In Vivo Criteria To Differentiate Monoamine Reuptake Inhibitors from Releasing Agents: Sibutramine Is a Reuptake Inhibitor

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ABSTRACT

Because monoamine reuptake inhibitors and releasing agents both increase extracellular neurotransmitter levels, establishing in vivo experimental criteria for their classification has been difficult. Using microdialysis in the hypothalamus of unanesthetized rats, we provide evidence that serotonin- (5-HT) selective and nonselective reuptake inhibitors can be distinguished from the 5-HT-releasing agent fenfluramine by four criteria: 1) Systemic fenfluramine produces a much greater increase in 5-HT than the reuptake inhibitors. 2) The 5-HT somatodendritic autoreceptor agonist, (-)-8-hydroxy-(dipropylamino)tetralin (8-OH-DPAT), attenuates the increase in 5-HT produced by reuptake inhibitors, but not by fenfluramine. 3) The large increase in 5-HT produced by infusion of reuptake inhibitors into the hypothalamus is attenuated by their systemic administration.

Many compounds have high affinity, without being substrates, for the 5-HT reuptake carrier and thereby increase extracellular levels of 5-HT in the CNS by blocking reuptake. The increase in extracellular 5-HT after inhibition of reuptake is dependent on impulse-mediated release (Fuller and Wong, 1990; Fuller, 1993). In contrast, other compounds can evoke 5-HT release by a mechanism that is mainly independent of neuronal activity (Kuhn et al., 1985; Sharp et al., 1986; Carboni and DiChiara, 1989; Raiteri et al., 1995). These are termed “releasing agents.” Some releasing agents exert their effects by entering the nerve terminal via the reuptake carrier where they displace 5-HT from its storage pool (Mennini et al., 1981; Rudnick and Wall, 1992; Berger et al., 1992). Because releasing agents such as fenfluramine and PCA act as substrates for the 5-HT transporter, they also appear to block reuptake at low concentrations (Garattini et al., 1986; Berger et al., 1992).

However, systemic injection of fenfluramine during its local infusion does not attenuate this increase. 4) Reuptake inhibitor pretreatment attenuates fenfluramine-induced increases in 5-HT. According to these criteria, the in vivo effects of the novel antiobesity drug sibutramine are consistent with its characterization as a 5-HT reuptake inhibitor and not a 5-HT releaser. Thus, sibutramine produced increases in hypothalamic 5-HT similar in magnitude to the effects of the known reuptake inhibitors, and the increase was attenuated by 8-OH-DPAT. Also, sibutramine attenuated fenfluramine-induced 5-HT release. Systemic administration of sibutramine failed to attenuate the increase in 5-HT produced by its local infusion, suggesting that this criterion is not applicable to compounds with low affinity for the 5-HT transporter.

