Differential Effect of Local Infusion of Serotonin Reuptake Inhibitors in the Raphe versus Forebrain and the Role of Depolarization-Induced Release in Increased Extracellular Serotonin

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ABSTRACT
Systemic administration of selective serotonin reuptake inhibitors (SSRIs) elicits larger increases in serotonin (5-HT) in raphe than in forebrain sites. Because serotonergic neuronal activity is suppressed, the mechanism underlying SSRI-induced increases in extracellular 5-HT is unclear. This study determined whether local infusion of SSRIs also elicited regionally selective increases in extracellular 5-HT, and whether changes depended on serotonergic neuronal depolarization. Conventional microdialysis methods were used to measure 5-HT in dorsal raphe (DRN), median raphe, nucleus accumbens (NAcc), and frontal cortex of unanesthetized rats. During infusion of SSRIs into each site, the maximum response was an 6- to 7-fold increase in 5-HT in NAcc and frontal cortex, and a 20-fold increase in DRN and median raphe. The larger increase in 5-HT in raphe was confirmed using zero-net-flux microdialysis. In NAcc, baseline 5-HT was 0.7 nM, and levels increased to a maximum of 3.1 nM during infusion of the SSRI citalopram. Baseline 5-HT in DRN was greater, 1.3 nM, and increased to 12.4 nM in response to citalopram. Consistent with evidence that autoreceptor activation inhibits serotonergic neuronal discharge, SSRI infusion into DRN produced a moderate decrease in 5-HT in NAcc. However, increases in 5-HT in DRN elicited by SSRI infusion were attenuated by 8-hydroxydipropylaminotetralin and tetrodotoxin. These data indicate that depolarization-dependent 5-HT release was not fully inhibited during SSRI infusion into DRN. In summary, SSRIs produce larger increases in extracellular 5-HT in raphe than in forebrain sites. Increases depend in part on depolarization-induced release, which may be greater in raphe than in forebrain.

Serotonin (5-HT) in extracellular space is inactivated primarily by high-affinity reuptake. Hence, drugs that block this process, such as the selective serotonin reuptake inhibitors (SSRIs), can produce increases in extracellular 5-HT (Invernizzi et al., 1992; Perry and Fuller, 1992) and have been used in treatment of depression (Blier and de Montigny, 1994). However, the efficacy of SSRIs is limited by an auto-inhibitory mechanism. Serotonergic neuronal discharge (Chaput et al., 1986; Gartside et al., 1995) and 5-HT release in forebrain sites (Rutter et al., 1995) is inhibited after systemic administration of SSRIs. This is attributable in part to elevation of extracellular 5-HT in the raphe and the consequent activation of somatodendritic autoreceptors (Adell and Artigas, 1991; Invernizzi et al., 1992). Nerve terminal autoreceptors also contribute to inhibition of 5-HT release and restrain the increase in extracellular levels after SSRI administration (Hjorth, 1993). The clinical efficacy of SSRIs may depend in part on autoreceptor desensitization during prolonged inhibition of reuptake (Blier and de Montigny, 1994). Thus, the interaction between reuptake and autoreceptors in regulation of serotonergic neurotransmission has been studied intensively.

Reuptake inhibitors may have a differential effect on extracellular 5-HT in the raphe and forebrain (for review, see Gardier et al., 1996). For example, at a dose just sufficient for suppressing serotonergic neuronal activity, the SSRI paroxetine elicited about a 2-fold increase in extracellular 5-HT in the raphe, with no change in extracellular levels in the frontal cortex (FCx) of rats (Gartside et al., 1995). Paradoxically, at a dose supramaximal for suppressing neuronal discharge, paroxetine produced about a 4-fold increase in extracellular 5-HT in the raphe and 2-fold increase in the FCx. Thus, some of these results were presented in preliminary form to the Society for Neuroscience (Tao et al., 1997).

