declined to about 800% in the second 30-minute interval, then gradually decreased further to about 300% above basal levels 3 hours after administration of duloxetine. No consistent change in levels of DA was observed. However, a trend toward an increase was noted (Figure 5A).

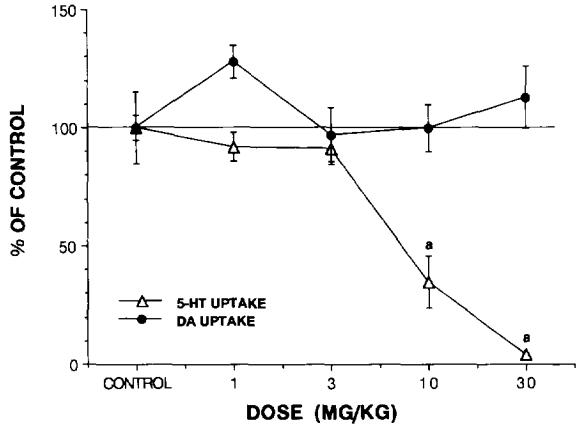

Figure 2. Inhibition of 5-HT and DA uptake by duloxetine. Administration of duloxetine 1, 3, 10, or 30 mg/kg PO lowered 5-HT uptake in platelets but was without effect on DA uptake in striatal homogenate ex vivo in rats.

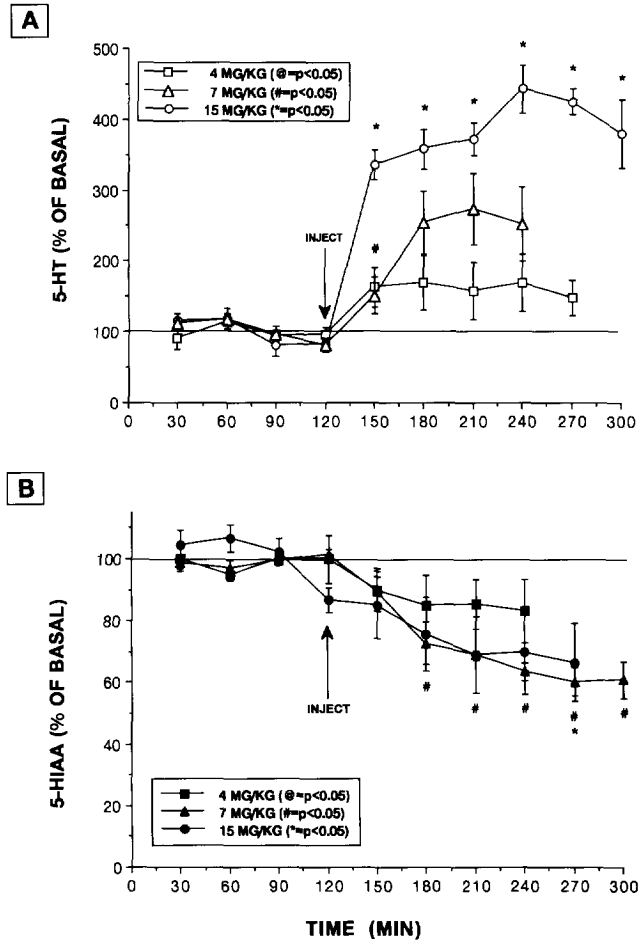

Figure 3. Dose effect of duloxetine on 5-HT and 5-HIAA levels in rat hypothalamus dialysate. Duloxetine at 4, 7, and 15 mg/kg, IP dose-dependently increases serotonin levels (**A**) while decreasing 5-HIAA levels (**B**) in dialysate from rat hypothalamus. Values are in percentages of basal levels (mean ± SEM for three or more experiments at each dose of duloxetine: * Significantly different from basal at $p < .05$, paired t-test.

At the 7-mg/kg dose of duloxetine, extracellular 5-HT levels gradually rose to 150% above basal within 1 hour, but this increase did not reach statistical significance (Figure 3A). The increase of NE levels reached nearly 200% above basal levels in 1 hour, and levels remained significantly elevated for up to 2 hours after administration (Figure 4A). Dialysate levels of 5-HT, NE, and DA did not vary appreciably from basal values after the 4-mg/kg dose of duloxetine (Figures 3A, 4A, and 5A).