Monoamine reuptake inhibitors and releasing agents both produce increases in extracellular neurotransmitter levels. Thus, it is difficult to establish experimental criteria for classification of these compounds in vivo (Fuller et al., 1988). However, previous observations suggest four distinguishing criteria: 1) The increase in extracellular 5-HT in response to systemic administration of reuptake inhibitors (Perry and Fuller, 1992; Rutter and Auerbach, 1993) is relatively small compared with the effect of releasing agents (Kalén et al., 1988; Sabol et al., 1992b). This is because the discharge of 5-HT neurons is largely inhibited by acute systemic administration of a reuptake inhibitor and the increase in extracellular 5-HT is dependent on depolarization-induced release (Blier et al., 1987; Adell and Artigas, 1991; Rutter and Auerbach, 1993). These autoinhibitory mechanisms should not modify the actions of 5-HT-releasing agents. 2) The effect of reuptake inhibitors (Carboni and DiChiara, 1989; Perry and Fuller, 1992; Rutter and Auerbach, 1993), but not releasing agents (Carboni and DiChiara, 1989; Gobbi et al., 1993), is attenuated by agents that inhibit 5-HT neuronal discharge, e.g., 8-OH-DPAT or TTX. 3) The large increase in extracellular 5-HT occurring during local infusion of these drugs into a terminal field is attenuated by their acute peripheral administration (Rutter and Auerbach, 1993; Hjorth and Auerbach, 1992b). This is because the discharge of 5-HT neurons is largely inhibited by acute systemic administration of a reuptake inhibitor and the increase in extracellular 5-HT is dependent on depolarization-induced release (Blier et al., 1987; Adell and Artigas, 1991; Rutter and Auerbach, 1993). These autoinhibitory mechanisms should not modify the actions of 5-HT-releasing agents. 2) The effect of reuptake inhibitors (Carboni and DiChiara, 1989; Perry and Fuller, 1992; Rutter and Auerbach, 1993), but not releasing agents (Carboni and DiChiara, 1989; Gobbi et al., 1993), is...
bath, 1994). This attenuation is due to the indirect activation of somatodendritic autoreceptors after systemic administration of a reuptake inhibitor (Auerbach et al., 1995; Rutter et al., 1995). It is unlikely that this would be a property of releasing agents, assuming that they produce depolarization-independent release of 5-HT. 4) Because both reuptake inhibitors and releasing agents such as fenfluramine bind to the 5-HT carrier, pretreatment with reuptake inhibitors can attenuate the increase in extracellular 5-HT produced by fenfluramine (Sabol et al., 1992a; Gobbi et al., 1992).

The primary aim of our research was to determine the validity of these four criteria for in vivo differentiation of monoamine reuptake inhibitors from releasing drugs. Although there are in vitro tests available, because the pharmacological profile of compounds can be altered by metabolism, it is important to perform in vivo tests. In order to achieve this aim, microdialysis in the hypothalamus of unanesthetized rats was used to compare fenfluramine, which is a 5-HT releasing agent (Berger et al., 1992), with the reuptake inhibitors fluoxetine, paroxetine and imipramine.

The second aim of this study was to then use these criteria to determine whether sibutramine (BTS 54 524; N-1-(1-[4-chlorophenyl)cyclobutyl]-3-methyl-butyl-3-N, N-dimethylaniline hydrochloride monohydrate) and its primary amine metabolite, BTS 54 505, act as reuptake inhibitors or releasing agents in vivo. Previous studies have established that sibutramine weakly inhibits NE and 5-HT reuptake in vitro (Buckett et al., 1988). However, the primary and secondary amine metabolites of sibutramine are potent NE and 5-HT reuptake inhibitors in vitro (Luscombe et al., 1989). Thus, the in vivo effects of sibutramine and BTS 54 505 were compared with those of fenfluramine and known reuptake inhibitors. Some of these results were previously presented in abstract form (Gundlah et al., 1996).

Methods

Animals and guide cannula implantation. Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) (250-400 g) were housed singly on a reversed 12:12 hr light-dark cycle (lights off at 9:30 a.m.) from the day of arrival, at least 10 days before experimentation, with free access to food and water. All experiments were performed during the dark cycle. All animal use procedures were in strict accordance with National Institutes of Health guidelines for the care and use of animals and were approved by the Rutgers University Institutional Review Board. Rats were anesthetized with xylazine (Rompun; 4 mg/kg i.p., Mobay Corp., Shawnee, KS) and ketamine (Ketaset; 100 mg/kg i.p., Fort Dodge Laboratories, Fort Dodge, IA) and guide cannulae were implanted as described previously (Auerbach et al., 1989). After surgery, the guide cannulae were plugged with styrels and the rats were allowed a minimum recovery period of 5 days.