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine, serotonin; SSRI, selective serotonin reuptake inhibitor; SERT, 5-HT reuptake transporter; TTX, tetrodotoxin; 8-OH-DPAT, 8-hydroxydipropylaminotetralin; DRN, dorsal raphe nucleus; MRN, median raphe nucleus; NAcc, nucleus accumbens; FCx, frontal cortex; aCSF, artificial cerebrospinal fluid; HPLC-EC, HPLC with electrochemical detection.
despite sustained and apparently complete inhibition of serotonergic neuronal discharge, synaptic 5-HT in the forebrain may be enhanced in response to acute administration of an SSRI (Gartside et al., 1995). This might indicate that increases in extracellular 5-HT produced by SSRIs do not depend on depolarization-induced release. However, tetrodotoxin (TTX) and autoreceptor agonists can attenuate increases in forebrain 5-HT elicited by systemic administration of reuptake inhibitors (Carboni and DiChiari, 1989; Perry and Fuller, 1992; Rutter and Auerbach, 1993). Furthermore, the possibility that SSRI-elicted increases in 5-HT in the raphe may be sustained by depolarization-independent release has not been directly tested. Thus, the mechanism underlying the ability of SSRIs to preferentially increase 5-HT in the raphe is unclear.

The major aims of this study were to compare the effect of local SSRI infusion into the raphe and forebrain and to directly test the hypothesis that SSRI-induced increases in extracellular 5-HT do not depend on depolarization-induced release. For this purpose, we used both conventional in vivo microdialysis to measure the percentage of change in extracellular 5-HT elicited by SSRIs and zero-net-flux microdialysis for determining actual concentrations of 5-HT. Changes in 5-HT were measured in response to reverse dialysis infusion of SSRIs into the dorsal raphe nucleus (DRN), median raphe nucleus (MRN), nucleus accumbens (NAcc), and FCx of unanesthetized rats. The DRN and MRN contain almost all of the serotonergic cell bodies with projections to the forebrain. The FCx is of particular interest because 5-HT release here is more tightly regulated than in other forebrain sites (for review, see Gardier et al., 1996). The NAcc is preferentially innervated by DRN serotonergic neurons (Azmitia and Segal, 1978). Thus, the role of somatodendritic autoreceptors in regulation of forebrain release could be characterized by infusion of citalopram into DRN while measuring 5-HT in the NAcc. Dependence of 5-HT release on depolarization was further tested by administration of the 5-HT1A receptor agonist 8-hydroxydipropylaminotetralin (8-OH-DPAT) to stimulate somatodendritic autoreceptors and TTX to block action potential conduction. The results provide evidence of differential regulation of extracellular 5-HT in the raphe compared with forebrain sites. However, even when reuptake was maximally inhibited, increases in extracellular 5-HT remained partly dependent on depolarization-induced release.

**Experimental Procedures**

**Animal Preparation.** Male Sprague-Dawley rats purchased from Harlan Sprague-Dawley Inc. (Indianapolis, IN) were individually housed with food and water available ad libitum. The animals were kept at least 2 weeks on a reversed light/dark cycle (lights off from 9:30 AM to 9:30 PM) and were briefly handled three to four times a week.

All animal-use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Rutgers University Institutional Review Board. Rats weighing 300 to 350 g were anesthetized with a combination of xylazine (4 mg/kg, i.p.) and ketamine (80 mg/kg, i.p.) and then mounted in a Kopf stereotaxic frame in the flat skull position. Guide cannulas (22-gauge stainless steel tubing) were implanted above the dura (0.9 mm ventral to the skull surface). According to a rat brain atlas (Paxinos and Watson, 1986), the coordinates for the guide cannulas relative to interaural zero were DRN, AP 1.2, ML 4.0, at a 32° angle lateral to midline; MRN, AP 1.2, ML 4.0, at an angle of 26° lateral to midline; NAcc, AP 10.7, ML 1.4; and FCx, AP 12.2, ML 3.2. Rats were allowed at least 1 week for recovery from surgery.

**Microdialysis Procedures.** Microdialysis was performed with an I-shaped probe constructed from 26-gauge stainless steel tubing and glass silica as previously described in detail (Auerbach et al., 1989). The dialysis tubing was hollow nitrocellulose fiber (0.2 mm o.d., 6000 mol. wt. cut-off, Spectrum Medical Industries, Los Angeles, CA). The length of the steel shaft was adjusted to place a 1.0-mm-long segment of dialysis tubing in the DRN (DV 5.5–6.4, 32° angle) or MRN (DV 7.7–8.6, 26° angle). Similarly, the length of the probe was adjusted to place 2.5-mm-long segments of dialysis tubing in the NAcc (DV 6.0–8.5) or FCx (DV 2.0–4.5).