After the 7- or 15-mg/kg doses of duloxetine, 5-HIAA and MHPG levels decreased to 40% below basal levels (Figures 3B and 4B). The DOPAC levels did not

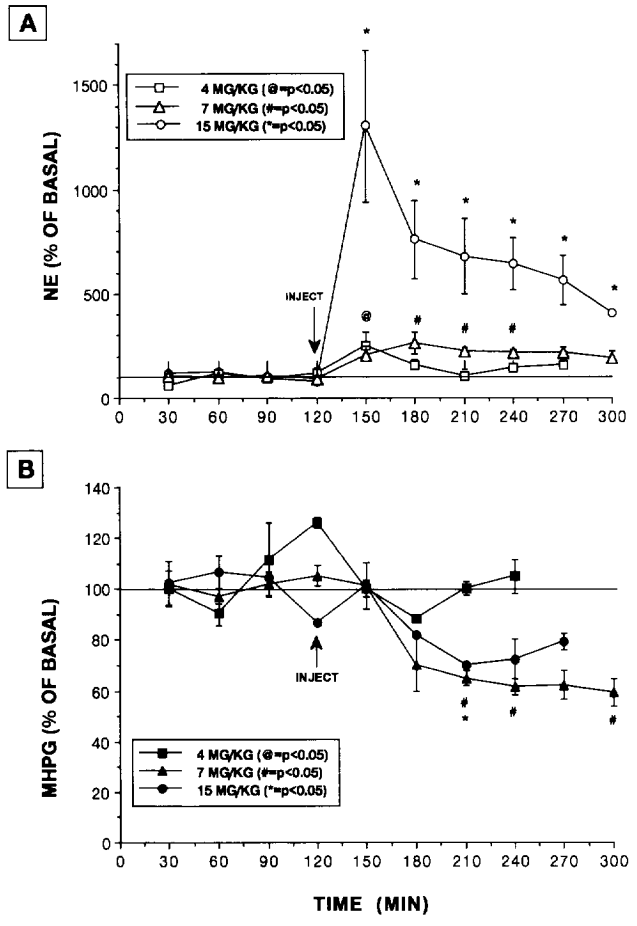

Figure 4. Dose effect of duloxetine on NE and MHPG levels in rat hypothalamus dialysate. Duloxetine at 4, 7, and 15 mg/kg, IP dose-dependently increases norepinephrine levels (**A**) while decreasing MHPG levels (**B**) in dialysate from rat hypothalamus. Values are in percentages of basal levels (mean ± SEM for at least three experiments at each dose of duloxetine. * Significantly different from basal at $p < .05$, paired t-test.