Dialysis probe implantation and perfusion. The dialysis probe was an I-shaped concentric design. The working surface was a 3.5 mm length of permeable nitrocellulose dialysis tubing of 0.25 mm outer diameter and 6000 MW cutoff (Spectrum Medical Industries, Los Angeles, CA). Fluid entered through an outer tube of 26-gauge stainless-steel and exited through an inner tube of hollow glass silica fiber (Polymicro Technologies, Phoenix, AZ). The probe length was adjusted to place the tip 0.2 mm above the base of the hypothalamus [flat skull position: 6.2 mm anterior and 0.9 mm lateral relative to intra-aural 0; and 9.2 mm below dura (Paxinos and Watson, 1982)]. The evening before an experiment, rats were briefly immobilized with a volatile anesthetic, methoxyflurane, and a dialysis probe was lowered slowly through the guide cannula and cemented in place. Rats were then placed in a cylindrical enclosure 12 inches in diameter, and attached to a counterweighted cable and fluid swivel that allowed the animals to move freely and have free access to food and water.

The dialysis solution (in millimolar: NaCl, 147; KCl, 4; and CaCl2, 1.8, unadjusted pH 6.3) was perfused through the probe at a rate of 1.1 μl/min by a syringe pump. The perfusate was collected at 30-min intervals and analyzed immediately for 5-HT content, as previously described in detail (Auerbach et al., 1989). Extracellular 5-HT was very high immediately after probe implantation, but fell to steady levels within 8 h (Auerbach et al., 1989). These initially high levels were most probably derived from plasma (Kalén et al., 1988). Therefore, all experiments were started no sooner than 8 hr after probe implantation.

Analysis of 5-HT. High-performance liquid chromatography with electrochemical detection was used for analysis of dialysis samples. Separation of 5-HT from other electroactive compounds was achieved on a 10 cm × 3.2 mm column with ODS 3 μm packing (BAS Inc., West Lafayette, IN) and a mobile phase of 0.15 M chloroacetic acid, 0.12 M sodium hydroxide, 0.18 mM EDTA, 60 μl/liter of acetonirole and 1.0 mM sodium octane sulfonic acid. Monoamines were measured by using a dual potentialstat electrochemical detector (EG&G PARC, Princeton, NJ) and dual glassy carbon working electrodes in the parallel configuration. Applied potentials, relative to an Ag/AgCl reference electrode, were set at approximately maximal and half-maximal for oxidation of 5-HT. These values were checked frequently and were usually about 590 and 530 mV. The detection limit for 5-HT was approximately 300 fg, based on a signal-to-noise ratio of 3:1.

Procedures and data analysis. The experimental protocol involved either systemic injection or local delivery of drugs by reverse dialysis into the hypothalamus. Mean baseline 5-HT levels were calculated as the average of four consecutive 30-min samples immediately before drug administration. In calculating and presenting the results, levels (predrug baseline and postdrug samples) were expressed as a percentage of the mean baseline value ± S.E. This preserves variability associated with random changes in extracellular levels across time although normalizing between animal differences in absolute baseline levels. Within dose, data were analyzed by repeated measures analysis of variance (general linear model), whereas between dose data were analyzed by repeated measures multivariate analysis of variance (general linear model). Factorial analysis, using Scheffe’s F test was applied for post hoc determination of significant differences (P < .05).

Histology. After probe sites were used, the rats were anaesthetized deeply with pentobarbital and perfused intracardially with physiological saline followed by 10% formalin. The brains were removed and 80-μm sections were mounted on slides and stained with cresyl violet. Probe location was determined with the aid of a microscope.

Materials. All chemicals and solvents were of at least analytical grade. Drugs were obtained from the following sources: d,l-fenfluramine and imipramine (Sigma Chemical Co., St. Louis, MO); fluoxetine and nisoxetine (Lilly Research Laboratories, Indianapolis, IN); sibutramine and BTS 54 505 (Knoll Pharmaceuticals Research and Development, Nottingham UK); paroxetine (SmithKline Beecham Pharmaceuticals, Surrey, UK); 8-OH-DPAT (Research Biochemicals Inc., Natick, MA). All drugs were weighed as the salt and administered in a volume of 2.0 ml/kg. For systemic administration, all drugs were dissolved in water and heated and sonicated until fully dissolved. For systemic administration, fenfluramine, fluoxetine, nisoxetine, imipramine, sibutramine, BTS 54 505 and paroxetine were injected i.p., and 8-OH-DPAT was injected s.c. For local administration into the hypothalamus, the drugs were dissolved in, and diluted with, the dialysis solution.
Results