The night before an experiment, rats were briefly anesthetized with methoxyflurane, and dialysis probes were inserted and secured with dental cement. Animals were attached to a fluid swivel, allowing unrestricted behavior within the testing chamber. Before collecting samples, dialysis probes were perfused overnight with a modified buffered Ringer’s solution containing 140 mM NaCl, 3.0 mM KCl, 1.5 mM CaCl2, 1.0 mM MgCl2, 0.27 mM NaH2PO4, and 1.2 mM Na2HPO4, pH 7.4. This Ringer’s solution [artificial cerebrospinal fluid (aCSF)] was pumped at a rate of 1.0 μl/min. Sample collection began at the beginning of the lights-off period under dim red light conditions. Samples were collected every 30 min and analyzed within 30 min of collection by HPLC with electrochemical detection (HPLC-EC) as previously described in detail (Auerbach et al., 1989). The HPLC-EC mobile phase composition was 0.12 M NaOH, 0.18 mM EDTA, 0.15 M monochloroacetic acid, 1.0 mM sodium octane sulfonic acid, and 96 μM acetonitrile, pH 3.4, and was pumped at a rate of 0.90 ml/min. Detection limit of the assay was 0.3 pg per sample.

**Experimental Protocol.** Drugs were administered to rats after 5-HT levels in three or four successive samples were stable (less than ±10% fluctuation of baseline). In some experiments, to study the effect of somatodendritic autoreceptor activation on 5-HT release in the forebrain, 8-OH-DPAT or citalopram was administered by reverse dialysis in the DRN while 5-HT was measured by a second dialysis probe in the NAcc. Other experiments involved drug administration by reverse dialysis and measurement of 5-HT in the same site. These conventional microdialysis experiments provided data concerning changes relative to baseline 5-HT during local infusion of an SSRI. In addition, we used the zero-net-flux method to estimate actual concentrations of 5-HT in extracellular space. Details of this method have been described previously by others (Lonnroth et al., 1989; Justice, 1993). In brief, 5-HT was added to the aCSF at concentrations above and below the expected concentration in extracellular space (0–30 nM), and 5-HT was measured in the aCSF effluent from the brain. To minimize loss of 5-HT due to decomposition across time at neutral pH, paired samples were collected simultaneously from the inlet and outlet lines and analyzed within 30 min.

After experiments, rats were deeply anesthetized with chloral hydrate (400 mg/kg, i.p.), and a 2% fast green solution was perfused through the dialysis probes to stain the surrounding tissue. The brain was removed, frozen, and sliced free-hand using a razor blade. Data were excluded if the probe track was not in the targeted site.

**Data Analysis.** Data from the zero-net-flux experiments were plotted as gain or loss of 5-HT in the dialysis probe effluent against the concentration of exogenous 5-HT infused through the probe inlet. Linear regression was used to interpolate the point of no net flux. This provides an estimate of the concentration of 5-HT in extracellular space (Lonnroth et al., 1987). For all other experiments, the mean of three or four successive samples before drug administration was taken as the baseline level and reported in the figure legends as picograms per sample, uncorrected for probe recovery. Also, the data were normalized and presented in figures as mean ± S.E. percentage of change from the averaged baseline measurements. Significance (P < .05) was determined using repeated-measures ANOVA followed by Scheffé’s test.

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Materials. All chemicals were reagent grade or better. Citalopram hydrobromide was provided courtesy of Lundbeck A/S (Copenhagen-Valby). Fluoxetine was a gift of Lilly Research Laboratories (Indianapolis, IN) and (-)-penbutolol was generously provided by Hoechst-Roussel (Somerville, NJ). (+)-8-OH-DPAT hydrobromide was purchased from Research Biochemicals International (Natick, MA), and TTX was obtained from Calbiochem-Novabiochem Corp. (La Jolla, CA). Drugs were dissolved in aCSF to produce specific concentrations for local infusion by reverse microdialysis. For systemic administration, 8-OH-DPAT was dissolved in water and injected in a volume of 1 ml/kg. The systemic dose of 8-OH-DPAT refers to the salt form.

Results

Microdialysis probes with a 1-mm-long exchange surface were implanted in the DRN and MRN. Probes with a 2.5-mm-long exchange surface were implanted in the NAcc and FCx. After overnight perfusion with aCSF containing no SSRI, baseline 5-HT levels in these sites were low but usually above the detection limit of our HPLC-EC assay. Averaged across experiments, basal 5-HT (pg/sample; uncorrected for probe recovery) in the absence of reuptake inhibitor was 0.7 ± 0.1 in the DRN (n = 61), 0.8 ± 0.1 in the MRN (n = 34), 0.9 ± 0.1 in the NAcc (n = 45), and 0.6 ± 0.1 in the FCx (n = 14).

