In Vivo Chronoamperometric Measures of Extracellular Serotonin Clearance in Rat Dorsal Hippocampus: Contribution of Serotonin and Norepinephrine Transporters

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ABSTRACT

The effects of blockade of serotonin (5-HT) and norepinephrine (NE) transporters (SERT and NET, respectively) on the removal of locally applied 5-HT from extracellular fluid (ECF) were examined using in vivo chronoamperometry. Male Sprague-Dawley rats were anesthetized with chloralose/urethane, and a nanofiber-coated, carbon fiber electrode attached to a multibarrel micropipette was positioned into either the dentate gyrus or CA3 region of the dorsal hippocampus. Pressure ejection of 5-HT elicited reproducible electrochemical signals of similar peak amplitude and time course in both structures. Local application of the selective serotonin reuptake inhibitors (SSRI) fluvoxamine and citalopram prolonged the clearance of 5-HT in both brain regions and also increased signal amplitude in the CA3 region. These effects were abolished in rats pretreated with 5,7-dihydroxytryptamine (5,7-DHT), a selective 5-HT

The SERT is responsible for terminating serotonergic neurotransmission by high affinity uptake of serotonin (5-HT) from the synaptic cleft (Amara and Kuhar, 1993). Blockade of 5-HT transport by SSRIs ameliorates a variety of neuropsychiatric disorders, including depression and certain anxiety disorders (Owens and Nemeroff, 1994; Frazer, 1997). Repeated administration of these drugs cause time-dependent changes in many aspects of serotonergic neuronal function (Blier and de Montigny, 1994). Drugs with selective effects on noradrenergic neurons in vitro are also antidepressants (Frazer, 1997), although some attribute the efficacy of such drugs (e.g., DMI) to actions on serotonergic function (e.g., enhancement of postsynaptic serotonergic responsiveness) (Blier and de Montigny, 1994).

There may be other mechanisms by which noradrenergic drugs could affect serotonergic function. For example, Shaskan and Snyder (1970) described the uptake of 5-HT into hypothalamic synaptosomes by at least two transport processes. One process, termed uptake 1, exhibits high affinity for 5-HT but a low capacity to transport 5-HT into the intracellular space and is thought to be localized to 5-HT neurons. The other process is termed uptake 2 and shows low affinity for 5-HT but high transport capacity. This latter transport mechanism reflects uptake into nonserotonergic, presumably catecholaminergic, neurons (Shaskan and Snyder, 1970). This phenomenon raises the possibility that selective inhibitors of the NET, such as DMI, may raise extracellular concentrations of 5-HT by blocking the uptake of this indoleamine into noradrenergic neurons.

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ABBREVIATIONS: DMI, desipramine; 5,7-DHT, 5,7-dihydroxytryptamine; ECF, extracellular fluid; Fluvox, fluvoxamine; HPLC, high-performance liquid chromatography; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine (serotonin); i.c.v., intracerebroventricularly; i.p., intraperitoneally; NE, norepinephrine; NET, norepinephrine transporter; PBS, phosphate-buffered saline; 6-OHDA, 6-hydroxydopamine; SERT, serotonin transporter; SSRIs, selective serotonin reuptake inhibitors.
Previous studies examining the uptake of 5-HT by catecholaminergic neurons have been carried out in vitro (Lichtensteiger et al., 1967; Shaskan and Snyder, 1970; Kuhar et al., 1972). More recent work has used the technique of in vivo microdialysis to demonstrate that noradrenergic uptake inhibitors such as DMI can, under certain conditions, raise the concentration of 5-HT in ECF (Bel and Artigas, 1996; Li et al., 1996). However, the mechanism or mechanisms by which this occurs were not studied. To address this issue more directly, we used the technique of in vitro chronoamperometry. In contrast to the considerable body of work measuring dopamine in vivo by fast voltammetric techniques, there has been relatively little application of this methodology to the analysis of 5-HT. The majority of voltammetric studies measuring extracellular 5-HT in vivo have used slower recording (e.g., 2–5 min) procedures such as differential pulse voltammetry (e.g., Pineyro et al., 1994; Rivot et al., 1995). We recently verified that high-speed chronoamperometry is a technique that can reliably measure the clearance of 5-HT from ECF in a millisecond time range (e.g., 100–200 msec) and that such clearance is a direct quantitative measure of SERT activity in vivo (Daws et al., 1997; Luthman et al., 1997). Thus chronoamperometric measures of 5-HT clearance offer a unique approach to examining functional changes in SERT activity in discrete brain nuclei.

The present study aimed to further explore the mechanism or mechanisms by which locally applied 5-HT is removed from the ECF in the dorsal hippocampus. The hippocampus was selected because it is part of the limbic system, which has been viewed as the “mood-controlling” system in the brain. Moreover, it is a structure where antidepressant drugs have been shown to cause both acute and regulatory effects (Blier et al., 1990). This aim was approached by characterizing the effect of selective 5-HT or NE uptake inhibitors on the clearance of 5-HT in the dentate gyrus and CA3 region of intact rats and in rats whose serotonergic or noradrenergic neurons had been destroyed by neurochemical lesions. The results provide a demonstration of the involvement of both the SERT and NET in removing exogenously applied 5-HT from the ECF in some but not all regions of the brain.