Effect of reuptake inhibitors, fenfluramine, sibutramine, and BTS 54 505 on extracellular 5-HT. The 5-HT selective reuptake inhibitor paroxetine produced an increase in extracellular 5-HT in the hypothalamus after systemic (i.p.) administration (fig. 1A). This effect was dose dependent, with injections of 0.1, 1, 10 and 30 mg/kg i.p. or vehicle was administered at time 0 (arrow) and extracellular 5-HT was measured for 2 hr. Fenfluramine produced a dose-dependent increase in 5-HT [F(4,25) = 73.65, P < .0001] (n = 5-7 for each dose). *Significantly different from vehicle (Scheffé’s post hoc test, P < .05). Fig. 2 is plotted on a different scale from figures 1A and 1B.

Fig. 1. Effect of paroxetine (A) and imipramine (B) on extracellular 5-HT in the hypothalamus. Values (mean ± S.E.) are expressed as a percent of the average of four baseline samples. The mean baseline levels for the five paroxetine treatment groups were not significantly different and the combined value was 1.1 ± 0.07 pg/30-min sample (n = 40). Similarly, the mean baseline levels for the four imipramine groups were not significantly different and the combined value was 2.0 ± 0.11 pg/30-min sample (n = 23). Drugs or vehicle were administered at time 0 (arrow). Paroxetine (0.1, 1, 10, 30 mg/kg i.p.) produced a significant dose-dependent increase in 5-HT [F(4,35) = 12.9, P < .0001] (n = 4-11 for each dose). *Significantly different from vehicle (Scheffé’s post hoc test, P < .05).

Fig. 2. Effect of fenfluramine on extracellular 5-HT in the hypothalamus. Values (mean ± S.E.) are expressed as a percent of the average of four baseline samples. The mean baseline levels for the three treatment groups were not significantly different and the combined value was 1.12 ± 0.08 pg/30-min sample (n = 30). d,l-Fenfluramine (1, 3, 10, 20 mg/kg i.p.) or vehicle was administered at time 0 (arrow) and extracellular 5-HT was measured for 2 hr. Fenfluramine produced a dose-dependent increase in 5-HT [F(4,25) = 73.65, P < .0001] (n = 5-7 for each dose). *Significantly different from vehicle (Scheffé’s post hoc test, P < .05). N.B. Figure 2 is plotted on a different scale from figures 1A and 1B.
doses increased 5-HT to a maximum of ~180, 580, 1000 and 2600% above baseline, respectively (fig. 2). Peak levels were observed within 60 min after injection.

Sibutramine produced a dose-dependent increase in hypothalamic 5-HT (fig. 3A) similar in magnitude to the effect of the reuptake inhibitors (fig. 1) but not fenfluramine (fig. 2). Systemic injection of 1, 3, 10 and 30 mg/kg i.p. increased 5-HT to a maximum of ~40, 60, 100 and 200% above baseline, within 1 hr after injection. The sibutramine metabolite, BTS 54 505, produced a similar dose-dependent increase in hypothalamic 5-HT (fig. 3B). Systemic administration of 1, 3 and 10 mg/kg i.p. increased extracellular 5-HT to a maximum of ~20, 220 and 280% above baseline, within 2 hr after administration.

Effect of 8-OH-DPAT on the increase in extracellular 5-HT produced by reuptake inhibitors, fenfluramine or sibutramine. The 5-HT1A receptor agonist, 8-OH-DPAT, was used to determine if increases in 5-HT were dependent on 5-HT neuronal discharge. At a low dose, 8-OH-DPAT (0.1 mg/kg s.c.) maximally stimulates somatodendritic autoreceptors (Sharp et al., 1989) without having significant effects on postsynaptic 5-HT1A receptors (Goodwin et al., 1987). In the absence of reuptake inhibitors, this results in decreases in extracellular 5-HT to ~30 to 40% of baseline levels. Because 8-OH-DPAT is rapidly metabolized, its effect on 5-HT is transient, lasting about 1 hr (Sharp et al., 1989; Auerbach et al., 1989).