Effect of Local SSRI Infusion on 5-HT in the Raphe and Forebrain Sites. SSRIs were infused locally by reverse dialysis into the area of serotonergic cell bodies in the DRN and MRN and into two forebrain sites, NAcc or FCx. As shown in Fig. 1, SSRI infusion produced larger increases in the raphe than in forebrain sites. Reverse dialysis infusion of citalopram (0.3–1000 μM in aCSF) or fluoxetine (10–1000 μM in aCSF) into the DRN produced similar dose-dependent increases in 5-HT in the DRN (Fig. 1, a and b). The maximum effect was an ~20-fold increase. The effect of infusing citalo-

![Fig. 1. The effect of infusing SSRIs into the raphe and forebrain sites on extracellular 5-HT. Results are expressed as mean (±S.E.) percentage of change from the average of three consecutive baseline 5-HT samples. Horizontal bars indicate the period of SSRI infusion. a, infusion of citalopram into DRN produced a dose-dependent increase in DRN 5-HT [F(6,39) = 26.069, P < .0001]. b, infusion of fluoxetine into the DRN produced a dose-dependent increase in DRN 5-HT [F(4,21) = 10.196, P < .0001]. c, infusion of citalopram into the MRN produced a dose-dependent increase in MRN 5-HT [F(6,33) = 10.184, P < .0001]. d, citalopram infusion into NAcc produced a dose-dependent increase in NAcc 5-HT [F(6,29) = 4.281, P < .0033]. e, citalopram infusion into FCx produced a dose-dependent increase in FCx 5-HT [F(2,12) = 13.181, P < .0009].]
pram into the MRN is shown in Fig. 1c. Similar to the DRN, 5-HT was dose dependently elevated with a maximal increase of nearly 20-fold.

The effect of infusing citalopram (0.3–1000 μM in aCSF) into forebrain sites was smaller than in the raphe. The maximal response to citalopram infusion into the NAcc was an ~7-fold increase in 5-HT (Fig. 1d). As shown in Fig. 1e, citalopram infusion had a similar effect on 5-HT in the FCx. Thus, 1 and 1000 μM citalopram produced ~4- and ~5-fold increases, respectively, in 5-HT in the FCx.

Figure 2 compares dose-response curves for the effect of local SSRI infusion into 5-HT in the DRN, MRN, and NAcc. It is apparent that the maximum increases in 5-HT in the raphe were produced at SSRI concentrations between ~300 and 1000 μM in the perfusion medium. In contrast, maximum increases in 5-HT in the forebrain were produced at SSRI concentrations between ~1 and 10 μM in the perfusion medium. The EC50 value for the effect of citalopram on 5-HT in NAcc was ~0.5 μM. The EC50 values for the effect of citalopram and fluoxetine on 5-HT in the raphe were ~35 and 70 μM, respectively. Furthermore, there appeared to be a biphasic response to SSRIs in the raphe compared with the NAcc.

The inhibitory influence of nerve terminal autoreceptors in forebrain sites might be a factor in the smaller response to infusion of SSRIs into the NAcc and FCx. To test this possibility, beginning 2 h before infusion of citalopram, we infused (−)-penbutololo into the NAcc to block terminal 5-HT autoreceptors (Hjorth and Sharp, 1993). As shown in Fig. 3, (−)-penbutololo (300 μM in aCSF) alone did not produce a significant increase in 5-HT in the NAcc. However, (−)-penbutololo significantly enhanced the effect of infusing citalopram (1000 μM) into the NAcc on 5-HT in this site. Thus, 5-HT in the NAcc was increased ~6-fold with citalopram alone, significantly less (P < .05) than the ~12-fold increase with (−)-penbutololo pretreatment. Presumably, (−)-penbutololo infusion at a concentration of 300 μM produced a maximal blockade of terminal autoreceptors. This is based on evidence that the effect of SSRI infusion on extracellular 5-HT was similarly enhanced using a 30-fold lower concentration of (−)-penbutololo (Rutter et al., 1995). Nevertheless, despite terminal autoreceptor blockade, infusing citalopram into the forebrain had a significantly smaller effect than in the raphe (F(1,12) = 7.764, P = .0165).