**Materials and Methods**

**Animals.** Male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 280 to 380 g, were used for all experiments. They were housed in either groups of three or individually, after recovering from surgery. The rats were maintained under a 12:12-hr light/dark cycle with food and water provided ad libitum. All animal procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize both the number of animals used and stress or discomfort to the animals during experimental procedures.

**Electrochemical recordings.** High-speed chronoamperometric recordings were made using an IVEC-10 system (Medical Systems, Greenvane, NY). Oxidation potentials consisted of 100-msec pulses of +0.55 V. Each pulse was separated by 100-msec interval during which the resting potential was maintained at 0.0 V. Voltage at the active electrode was applied with respect to a Ag/AgCl reference electrode positioned in the ECF of the ipsilateral superficial cortex. The oxidation and reduction currents were digitally integrated during the last 80 msec of each 100-msec voltage pulse.

**Electrode preparation and in vitro calibration.** Single carbon fiber electrodes with a 30-μm tip diameter and an active recording area ranging from 95 to 175 μm in length (Quanteen, Denver, CO) were used in all experiments. Before use in vivo, each electrode was coated with Nafion (5% solution, Aldrich Chemical, Milwaukee, WI), a perfluorosulfonated ion exchange resin that minimizes signal detection of and interference from anions such as monomeric metabolites, uric acid and ascorbate (Gerhardt et al., 1984). Seven coats of Nafion were applied, with electrodes heated for 3 min at 200°C after each coat. Electrodes were then tested for sensitivity to ascorbate (250 μM) and calibrated with six accumulating concentrations of 5-HT ranging from 0.5 to 3.0 μM. All calibrations were performed at room temperature using 0.1 M PBS (pH 7.4). Only electrodes displaying a selectivity ratio for 5-HT over ascorbate of >1000:1 and a linear response ($r^2 > 0.997$) to 5-HT (0.5–3.0 μM) were used. The detection limit for the measurement of 5-HT was defined as the concentration that produced a response with a signal-to-noise ratio of 3. In these experiments averaged $27±2$ nM ($n=70$). Electrodes were also intermittently tested for sensitivity to 5-HIAA (10 μM) and uric acid (50 μM). These compounds oxidize at similar potentials to 5-HT and therefore are capable of contributing to the 5-HT signal (Cespuglio et al., 1986; Rivot et al., 1995). As previously reported (Daws et al., 1997) these electrodes have excellent selectivity for 5-HT over these compounds (e.g., $3082±284.1$ and $5116±362.1$, for 5-HIAA and uric acid respectively; $n=10$ electrodes).

**In vivo experimental protocols.** Rats were anesthetized by i.p. injection of chloralose (85 mg/kg) and urethane (850 mg/kg) and, after tracheal intubation, placed into a stereotaxic frame. Body temperature was maintained at 37 ± 1°C by a water circulating heated pad. The scalp was incised and reflected, and the skull and dura overlaying the dorsal hippocampus were removed for electrode placement into the dentate gyrus (stereotaxic coordinates, AP, −3.8 mm from bregma; ML, 1.5 mm from midline; DV, −3.5 mm from dura) or CA3 region (AP, −4.1; ML, +3.3; DV, −3.6) of the dorsal hippocampus. A small burr hole was made over the anterior cortex, and a Ag/AgCl reference electrode was placed in contact with brain tissue.

The electrochemical recording assembly consisted of a Nafion-coated, single carbon fiber electrode attached to a seven-barreled micropipette. The assembly was constructed such that the electrode and micropipette tips were separated by 250 to 350 μm. The tip diameter of each barrel of the multibarrelled micropipette was 8 to 12 μm. Barrels were filled with 5-HT (200 μM), the SSRIAs fluvoxamine (10–400 μM) and citalopram (100 μM), the norepinephrine reuptake inhibitors DMI (10–400 μM) or protriptyline (400 μM) or vehicle. Serotonin, fluvoxamine and citalopram were dissolved in PBS with 100 μM ascorbic acid added as an antioxidant. Due to solubility problems, DMI and protriptyline were dissolved in ultrapure water with 100 μM ascorbic acid. The pH of all solutions was adjusted to 7.3 to 7.4. Serotonin was pressure ejected (5–25 psi for 0.25–3 sec) at 5- to 10-min intervals until a reproducible signal was obtained (usually three or four applications) (fig. 1). Unless specified otherwise, the mean ± S.E.M. number of picomoles of 5-HT delivered was 12 ± 2 in a volume of 60 ± 10 nl as measured by determining the amount of fluid displaced from the micropipette using a dissection microscope fitted with an eyepiece reticle.

Once the 5-HT signal was reproducible, vehicle or drug was applied 60 to 90 sec before the next application of 5-HT. These solutions were pressure ejected over 10 to 20 sec to minimize disturbances to the base-line electrochemical signal. The volume of drug or vehicle ejected was 2 to 3 times the volume of 5-HT. At the conclusion of each experiment, the rat was decapitated while still anesthetized, and the brain was removed and stored in formalin (10%) for 24 hr. The brains were frozen and sliced into 20-μm-thick sections for histological verification of electrode tract localization. Only data from rats where the electrode tip was confirmed to be in the dentate gyrus or CA3 region are shown.