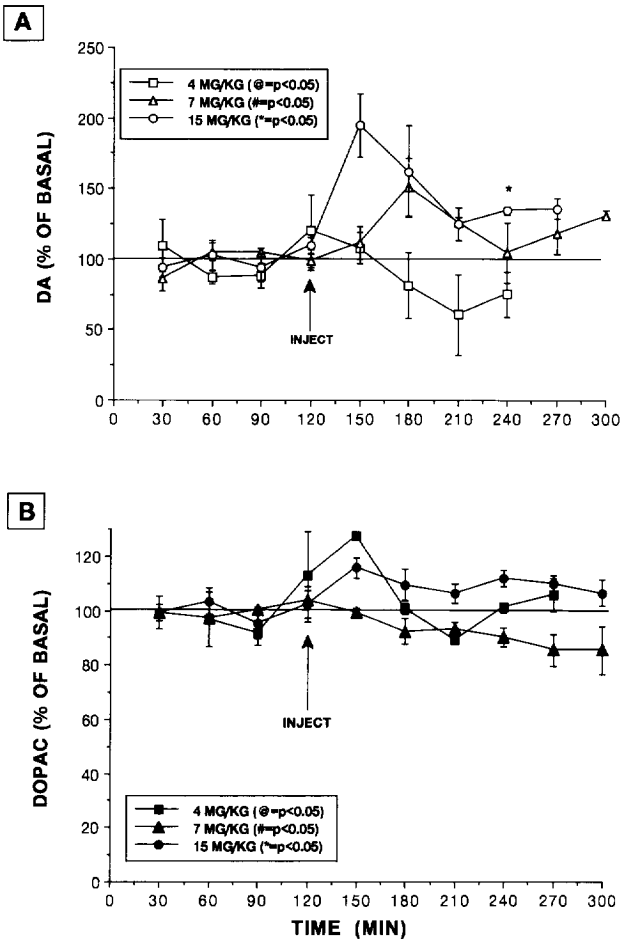

Figure 5. Extracellular levels of DA (**A**) and DOPAC (**B**) in hypothalamus as measured by in vivo microdialysis in unanesthetized rats. Duloxetine was administered at 4, 7, or 15 mg/kg IP after at least four 30-minute periods basal dialysate measurement. Values are presented as percentages of basal values from at least three experiments at each dose of duloxetine. * Significantly different from basal at $p < .05$, paired t-test.

significantly vary from basal levels after administration of duloxetine at 4, 7, or 15 mg/kg (Figure 5B).

DISCUSSION

Duloxetine, a secondary amine, potently inhibits 5-HT and NE uptake with a three times greater potency for inhibiting 5-HT uptake. The primary amine of duloxetine is also an effective inhibitor of 5-HT and NE uptake at one-fourth to one-fifth the potency of duloxetine. Thus the potential primary amine metabolite of duloxetine might contribute, in part, to the inhibition of 5-HT and NE uptake in vivo. The potency to inhibit NE uptake by each of these compounds was greater in hypothalamic than cortical preparations and may reflect the higher concentrations of NE uptake carriers in the hypothalamus (Gehlert et al. 1993). Most significantly, the present study shows that duloxetine in vivo produced robust increases in extracellular 5-HT and NE in hypothalamus matching or surpassing the increases elicited separately with other potent inhibitors of 5-HT uptake and inhibitors of NE uptake.

In the current study duloxetine at 15 mg/kg IP increased extracellular 5-HT to nearly fourfold above the baseline in dialysis samples from hypothalamus. The magnitude of increase after duloxetine treatment was similar to that observed in hypothalamus after the systemic administration of a comparable dose of fluoxetine (Perry and Fuller 1993a). Fluoxetine treatment consistently increased 5-HT levels in most other brain areas: Nearly fourfold in striatum (Perry and Fuller 1992), 2.5-fold in thalamus (Dailey et al. 1992), and twofold in diencephalon (Rutter and Auerbach 1993). Among other selective 5-HT uptake inhibitors, indalpine increased

5-HT levels threefold in caudate-putamen (Kalen et al. 1988). Sertraline (Invernizzi et al. 1991), citalopram (Invernizzi et al. 1992), or clomipramine (Adell and Artigas 1991) produced five-, four-, and threefold increases, respectively, in the raphe areas. However, even after chronic administration of amitriptyline (10 mg/kg PO), a putative dual inhibitor of 5-HT and NE uptake, daily for up to 21 days, the extracellular levels of 5-HT in rat frontal cortex were unchanged (Sleight et al. 1989). Acutely, amitriptyline also failed to significantly change 5-HT levels at rat frontal cortex (Kihara and Ikeda 1995). In hypothalamus, however, amitriptyline at 30 mg/kg IP significantly increased 5-HT levels, although only by 100% (Engleman and Wong 1994).