**Zero-Net-Flux Method for Estimating the Concentration of 5-HT in Extracellular Fluid.** Conventional microdialysis provided evidence of differences in the effect of infusing uptake blockers into the raphe compared with forebrain. However, differences in dialysis probe recovery of neurotransmitter in these sites may have been a factor in the apparently larger effect of blocking 5-HT reuptake in the raphe. To examine this possibility, we used zero-net-flux microdialysis to estimate the concentration of 5-HT in extracellular space. Varying concentrations of 5-HT were added to the aCSF, and the amount of 5-HT gained or lost during dialysis in the DRN or NAcc was measured. Net gain or loss of 5-HT in the aCSF effluent is plotted against the concentration of exogenous 5-HT added to the aCSF (Fig. 4). The interpolated point of no net flux is an estimate of the actual concentration of 5-HT in extracellular fluid that does not depend on probe length or other factors that can affect in vivo recovery of neurotransmitters (Justice, 1993). We estimated basal 5-HT and the increase in response to 1 and 300 μM citalopram. These concentrations were chosen because they produced, respectively, submaximal and maximal increases in extracellular 5-HT as determined by conventional microdialysis (see Fig. 1).

As shown in Fig. 4, the basal concentration of 5-HT was 1.3 nM in the DRN and 0.7 nM in the NAcc. During infusion of 1 and 300 μM citalopram into the NAcc, 5-HT was increased to 2.9 and 3.1 nM, respectively. During infusion of 1 μM citalopram into the DRN, 5-HT was increased to 3.4 nM. In contrast to the NAcc, there was an additional significant increase in 5-HT concentration to 12.4 nM during infusion of 300 μM citalopram into the DRN. Thus, the maximum concentration of 5-HT in the DRN was ~4-fold greater than in the NAcc.

**Influence of Direct and Indirect Stimulation of Somatodendritic Autoreceptors in the DRN on 5-HT Release in the NAcc.** By eliciting increased extracellular 5-HT, reuptake inhibitors indirectly activate somatodendritic autoreceptors and thus inhibit serotonergic neuronal discharge (Sheard et al., 1972). Hence, we did not anticipate the very large increases in extracellular 5-HT during infusion of citalopram and fluoxetine into the raphe. To evaluate the influence of somatodendritic autoreceptors on 5-HT release, we infused 8-OH-DPAT, a 5-HT1A receptor agonist (Sharp et al., 1989), or citalopram into the DRN while measuring extracellular 5-HT with a second dialysis probe in the NAcc. As shown in Fig. 5a, infusion of 8-OH-DPAT (100 μM in aCSF) produced a significant, ~40%, decrease in 5-HT in the NAcc. Similarly, infusion of citalopram (1–1000 μM in aCSF) into the DRN caused a dose-dependent decrease in extracellular 5-HT in the NAcc (Fig. 5b). Figure 5b (inset) shows the dose-response curve for the inhibitory effect of citalopram infusion. There was no significant effect at the two lowest doses, 1 and 10 μM citalopram. However, 100, 300, and 1000 μM citalopram produced significant reductions of ~15, ~20, and ~30%, respectively. As calculated from these data, the EC50 value for the inhibitory effect of citalopram in the DRN on 5-HT in the NAcc was ~100 μM in the

![Fig. 2. Dose-response curves for the effect of local SSRI infusion into DRN, MRN, and NAcc. Mean (±S.E.) percentages of elevation in 5-HT (four successive samples collected from 1.5 to 3 h after the start of SSRI infusion; values from Fig. 1) are plotted against the log of citalopram concentration in aCSF. © DRN citalopram; □, DRN fluoxetine; △, MRN citalopram; ∨, NAcc citalopram.](image-url)
perfusion medium. Presumably, citalopram infusion into the DRN indirectly activates somatodendritic autoreceptors, but decreases in extracellular 5-HT in the NAcc were significant only at relatively high perfusate concentrations.