**5,7-DHT and 6-OHDA lesions.** Rats were administered i.c.v. either 5,7-DHT to destroy serotonergic neurons or 6-OHDA to destroy noradrenergic neurons. Animals lesioned with 5,7-DHT were given nomifensine (30 mg/kg i.p.) 30 min before the administration of
5,7-DHT to prevent destruction of noradrenergic and dopaminergic neurons (Gershnik et al., 1979); those lesioned with 6-OHDA were given zimelidine (25 mg/kg i.p.) (Gravel and de Montigny, 1987) and GBR 12909 (30 mg/kg i.p.) (Nissbrandt et al., 1991) 30 min before administration of 6-OHDA to prevent destruction of aerotergenic and dopaminergic neurons. Animals were anesthetized with sodium pentobarbital (65 mg/kg i.p.) and placed into a stereotaxic frame. Neurotoxin was delivered bilaterally into the lateral ventricles using a 28-gauge stainless steel injector, connected to a microsyringe pump (stereotaxic coordinates: AP, −0.8 from bregma; ML, 1.5 from mid-line; DV, −3.7 from skull). Ten microliters of PBS containing 100 μg of neurotoxin and 100 μM ascorbic acid was infused, in series, into each lateral ventricle. The infusion rate was 1 μl/min. The total amount of 5,7-DHT or 6-OHDA delivered therefore was 200 μg. After completion of the i.e.v. infusion, the injector was left in place for 5 min to allow diffusion of the neurotoxin from the injector. Control rats were subjected to the same procedure but were injected with vehicle containing 100 μM ascorbic acid alone. The control rats were also pretreated with nomifensine (5,7-DHT sham) or zimelidine and GBR 12909 (6-OHDA sham) to mimic the pretreatment of the lesioned group. In vivo electrochemical recordings were performed 2 to 4 weeks after the lesioning procedure.

At the conclusion of the electrochemical recording experiment, brains were removed as described above. The brain hemisphere where recordings had been made was stored for histological verification of the electrode placement. Hippocampi from the contralateral hemispheres were rapidly removed over ice, weighed, frozen in chilled isopentane and stored at −70°C. This tissue was then used to determine hippocampal levels of 5-HT and NE by HPLC coupled with electrochemical detection as described by Hall et al. (1989). Three-micron particle (4.6 × 100 mm) C18 Hypersil-ODS columns (Keystone Scientific) were used for high sensitivity as well as rapid sample throughput. Flow rates of 2 ml/min were used with a 0.17 M citrate-acetate buffer (pH 4.1). An ESA 5100A Coulochem system with a model 5011 analytical cell was used for electrochemical detection of the eluting species. Dihydroxybenzylamine was added to each of the tissue samples to calculate recovery. The levels of monoamines are expressed as nanograms per gram wet weight of tissue.

**Data analyses.** In most instances, two signal parameters were analyzed: (1) the maximal amplitude of the signals resulting from local application of 5-HT and (2) t 40−80 , the time (in sec) for the signal to decline between 40% and 80% of the maximal amplitude. The t 40−80 value was used to demonstrate drug-induced changes in the 5-HT signal as this parameter is markedly affected by inhibitors of the uptake of dopamine (Cass et al., 1993) and 5-HT (Daws et al., 1997). Only oxidation currents were used for data analyses. Amplitude and time course data were analyzed with paired, two-tailed t tests (pre-application vs. post-application of drug) or t tests for independent samples (sham vs. lesioned). The percentage change from predrug value for these parameters was analyzed by Mann-Whitney U tests. A two-tailed probability level of P < .05 was accepted as statistically significant for all tests.

**Drugs.** Serotonin hydrochloride, desipramine hydrochloride, and 5,7-DHT creatinine sulfate were purchased from the Sigma Chemical (St Louis, MO). GBR 12909 dihydroxyamine, zimelidine hydrochloride and 6-OHDA hydrobromide were purchased from Research Biochemicals International (Natick, MA). Citalopram hydrobromide was a gift from Lundbeck (København-Valby), fluvoxamine maleate was generously donated by Pharmacia and Upjohn (Kalamazoo, MI), nomifensine maleate was a gift from Hoechst-Roussel Pharmaceuticals (North Somerville, NJ) and protriptyline hydrochloride was kindly provided by Merck Research Labs (West Point, PA).

**Results**

**Intact rats.** Figure 1 shows the oxidation and reduction currents for three applications of the same amount of exogenous 5-HT (12 ± 2 pmol) given at 7-min intervals into either the hilar region of the dentate gyrus or the CA3 region (strata radiatum) of the dorsal hippocampus. It is evident that reproducible electrochemical signals were obtained and that PBS had no effect on the electrochemical signal evoked by 5-HT. The oxidation and reduction currents, converted to micromolar concentrations using a calibration factor determined in vitro, are shown for three consecutive applications of 5-HT. Two consecutive applications of 5-HT were followed by PBS. Various parameters are obtained from the electrochemical signal produced by exogenous application of 5-HT. Peak amplitude is presented in micromolar units, whereas those parameters used to provide an index of the rate of removal of 5-HT are given in units of seconds. Such parameters include t 50 and t 90 , the time it takes for the peak amplitude to be reduced by 50% or 90% respectively; t 40−80 , the time for the signal to decrease from 60% of its peaks value to 20%; and total time, the total time course of the signal. When the same amount of 5-HT was pressure ejected into either the dentate gyrus or the CA3 region, the electrochemical signals were produced by consecutive applications of 5-HT. Fluvoxamine and DMI inhibited the rate of disappearance of