Administration of 8-OH-DPAT (0.1 mg/kg s.c.) 2.5 hr after injection of the reuptake inhibitors paroxetine (10 mg/kg i.p.) or 2 hr after fluoxetine (10 mg/kg i.p.) produced a significant but transient attenuation of the increase in hypothalamic 5-HT (fig. 4A, 4B). Administration of 8-OH-DPAT (0.1 mg/kg s.c.) 2.5 hr after sibutramine (10 mg/kg i.p.) also produced a significant, transient attenuation (fig. 4C).

The peak increase in extracellular 5-HT after systemic fenfluramine was short-lasting. Therefore, 8-OH-DPAT (0.1 mg/kg s.c.) was administered either simultaneously (fig. 5A), or 30 min after d,l-fenfluramine (10 mg/kg i.p.) (fig. 5B) to determine if the increase in 5-HT was dependent on neuronal discharge. In both cases, 8-OH-DPAT had no significant effect on the magnitude or time course of fenfluramine-induced changes in 5-HT.

Effect of peripheral injection of reuptake inhibitors, fenfluramine, sibutramine and BTS 54 505 on extracellular 5-HT during their local infusion. The increase in extracellular 5-HT during local infusion of a 5-HT reuptake inhibitor into a forebrain site is attenuated by subsequent systemic administration of fluoxetine, citalopram or sertraline (Rutter and Auerbach, 1993; Auerbach et al., 1995). Antagonists of 5-HT1A receptors block the decrease induced by systemic administration of these high affinity, selective 5-HT reuptake inhibitors. This suggests that the decrease is mediated by somatodendritic autoreceptors and consequent inhibition of 5-HT release (Rutter et al., 1995; Auerbach et al., 1995).
However, the nonselective reuptake inhibitors imipramine, clomipramine and amitriptyline had low efficacy in this “local-peripheral” experimental paradigm (Auerbach et al., 1995). The correlation between low selectivity and low efficacy suggested the possibility that an enhancement of extracellular NE uptake inhibition may offset autoreceptor-mediated inhibition of 5-HT release. The following experiments were designed to test this hypothesis using drugs that alone, or in combination block the reuptake of 5-HT and NE to compare the efficacy of known reuptake inhibitors to fenfluramine and sibutramine in the local-peripheral experimental paradigm. Doses of these drugs were chosen according to their ability to produce similar maximal increases in 5-HT.

As shown in figures 6 and 7, reverse dialysis infusion of either paroxetine (10 μM), imipramine (80 μM), fluoxetine plus the NE reuptake inhibitor nisoxetine (each 10 μM) or d,l-fenfluramine (100 μM) into the hypothalamus produced a maximum increase in 5-HT to ~400 to 600% above baseline. During the period of local infusion of paroxetine or nisoxetine plus fluoxetine, systemic injection of the corresponding drug(s) (each 10 mg/kg i.p.) resulted in a significant decrease in 5-HT to ~150% above baseline (fig. 6A, C). Systemic injection of imipramine (10 mg/kg i.p.; fig. 6B) during its local infusion had no effect on extracellular 5-HT. Systemic d,l-fenfluramine (10 mg/kg i.p.; fig. 7) during its local infusion slightly increased 5-HT levels.

Local infusion of sibutramine (2 mM) or its metabolite BTS 54 545 (10 μM) increased extracellular 5-HT to a maximum of ~400% above baseline. Although there was a tendency toward a decrease, systemic sibutramine (10 mg/kg i.p.) had no significant effect on hypothalamic 5-HT concentrations during its local infusion (fig. 8A). In contrast, systemic injection of BTS 54 545 (10 mg/kg i.p.) during its local infusion significantly decreased extracellular 5-HT in the hypothalamus to ~120% above baseline (fig. 8B).