Interaction between Citalopram and 8-OH-DPAT in the DRN: To What Extent Are Somatodendritic Autoreceptors Activated by Local Infusion of an SSRI into the Raphe? Citalopram infusion into the DRN produced relatively small decreases in 5-HT in the NAcc. Thus, it is possible that 5-HTT receptors in the DRN were not maximally activated by endogenous 5-HT even during local infusion of high concentrations of citalopram. In contrast, the direct acting 5-HT receptor agonist 8-OH-DPAT might produce a more complete suppression of serotonergic neuronal activity. To test these inferences, we measured the effect of citalopram followed by 8-OH-DPAT infusion on 5-HT in the DRN. Figure 6 shows that, in the presence of 1 μM citalopram, 8-OH-DPAT decreased extracellular 5-HT. At concentrations of 10 and 100 μM in the aCSF, 8-OH-DPAT infusion into the DRN produced similar, ~40%, reductions in 5-HT in DRN (Fig. 6a). As shown in Fig. 6b, during infusion of 1000 μM citalopram into the DRN, higher doses of 8-OH-DPAT were necessary for reducing 5-HT in the DRN. With the SSRI present at a high concentration in the DRN, the maximal reduction induced by 100 and 1000 μM 8-OH-DPAT was ~25 and ~40%, respectively. Thus, the potency but not the efficiency of 8-OH-DPAT infused into the DRN was attenuated during infusion of citalopram at a high concentration.

Interaction between Citalopram and TTX in DRN and NAcc: Is Depolarization-Dependent Release Necessary for SSRI-Elicited Increases in Extracellular 5-HT? Even during citalopram infusion at a high concentration, 8-OH-DPAT produced a decrease in extracellular 5-HT. Presumably, 8-OH-DPAT, via direct stimulation of somatodendritic autoreceptors, inhibited serotonergic neuronal activity. This result provides evidence that citalopram alone did not completely inhibit serotonergic neuronal activity and that the SSRI-induced increases in 5-HT were dependent at least in part on depolarization-induced release. To further test this inference, we infused TTX to block the generation and conduction of action potentials. Two sets of experiments were carried out. In the first, citalopram (1 or 1000 μM in aCSF) was infused into the DRN followed by TTX (1 μM in combination with citalopram). As shown in Fig. 7a, during infusion of 1 μM citalopram, TTX elicited a ~60% reduction in 5-HT in the DRN, and during infusion of 1000 μM citalopram, TTX elicited an ~40% reduction. Although the maximal effect of TTX was apparently smaller during infusion of 1000 than of 1 μM citalopram, this difference was not significant (P > .05). In the second set of experiments, citalopram (1 or 1000 μM) and TTX were infused into the NAcc. As shown in Fig. 7b, TTX infused into the NAcc elicited similar, ~60%, maximal reductions in 5-HT in NAcc during infusion of 1 and 1000 μM citalopram.

Discussion

Consistent with the effect of systemic administration of reuptake inhibitors (Adell and Artigas, 1991; Invernizzi et al., 1992), local SSRI infusion produced greater increases in extracellular 5-HT in the raphe than in forebrain. Thus, citalopram infusion produced a 3-fold greater increase relative to baseline levels in the raphe compared with forebrain (Fig. 1). Also, we determined absolute 5-HT concentrations using zero-net-flux microdialysis, and these results support the conclusion that reuptake inhibition has an ~3-fold greater effect on 5-HT in raphe (Fig. 4). Moreover, the pre-drug concentration of 5-HT in DRN, ~1.3 nM, was about twice the level in NAcc at ~0.7 nM. Our estimate of basal 5-HT in NAcc is consistent with the concentration determined using a similar approach (Smith and Weiss, 1999). However, as far as we are aware, there are no previous studies comparing absolute concentrations of 5-HT in raphe with forebrain.