![Fig. 1. Reproducibility of electrochemical signals recorded in (a) the dentate gyrus and (b) CA3 region of the dorsal hippocampus in vivo. Serotonin (12 ± 2 pmol) was applied at 5 min intervals by pressure ejection. The peak amplitude, t 50 , t 90 , t 40−80 and total time course are defined on the first signal in (a).](image-url)
5-HT without significantly affecting the peak signal amplitude. By contrast, in the CA3 region, only fluvoxamine altered the signal, affecting both the maximal amplitude as well as the rate of removal of 5-HT. The effects of these drugs on the various parameters obtained from the electrochemical signal are shown in Table 1. In the dentate gyrus, both fluvoxamine and DMI increased significantly the $t_{50}$, $t_{80}$, $t_{40–80}$ and total time values. No significant effect was observed on the value for maximal amplitude. A different pattern of results was observed in the CA3 region. With the exception of $t_{50}$, fluvoxamine significantly increased the same signal parameters in this region as it did in the dentate gyrus. In addition, fluvoxamine increased significantly the peak signal amplitude. By contrast, DMI produced no significant alteration of the electrochemical signal caused by 5-HT in the CA3 region.

In subsequent experiments, only signal amplitude and $t_{40–80}$ data are presented. The $t_{40–80}$ was selected as this signal parameter has previously been shown to be particularly sensitive to high-affinity uptake inhibitors (Cass et al., 1993; Daws et al., 1997). The above experiment confirmed that fluvoxamine (dentate gyrus and CA3) and DMI (in the dentate gyrus) have robust effects on this parameter. In addition, subsequent data are presented as percent change from predrug base-line to facilitate comparison of the effects of the different drugs in the two hippocampal regions.

In the next experiment, the effect of two additional inhibitors of monoamine uptake were compared with the effects of fluvoxamine or DMI. Selected for study were the SSRI citalopram (18 ± 11 pmol) or the selective inhibitor of NE re-uptake protriptyline (96 ± 13 pmol). Results obtained in both the dentate gyrus and CA3 region are shown in Figure 4. The effect of citalopram on the $t_{40–80}$ in both areas of brain was comparable to that of fluvoxamine. Comparable increases in signal amplitude were produced by both drugs in the CA3 region, but only the effect due to fluvoxamine was statistically significant. Similarly, protriptyline’s effects were identical to those of DMI in that both drugs significantly increased $t_{40–80}$ only in the dentate gyrus and had no significant effect on the 5-HT signal in the CA3 region.

In a separate experiment, the dose dependency of the effect of fluvoxamine and DMI on the $t_{40–80}$ value of the 5-HT signal in the dentate gyrus was investigated. As before, 5-HT (12 ± 2 pmol) was locally applied at 5-min intervals until a consistent response was established. Fluvoxamine (0–100 pmol) or DMI (0–100 pmol) was pressure ejected 60 to 90 sec before the next application of 5-HT. The barrel concentrations of drug ranged from 10 to 400 mM, so the volume ejected remained constant. As illustrated in Figure 5, neither fluvoxamine nor DMI altered the $t_{40–80}$ of the 5-HT signal when <25 pmol was delivered. At ≥25 pmol, fluvoxamine and DMI increased the $t_{40–80}$ of the 5-HT signal in a comparable manner. Signal amplitude was not altered by any of the “doses” of fluvoxamine or DMI used.

In all instances when a drug effect was observed (dentate gyrus or CA3 region), the 5-HT electrochemical signal returned to its predrug base-line value within 45 min of drug administration (i.e., four or five postdrug applications of 5-HT). The rate of recovery did not differ significantly between drug treatments (data not shown).

None of the drugs or PBS produced any effect on the base-line electrochemical signal when applied by themselves. In some instances, there was a slight decrease in the base-line signal that recovered within 60 sec [likely due to dilution of electroactive species by the drug solution (Gerhardt et al., 1987)].
Lesioned rats. To determine whether the ability of 5-HT and NE reuptake inhibitors to prolong the 5-HT signal in the dentate gyrus was due to the presence of serotonergic and/or noradrenergic terminals, these were destroyed with 5,7-DHT or 6-OHDA, respectively. Rats treated with 5,7-DHT had $90\%$ depletion of hippocampal 5-HT, whereas their content of NE was not different from that of sham-lesioned rats. Rats treated with 6-OHDA had a $86\%$ depletion of hippocampal NE, whereas their content of 5-HT was not different from that of sham-lesioned rats (table 2).

In rats lesioned with 5,7-DHT, pressure ejection of the same amount of 5-HT into either the dentate gyrus or CA3 region caused marked increases in both the peak signal amplitude and the $t_{40-80}$ value in comparison with the values obtained in sham-lesioned rats (figs. 6 and 7). Pretreatment of rats with 6-OHDA caused comparable changes in these parameters in the dentate gyrus to those caused by pretreatment with 5,7-DHT. In the CA3 region, by contrast, pretreatment of rats with 6-OHDA did not alter the electrochemical signal caused by 5-HT (figs. 6 and 7).