An 11-fold increase in extracellular NE levels in hypothalamus microdialysates is most robust among increases of monoamine levels after administration of duloxetine at 15 mg/kg IP. However, administration of desipramine (5 mg/kg IP), a selective inhibitor of NE uptake, produced a fourfold increase in NE levels in rat hypothalamus (Itoh 1990). In other brain areas, desipramine at 10 mg/kg IP has been shown to elevate NE levels by 2.5-fold in cerebral cortex (L'Heureux et al. 1986) and twofold in thalamus (Yan et al. 1993). Tomoxetine, another selective inhibitor of NE uptake, at the relatively high dose of 20 mg/kg SC, which is over five times the ED_{50} dose to inhibit NE uptake in vivo (Wong et al. 1982), increased NE levels fourfold in rat hypothalamic microdialysates (unpublished data). Thus, other factors in addition to NE uptake inhibition may contribute to the large increase of NE levels after duloxetine administration at 15 mg/kg as observed in the present study. The greater availability of 5-HT resulting from the inhibition of 5-HT uptake may contribute to the robust increase of NE concentrations. Indeed, systemic administration of fluoxetine (10 mg/kg IP), a selective serotonin uptake inhibitor, increases not only extracellular 5-HT (200%) but also NE levels (350%) in hypothalamus (Perry and Fuller 1993a, 1993b). Addition of duloxetine, fluoxetine (unpublished data), or amitriptyline (Engleman and Wong 1994) to the artificial cerebrospinal fluid (CSF) at up to 10 μM did not induce the robust increases in NE levels observed with systemic administration of the respective compounds. However, systemic administration of the direct 5-HT agonists 8-hydroxy-2-(di-n-propylamino) tetraline (8-OH-DPAT) Tian et al. 1993) or MK212 (Mayle et al. 1991) increased tissue levels of MHPG-SO_4 in the hypothalamus indicative of an increase in NE release. Together, these studies suggest a role for 5-HT heteroreceptors located outside of the hypothalamus on NE neurons in increasing extracellular NE levels as observed in the present study. Thus, the concomittant inhibition of 5-HT and NE uptake may afford a greater availability of NE than that resulting from NE uptake inhibition alone.

Milnacipran (Moret et al. 1985) and venlafaxine (Muth et al. 1986) are both tertiary amines and are also nontricyclic mixed inhibitors of 5-HT and NE uptake. Milnacipran inhibited 5-HT and NE uptake by 50% (IC_{50}) at 203 and 100 nM, respectively, while venlafaxine had IC_{50} values of 210 and 640 nM, respectively. However, similar neurochemical studies of milnacipran and venlafaxine using microdialysis techniques have not been reported.

In contrast to the distinct increases in 5-HT and NE levels, no significant increases in DA were observed. Unlike NE and 5-HT, DA metabolite levels (DOPAC) did not decrease after duloxetine. Indeed, at doses up to 30 mg/kg IP, duloxetine had no effect on DA uptake in striatum ex vivo (Figure 2). These results confirm that duloxetine administration at doses that adequately inhibit 5-HT uptake ex vivo in rat brain or platelets do not inhibit striatal DA uptake ex vivo.

The effects of duloxetine on monoamine levels in the current study support findings from behavioral studies. Duloxetine was shown to dose-dependently suppress food (Wong et al. 1993) and ethanol (Murphy et al. 1989) intake in rats. In mice (Katoh et al. 1993) duloxetine (1.6 to 12.5 mg/kg PO) prevented tetrabenazine-induced (50 mg/kg SC) ptosis and reserpine-induced hypothermia. At 12.5 to 25 mg/kg PO it induced head movements and tremor when coadministered with 5-hydroxytryptophan (100 mg/kg IP). It also potentiated both clonidine- and morphine-induced analgesia (0.6 to 20 mg/kg SC) and attenuated "despair" in forced swimming in mice. In the rat (Katoh et al. 1993) duloxetine (12.5 to 25 mg/kg PO) significantly decreased REM sleep, but at concentrations up to 200 mg/kg PO did not affect salivation and lachrymation induced by the cholinergic agonist oxotremorine (1 mg/kg SC). These findings demonstrate the behavioral effects of duloxetine resulting from simultaneous NE and 5-HT uptake inhibition and its poor cholinergic activity.

In summary, the data presented in the current study confirm and extend previous neuropharmacological and behavioral studies and demonstrate that duloxetine is a potent dual inhibitor of NE and 5-HT uptake in vivo. The ability of duloxetine to simultaneously increase extracellular levels of NE and 5-HT while not directly interacting with neurotransmitter receptors suggests that duloxetine may be a highly effective antidepressant agent with a low potential for untoward effects.

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