The EC50 value for SSRI-elicited increases in extracellular 5-HT in raphe was high compared with forebrain (Fig. 2). A semi-independent measure, the concentration of citalopram in the DRN necessary for inhibition of 5-HT release in the
NAcc, provides a similar estimate for SSRI affinity to transporters in the raphe (Fig. 5b). Because the dialysis membrane prevents free diffusion, drug concentrations in extracellular fluid are low compared with amounts added to the dialysis solution (Dykstra et al., 1992). Nevertheless, the very high concentration of citalopram necessary for producing a maximal increase in 5-HT release in forebrain was unexpected. We obtained similar results with citalopram in MRN and fluoxetine in DRN, suggesting that this is a robust finding. Several factors may be involved in the high EC50 values in the raphe. 1) Compared with forebrain, the higher extracellular concentration of 5-HT in raphe would provide more competition for SSRI binding to the 5-HT reuptake transporter (SERT). Furthermore, the density of SERT in raphe is high compared with forebrain (Hensler et al., 1994). Thus, binding to the transporter may effectively lower the concentration of SSRIs in the raphe. Both of these factors might cause an increase in the apparent affinity of an SSRI. 2) Another possibility is that the SSRIs at high concentrations in the raphe acted as 5-HT releasers. However, increases in extracellular 5-HT produced by releasing drugs are unaffected by inhibitors of serotoninergic neuronal discharge and nerve terminal depolarization (Carboni and DiChiara, 1989; Gundlah et al., 1997). Thus,
the decrease in 5-HT during infusion of 8-OH-DPAT or TTX (Figs. 6 and 7) suggests that the SSRIs were not acting as 5-HT-releasing agents. 3) SSRIs have measurable, albeit remarkably low, affinity for some neurotransmitter receptors (for review, see Sanchez and Hyttel, 1999). Thus, it is conceivable that citalopram and fluoxetine at high enough doses bind to, for example, α1-adrenergic receptors in the raphe and thus might stimulate 5-HT release. Finally, the high EC_{50} value and biphasic response to SSRIs infused into the DRN and MRN may indicate that there is a low-affinity, high-capacity mechanism for 5-HT uptake in the raphe. For example, multiple mRNA species resulting from alternative splicing of the SERT gene have been detected (Bengel et al., 1997). Thus, there could be heterogeneous SERT proteins with different affinities for 5-HT. Indeed, some evidence from in vitro binding studies supports the presence of low- and high-affinity binding sites on 5-HT transporter proteins (Sur et al., 1998), and there may be regional differences in the distribution of the two sites (Rothman et al., 1994). However, in vitro experiments to directly test for low-affinity uptake of 5-HT would be difficult because of the small amount of tissue obtainable from rat raphe.

The balance between the rates of release and clearance determines steady-state extracellular neurotransmitter levels. If a low-affinity form of SERT regulated 5-HT clearance, higher release may be the major factor in higher 5-HT in raphe compared with forebrain when reuptake was fully blocked by SSRIs. Nerve terminal autoreceptors might inhibit forebrain 5-HT release during local SSRI infusion. However, even during infusion of (-)-penbutolol to block terminal autoreceptors, the increase in 5-HT in forebrain during citalopram...
infusion was still less than in raphe (Fig. 3). These observations are consistent with other evidence (Héry and Ternaux, 1981) that 5-HT release is greater in raphe than in forebrain.

**Increased Extracellular 5-HT Elicited by SSRIs Is Dependent in Part on Depolarization.** SSRIs produce sustained increases in extracellular 5-HT at doses that suppress serotonergic neuronal discharge (Gartside et al., 1995). This suggests that SSRI-induced increases in extracellular 5-HT are mainly independent of depolarization-induced release. However, the direct somatodendritic autoreceptor agonist 8-OH-DPAT produced decreases in 5-HT during citalopram infusion into the DRN (Figs. 5 and 6). This agrees with reports that 8-OH-DPAT attenuated the increase in 5-HT evoked by systemic SSRI administration (Rutter and Auerbach, 1993). TTX infusion also attenuated the increases in extracellular 5-HT produced by citalopram infusion into the raphe or NAcc (Fig. 7). This is consistent with reports that TTX reversed the effect of systemic administration of reuptake inhibitors (Kalén et al., 1988; Carboni and DiChiara, 1989; Perry and Fuller, 1992). Thus, microdialysis results indicate that neuronal impulse activity is partly responsible for increased extracellular 5-HT elicited by reuptake inhibitors. With respect to this hypothesis, it is important to note that in contrast to the inhibitory effects of direct autoreceptor agonists such as 8-OH-DPAT, the decrease in serotonergic neuronal activity in response to SSRIs depends on the evoked increase in extracellular 5-HT. Moreover, because the elevation in extracellular 5-HT remains dependent on depolarization, our results suggest that the inhibitory effect of SSRI administration is self-limiting and in principle should not produce a total suppression of serotonergic discharge.

The apparent conflict between the electrophysiological and neurochemical results may be attributable to technical differences. Microdialysis samples extracellular neurotransmitter released from a large population of cells over a long time period. This contrasts with second-to-second electrophysiological recordings of single unit activity. Intermittent discharge of a few serotonergic neurons might be missed when recording one cell, but residual activity could sustain elevations in extracellular 5-HT when clearance is blocked. Thus, SSRI administration may elicit a new steady state with a very low rate of serotonergic neuronal discharge and depolarization-induced 5-HT release capable of maintaining elevated extracellular levels.