Due to the significant increase in base-line signals in lesioned rats compared with those in sham animals, the volume of 5-HT ejected was reduced in lesioned rats so the base-line signal amplitude and $t_{40-80}$ were equivalent to those measured in sham rats. In the dentate gyrus, sham rats received 13 $\pm$ 4 pmol 5-HT, whereas 5,7-DHT- and 6-OHDA-lesioned rats received 6 $\pm$ 1 and 5 $\pm$ 3 pmol, respectively. In the CA3 region, sham- and 6-OHDA-lesioned rats

### Table 1

The effect of serotonin and norepinephrine reuptake inhibitors on signal amplitude and time course parameters of the clearance of exogenously administered 5-HT in rat hippocampus

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amplitude</th>
<th>$t_{50}$</th>
<th>$t_{90}$</th>
<th>$t_{40-80}$</th>
<th>Total time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentate gyrus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1.15 $\pm$ 0.37</td>
<td>48 $\pm$ 5</td>
<td>97 $\pm$ 15</td>
<td>58 $\pm$ 10</td>
<td>171 $\pm$ 24</td>
</tr>
<tr>
<td>Fluvox</td>
<td>0.96 $\pm$ 0.26</td>
<td>87 $\pm$ 15$^a$</td>
<td>211 $\pm$ 49$^a$</td>
<td>151 $\pm$ 35$^a$</td>
<td>444 $\pm$ 96$^a$</td>
</tr>
<tr>
<td>None</td>
<td>1.31 $\pm$ 0.31</td>
<td>58 $\pm$ 8</td>
<td>120 $\pm$ 18</td>
<td>71 $\pm$ 12</td>
<td>165 $\pm$ 27</td>
</tr>
<tr>
<td>DMI</td>
<td>1.21 $\pm$ 0.40</td>
<td>85 $\pm$ 11$^b$</td>
<td>206 $\pm$ 29$^b$</td>
<td>151 $\pm$ 20$^b$</td>
<td>485 $\pm$ 74$^b$</td>
</tr>
<tr>
<td>CA3</td>
<td>None</td>
<td>0.75 $\pm$ 0.11</td>
<td>91 $\pm$ 11</td>
<td>129 $\pm$ 18</td>
<td>53 $\pm$ 11</td>
</tr>
<tr>
<td>Fluvox</td>
<td>1.01 $\pm$ 0.13$^a$</td>
<td>105 $\pm$ 14</td>
<td>172 $\pm$ 20$^b$</td>
<td>112 $\pm$ 13$^b$</td>
<td>298 $\pm$ 49$^b$</td>
</tr>
<tr>
<td>None</td>
<td>0.88 $\pm$ 0.19</td>
<td>84 $\pm$ 15</td>
<td>144 $\pm$ 24</td>
<td>72 $\pm$ 11</td>
<td>264 $\pm$ 42</td>
</tr>
<tr>
<td>DMI</td>
<td>0.71 $\pm$ 0.14</td>
<td>98 $\pm$ 17</td>
<td>169 $\pm$ 28</td>
<td>85 $\pm$ 14</td>
<td>320 $\pm$ 61</td>
</tr>
</tbody>
</table>

Data are expressed as mean $\pm$ S.E.M. $n = 7$ for all groups.
Value significantly different from predrug value ($^a$ $P < .05$, $^b$ $P < .01$, paired $t$ test).

**Fig. 4.** Summary of the effect of selective 5-HT and NE reuptake inhibitors on $t_{40-80}$ (A and B) and signal amplitude (C and D). Data represented on the left are for the dentate gyrus, and those on the right represent data derived from the CA3 region. PBS, fluvoxamine (Fluvox, 67 $\pm$ 9 pmol), citalopram (Cital, 18 $\pm$ 11 pmol), DMI (63 $\pm$ 10 pmol) or protriptyline (Protrip, 96 $\pm$ 13 pmol) was pressure ejected 60 to 90 sec before the next application of 5-HT. Data are expressed as percent (mean $\pm$ S.E.M.) of the pretreatment signal base-line. $n = 7$ for all groups with the exception of Cital and Protrip, where $n = 5$. *, $P < .05$, **, $P < .01$, Mann-Whitney $U$ test.
received 11 ± 2 and 10 ± 2 pmol 5-HT, respectively, whereas 7 ± 2 pmol 5-HT was given to 5,7-DHT-lesioned rats to yield comparable 5-HT signals. Once stable control responses to 5-HT had been established, fluvoxamine, DMI or PBS was pressure ejected 60 to 90 sec before the next application of 5-HT. As expected, in both brain regions fluvoxamine produced a 2-fold increase in the t₄₀–₈₀ value in control animals; however, it caused no effect in rats lesioned with 5,7-DHT (fig. 8). Also as expected, DMI doubled the t₄₀–₈₀ of the 5-HT signal in sham rats only in the dentate gyrus but, by contrast, was without effect on 5-HT signal parameters in rats pretreated with 6-OHDA. Pretreatment of rats with 5,7-DHT did not alter the ability of DMI to increase the the t₄₀–₈₀ value in the dentate gyrus. Similarly, pretreatment with 6-OHDA had no effect on the ability of fluvoxamine to increase the t₄₀–₈₀ values in either the dentate gyrus or CA3 (fig. 8).

As observed in intact rats, neither fluvoxamine nor DMI alone altered signal amplitude in the dentate gyrus in any neurotoxin treatment group. In the CA3 region, DMI did not alter the 5-HT signal amplitude in sham-, 6-OHDA- or 5,7-DHT-lesioned rats. However, fluvoxamine enhanced the 5-HT signal amplitude by 151 ± 20% and by 211 ± 33% (P < .05, Mann-Whitney U) in sham and 6-OHDA-lesioned rats, respectively. Fluvoxamine did not alter the signal amplitude caused by 5-HT in 5,7-DHT-lesioned rats. Pressure ejection of vehicle produced no change in signal amplitude or the t₄₀–₈₀ value of 5-HT in the dentate gyrus or CA3 region in any treatment group.

### Discussion

The present study demonstrated that the SERT is primarily responsible for the clearance of 5-HT in the CA3 region, whereas in the dentate gyrus both the SERT and the NET are capable of removing 5-HT from the ECF.

The clearance of 5-HT in the dentate gyrus or CA3 region of the dorsal hippocampus was examined by measuring the disappearance of locally applied, exogenous 5-HT. Although endogenously released transmitter can be tentatively identified by the ratio of its reduction and oxidation currents (Gratton et al., 1989), additional neuropharmacological methods are required to fully determine the identity of the released compound or compounds. The subgranular zone of the dentate hilar region receives primarily serotonergic innervation, whereas noradrenergic innervation is prominent throughout the remainder of the hilar region. The strata radiatum of the CA3 region is predominantly innervated by serotonergic neurons with comparatively little noradrenergic innervation (Swanson et al., 1987). However, regardless of this anatomic separation, it is not so distinct as to eliminate the possibility that the electrochemical signal resulting from, for example, the potassium-evoked release of endogenous neurotransmitter (Gerhardt et al., 1987) could be composed of 5-HT and NE. Because of this, it would be difficult to use this approach to

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**TABLE 2**

Hippocampal content of 5-HT and NE in sham-, 5,7-DHT- and 6-OHDA-lesioned rats

<table>
<thead>
<tr>
<th></th>
<th>5-HT (ng/g)</th>
<th>NE (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (n = 12)</td>
<td>151 ± 16</td>
<td>300 ± 23</td>
</tr>
<tr>
<td>5,7-DHT (n = 15)</td>
<td>15 ± 5*</td>
<td>252 ± 28</td>
</tr>
<tr>
<td>6-OHDA (n = 12)</td>
<td>157 ± 32</td>
<td>43 ± 11*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M.

* P < .05 from sham control.
make conclusions regarding clearance mechanisms for endogenously released 5-HT (or NE) specifically. In addition, when the release of endogenous transmitter is evoked, the measured response represents a combination of both release and reuptake, such that the two processes cannot be examined independently. Electrical stimulation of cells innervating the area from which voltammetric recordings are made has been successfully used to evoke the release of specific monoamines such that release and reuptake components can be distinguished (Mitchell et al., 1994; Jackson and Wightman, 1995; Garris et al., 1997). However, the present study used the local application of exogenous neurotransmitter paradigm because the source and identity of the electrochemical signal are well defined and clearance mechanisms alone can be studied without the need to electrically stimulate cells and verify the identity of the released substance.

Local application of 5-HT into the hilar region of the dentate gyrus or the CA3 region of the dorsal hippocampus resulted in reproducible electrochemical signals with reduction-oxidation ratios characteristic of those found in vitro (compare with Gratton et al., 1989; Daws et al., 1997; Luth- 
man et al., 1997). This suggests that the signal recorded in vivo is likely due exclusively to exogenously applied 5-HT. It is unlikely that NE would contribute to the signal as it has recently been reported that increases of extracellular 5-HT (either by exogenous application of 5-HT or by administration of fluoxetine) inhibits the release of NE in the hippocampus of the rat (Matsumoto et al., 1995). Signal amplitudes and clearance times of exogenous 5-HT were similar in the dentate gyrus and CA3 region of the hippocampus suggesting that the clearance mechanism or mechanisms in each region are comparably effective. Because there are fewer SERTs in the dentate gyrus than in the CA3 (Hensler et al., 1994), it is possible that another mechanism or mechanisms contribute to the removal of 5-HT from the ECF in the dentate gyrus.

To determine pharmacologically the involvement of the SERT in mediating clearance of exogenously applied 5-HT in the dentate gyrus and the CA3 region, the SSRIs fluvoxamine and citalopram and the NE uptake inhibitors DMI and protriptyline were used. These drugs show $25$-fold selectivity for inhibiting the reuptake of one of these neurotransmitters in comparison to that of other biogenic amines (see Frazer, 1997). Citalopram has a 5-fold greater affinity for the

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**Fig. 7.** Summary data for the peak amplitude and $t_{40-80}$ of the electrochemical signal produced by 5-HT (12 ± 2 pmol) locally applied in the dentate gyrus or CA3 region of sham-, 5,7-DHT- or 6-OHDA-lesioned rats. Data are expressed as the mean ± S.E.M. $n = 6$ per group. *, $P < .05$, significantly different from sham-lesioned rats, $t$ test for independent samples.