Although efficacy was not significantly decreased, higher concentrations of 8-OH-DPAT were necessary for attenuation of the increase in 5-HT elicited by infusion of 1000 compared with 1 μM citalopram into the DRN (Fig. 6). With reuptake maximally inhibited, the reduced potency of 8-OH-DPAT was presumably attributable to nearly complete occupancy of 5-HT₁A receptors by 5-HT. Thus, according to the law of mass action, binding to residual unoccupied receptors would require very high concentrations of 8-OH-DPAT. However, it is important to note that the maximum decreases in 5-HT produced by 8-OH-DPAT and TTX were not greater than 60% (Figs. 6 and 7). Together with other reports (Carboni and DiChiara, 1989; Perry and Fuller 1992), these results suggest that SSRI-induced increases in 5-HT are partly independent of impulse-induced release. A possible source of the depolarization-independent fraction of 5-HT in extracellular fluid is spontaneous vesicular release of neurotransmitter, a physiological process that has been demonstrated, for example, in recordings of miniature end-plate potentials at the neuromuscular junction. When depolarization is inhibited, the releasable pool of 5-HT is increased (Auerbach and Lipton, 1985). Thus, during SSRI infusion into the raphe and, consequently, autoreceptor-mediated attenuation of depolarization-induced exocytosis, there could be an increase in the amount of spontaneously released 5-HT.

**Other Methodological Considerations and Significance.** Maximal increases in 5-HT in raphe and forebrain were greater during SSRI infusion than those observed after systemic administration. Systemic SSRIs produce 2- to 4-fold increases in 5-HT (for review, see Fuller, 1994). In contrast, local infusion induced an ~6-fold increase in forebrain and an ~20-fold increase in midbrain levels (Fig. 1). Presumably, limited autoreceptor activation during local infusion is the explanation for this difference. Thus, during local SSRI infusion, increased 5-HT is confined to a small area around the microdialysis probe. In contrast, cell bodies that release 5-HT in the area of the probe may be located far away. Furthermore, some evidence suggests that part of the inhibitory effect of systemic SSRI administration is mediated by long-loop feedback (Ceci et al., 1994; Bosker et al., 1997; Hajós et al., 1998). Consistent with these inferences, local infusion of 8-OH-DPAT or citalopram into the DRN produced relatively small decreases in NAcc 5-HT (Fig. 5). In summary, the inhibitory effect of local SSRI or 8-OH-DPAT infusion may be limited because this route of administration would not cause widespread activation of somatodendritic autoreceptors or activate long-loop feedback inhibition of serotonergic neuronal discharge.

Reuptake is efficient in limiting the amount of neurotransmitter that spills over into extracellular space (Yang et al., 1998). Furthermore, because the dialysis probe membrane hinders diffusion of substances into the dialysate, 5-HT recovery is only a fraction of the amount in extracellular space. Thus, baseline levels may be near the sensitivity limit of detection methods. To increase recovery, a reuptake inhibitor is often added to the dialysis perfusion medium. For example, SSRI infusion was necessary for detecting changes in forebrain 5-HT during electrical stimulation in raphe (Brodin et al., 1990; Sharp et al., 1990). Our results suggest that at low concentrations of SSRI in the dialysate, 5-HT release remains predominantly dependent on depolarization. However, it is important to carry out appropriate control experiments and to be cautious in interpretation of microdialysis data when a reuptake inhibitor is used to enhance neurotransmitter recovery (for further discussion, see DeBoer and Abercrombie, 1996).

In summary, our results provide evidence that 5-HT release capacity is greater and suggest that SERT affinity is lower in the raphe compared with forebrain projection sites. Thus, physiological activation of serotonergic neuronal discharge would result in relatively large increases in extracellular 5-HT in the raphe, stimulation of somatodendritic autoreceptors, and consequently, restrained increases in extracellular 5-HT in the forebrain. These inferences are in line with evidence that serotonergic neuronal discharge and 5-HT release in forebrain are confined to a remarkably narrow range even during periods of strong behavioral activation in response to physiological challenges (for review, see Rueter et al., 1997). However, our results do not support the hypothesis that reuptake blocker-induced increases in fore-
brain extracellular 5-HT are completely independent of neuronal activity. Increases in 5-HT in the forebrain were smaller than in the raphe, but they remained largely dependent on depolarization during SSRI treatment.

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References


Ceci A, Baschirotto A and Borsini F (1994) The inhibitory effect of 8-OH-DPAT on the serotoninergic projection to the brain extracellular 5-HT are completely independent of neuronal activity. Increases in 5-HT in the forebrain were smaller than in the raphe, but they remained largely dependent on depolarization during SSRI treatment. Brain Res 566:201–208.