**Fig. 8.** Summary of the effects of locally applied fluvoxamine (61 ± 3 pmol) or DMI (65 ± 2 pmol) into sham, 5,7-DHT- or 6-OHDA-lesioned rats. To elicit signals with comparable amplitude and time course, 6 ± 1 and 5 ± 3 pmol of 5-HT were pressure ejected into the dentate gyrus of 5,7-DHT- and 6-OHDA-lesioned rats respectively, whereas sham-lesioned rats received 13 ± 4 pmol 5-HT. In the CA3 region, 7 ± 2 pmol of 5-HT was pressure ejected into 5,7-DHT-lesioned rats, whereas sham and 6-OHDA-lesioned rats received 11 ± 2 and 10 ± 2 pmol 5-HT, respectively. Data are expressed as percent (mean ± S.E.M.) of the pretreatment signal base-line. $n = 6$ per group. *, $P < .05$, Mann-Whitney $U$ test.
SERT than fluvoxamine does (Claassen, 1983; Milne and Ga, 1991); thus to yield comparable results, a lower concentration of citalopram than fluvoxamine was used. Similarly, protriptyline has a marginally lower affinity for the NET than DMI does (Frazer, 1997), and so a somewhat higher concentration was applied. In the CA3 region, only the SSRIs delayed the rate of removal of 5-HT from the ECF. This is shown by the statistically significant increase produced by these drugs on parameters used to measure the decline of the serotonin signal (e.g., t_{40-80}, total time) (table 1). Both drugs had less of an effect on peak signal amplitude than on the rate of removal of 5-HT, with only fluvoxamine producing a statistically significant effect. This SSRI-induced inhibition of 5-HT “clearance” indicates that the removal of 5-HT is regulated by the SERT in the CA3 region. By contrast, the NE uptake inhibitors had no significant effect on the rate of disappearance of locally applied 5-HT from the CA3 region, implying that the NET is not involved in the removal of 5-HT from the ECF in this area.

In the dentate gyrus, both the SSRIs and the selective NE uptake inhibitors inhibited the rate of removal of 5-HT. There are several possible explanations why selective norepinephrine uptake inhibitors prolonged the 5-HT signal. That DMI and protriptyline cause release of endogenous 5-HT can be eliminated as one possibility because local application of the drugs by themselves did not lead to an increase in the electrochemical signal. Also, it does not appear that the concentration of DMI used was sufficiently high to inhibit the SERT. The dose-response studies indicated comparable potency of DMI and fluvoxamine at prolonging the clearance of exogenous 5-HT, yet DMI is ~60-fold less potent than fluvoxamine at inhibiting the reuptake of 5-HT (Frazer, 1997). If DMI were inhibiting the uptake of 5-HT exclusively into serotonergic neurons, it would be expected that it would take much higher concentrations of DMI than fluvoxamine to do this. The inability of DMI to prolong the rate of removal of 5-HT in rats devoid of noradrenergic neurons is also not supportive of its inhibitory effect being due to an action on the SERT. Rather, the studies in rats with lesions of serotonergic or noradrenergic neurons provide strong evidence that selective NE uptake inhibitors exert their effect on the removal of 5-HT through their action on the NET. The ability of DMI to prolong the 5-HT signal was absent in rats lesioned with 6-OHDA- but retained in 5,7-DHT- or sham-lesioned rats. Similarly, the fluvoxamine-induced inhibition was not observed in rats treated with 5,7-DHT- but was readily apparent in 6-OHDA- or sham-lesioned rats. From these data it may be inferred that NE and 5-HT terminals are requisite for 5-HT uptake 1, with V_{max} values being 12- to 15-fold greater than the corresponding values for uptake 1. Despite the much lower affinity of uptake 2 for 5-HT than uptake 1, its much greater capacity means that appreciable accumulation of 5-HT, even at low concentrations, could be due to uptake 2 (Shaskan and Snyder, 1970). These studies have been supported by others (Kuhar et al., 1972). More recently, it has also been demonstrated that 5-HT can be taken up by the dopamine transporter in the striatum (Jackson and Wightman, 1995).

It may be noteworthy that there was no effect of fluvoxamine on peak signal amplitude in the dentate gyrus, whereas it significantly increased signal amplitude in the CA3 region. This observation may be explained by the existence of two transport processes in the dentate gyrus (SERT and NET) and only one (SERT) in the CA3 region. Given that 5-HT signal amplitudes and the rate of removal of 5-HT were similar in these brain regions, it is likely that uptake of 5-HT by both the SERT and the NET in the dentate gyrus is comparable to that produced by the SERT alone in the CA3. Given that SSRIs inhibit only the SERT, the continued ability of the NET to remove 5-HT in the dentate gyrus may reduce the likelihood of seeing an effect on amplitude. Alternatively, if less high affinity uptake is taking place in the dentate gyrus than in the CA3 region, then the effect of an uptake inhibitor would tend to be less pronounced. This finding is analogous to that reported in other electrochemical studies of the effect of the catecholamine uptake inhibitor, nomifensine, on the signal amplitude of locally applied catecholamines in the striatum and cerebellum. High affinity catecholamine uptake sites are less dense in the cerebellum than in the striatum (Javitch et al., 1985). Not only was the signal amplitude greater in the cerebellum than in the striatum per pmol catecholamine, but nomifensine increased signal amplitude in the striatum by >300%, whereas it produced a <50% increase in the cerebellum (Cass and Gerhardt, 1995, Cass et al., 1995). Similarly, the signal produced by an equivalent amount of 5-HT pressure ejected into the corpus callosum, a fiber tract relatively devoid of SERT (Swanson et al., 1987; Sur et al., 1996), produced electrochemical signals with 2-fold greater amplitudes and prolongation of the time for removal of 5-HT than those observed in the dentate gyrus; furthermore, fluvoxamine did not significantly alter the 5-HT signal in the corpus callosum (Daws et al., 1997). These data are consistent with models proposed by Cass et al. (1993) that (1) less transmitter is needed to produce a given signal amplitude in brain regions with fewer transporters and (2) for a particular signal amplitude, the time course of the signal is longer in a region with fewer transporters. According to Shaskan and Snyder (1970), because “uptake 2” (presumably the NET) has a low affinity but high capacity to remove 5-HT from the ECF and changes in signal amplitude are more dependent on high affinity uptake (Cass et al., 1993), increases in signal amplitude would not be predicted after blockade of the NET.

It could be argued that if 5-HT is being removed from the ECF in the dentate gyrus by both the SERT and the NET, then their simultaneous blockade should produce an increase
in the signal amplitude of locally applied 5-HT. Preliminary studies where fluvoxamine and DMI were pressure ejected sequentially into the dentate gyrus revealed a 212 ± 15% \((n = 5;\ \text{L. C. Daws, G. M. Toney, G. A. Gerhardt and A. Frazer, unpublished observations})\) increase in 5-HT signal amplitudes, providing support for this hypothesis.

It is not clear why lesioning neurons with either 5,7-DHT or 6-OHDA caused a much greater effect on the amplitude of the signal caused by exogenous 5-HT than the effect caused by local application of uptake inhibitors. Although lesioning neurons with specific neurotoxins may eliminate >90% of transport sites (as indicated by the marked 5,7-DHT-induced reduction of the content of 5-HT measured in the present study), the local application of uptake inhibitors may not achieve such an effective reduction of the transport process. If so, then for the same amount of exogenously applied 5-HT, a greater increase in signal amplitude would be predicted in a lesioned rat than in one given an uptake inhibitor. Clearly further studies are needed to fully understand why neurotoxic lesion and uptake inhibition have such different effects on the dynamics of the electrochemical signal produced by exogenously administered 5-HT.

The present results show that in brain regions where there is both serotonergic and noradrenergic innervation, 5-HT can be removed from the ECF by both the SERT and the NET. Of fundamental importance is whether the uptake of 5-HT by catecholaminergic neurons has either physiological or pharmacological importance or is merely an artifact of the “high” (>0.5 \(\mu\text{M}\)) concentrations used to demonstrate it. Although this question cannot be answered precisely, the most current estimate for a transmitter such as glutamate is that the synaptic concentration exceeds 1 mM and that transmitter can travel several micrometers away from the cleft so as to reach concentrations of >10 \(\mu\text{M}\) in the extrasynaptic space (Clements, 1996). If this is true at serotonergic synapses, then the concentrations of 5-HT could be sufficiently high to allow it to be taken up by adjacent catecholaminergic neurons. Furthermore, an SSRI may increase the amount of 5-HT entering the extrasynaptic space, increasing the likelihood of its uptake by adjacent catecholaminergic neurons. This scheme envisions, then, that SSRIs enhance serotonergic neurotransmission by, at least in part, a process akin to “spatial recruitment” and that such recruitment would be increased even further by blocking 5-HT uptake into catecholaminergic neurons. In other words, in areas of brain receiving both a dense serotonergic and noradrenergic innervation, inhibition of 5-HT reuptake may permit 5-HT to “escape” the synaptic space and come into contact with NE transporters so as to be taken up by noradrenergic neurons. Such a scheme is consistent with data presented by Bel and Artigas (1996), who used microdialysis to show that DMI has been reported to increase the basal level of 5-HT, but this is dependent on the brain region examined, the duration of drug treatment and the route of drug administration (e.g., Bel and Artigas, 1996; Li et al., 1996). These studies did not, however, evaluate whether the treatment paradigms in which DMI increased the extracellular concentration of 5-HT was due to its ability to block uptake of 5-HT into noradrenergic neurons. Given the limitations of spatial resolution with dialysis, it is possible that changes in the concentration of 5-HT in discrete nuclei may have been obscured due to the large area from which the dialysate is collected. By using the technique of \textit{in vivo} chronoamperometry with single carbon fiber electrodes, the present study has shown that there are differences in the contribution of noradrenergic neurons to the uptake of 5-HT within two discrete regions of the hippocampus.

In conclusion, chronoamperometry offers a unique approach for examining functional changes in transporter activity \textit{in vivo}. That the extracellular concentration of 5-HT is controlled in certain brain regions by the SERT and the NET may be of considerable pharmacological importance. This technology offers a useful tool for studying alterations in SERT function as a consequence of acute and chronic treatment with drugs (e.g., antidepressants). Moreover, the present results may be useful in better defining the mechanism of action of drugs with both 5-HT and NE uptake inhibiting abilities given recent reports that they may lead to more rapid and effective treatment of depressive disorders than SSRIs alone (Nelson et al., 1991; Anderson and Tomenson, 1994).

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