**Effect of paroxetine, fluoxetine or sibutramine on fenfluramine-induced 5-HT release.** Animals were treated with fluoxetine (10 mg/kg i.p.), paroxetine (10 mg/kg i.p.), sibutramine (10 mg/kg i.p.) or saline 2 hr before d,l-fenfluramine challenge (10 mg/kg i.p.). As shown in figure 9, fenfluramine alone produced a 1400% increase above baseline 5-HT. Pretreatment with fluoxetine or paroxetine significantly attenuated the effect of fenfluramine on 5-HT. The
largest attenuation was observed with paroxetine, which almost completely blocked the increase in 5-HT, whereas fluoxetine produced an attenuation to ~100% above baseline. Pretreatment with sibutramine significantly attenuated the fenfluramine-induced increase to 320% above baseline 5-HT (fig. 9).

Discussion

Unequivocal classification of 5-HT reuptake inhibitors and releasing agents is difficult because both drug types produce increases in extracellular 5-HT. Four criteria for in vivo differentiation of the effects of 5-HT reuptake inhibitors and releasing agents were evaluated using the extensively characterized 5-HT reuptake inhibitors paroxetine and fluoxetine, the NE and 5-HT reuptake inhibitor imipramine, and the 5-HT releasing agent fenfluramine. Using these criteria, experiments were then performed to determine whether sibutramine and its primary amine metabolite, BTS 54 505, act similarly to 5-HT reuptake inhibitors or releasing agents in vivo.

Magnitude of increase in extracellular 5-HT. The first criterion was the difference in the magnitude of the maximal increase in hypothalamic 5-HT after acute systemic administration of reuptake inhibitors compared with releasing agents. Thus, the 2- to 4-fold maximal increase in hypothalamic 5-HT in response to acute systemic administration of paroxetine or imipramine is in agreement with the effect of other reuptake inhibitors such as fluoxetine and citalopram (Rutter and Auerbach, 1993; Perry and Fuller, 1992; Hjorth and Sharp, 1993). Presumably, the small increase in 5-HT represents a balance between blocking reuptake and the strong but incomplete inhibition of release that is a consequence of autoreceptor stimulation (Rutter et al., 1995). The sustained effect of paroxetine and BTS 54 505 is probably due to the fact that they are not rapidly metabolized to inactive compounds (Preskorn, 1994; K. F. Martin and D. J. Heal, unpublished data). Similarly, the prolonged effect of sibutramine is due to the sustained presence of its primary amine metabolite BTS 54 505 (K. F. Martin and D. J. Heal, unpublished data). The decrease from peak levels of extracellular 5-HT relatively soon after imipramine administration could be a reflection of its rapid metabolism to the selective NE reuptake inhibitor desmethyl imipramine (Sato et al., 1994).

In contrast, moderate systemic doses of 5-HT releasing agents, e.g., fenfluramine, MDMA and PCA, produce much larger increases in extracellular 5-HT concentrations. In our study, systemic injection of fenfluramine increased hypothalamic 5-HT to a transient peak, ~20-fold above baseline levels. This is consistent with the previous observation that fenfluramine produced an ~30-fold increase in hippocampal 5-HT (Sabol et al., 1992a). Similarly, after systemic administration of MDMA or PCA, extracellular 5-HT was increased more than 10-fold in rat striatum (Kalén et al., 1988; Gudelisky and Nash, 1996). The relatively large increase in extracellular 5-HT is probably because the effect of fenfluramine...
at high doses is mainly independent of 5-HT neuronal activity and calcium influx (Fuller et al., 1988; Berger et al., 1992; Levi and Raiteri, 1993). As compared to the reuptake inhibitors, there was a rapid decline from peak 5-HT levels after fenfluramine administration. This may be due to partial depletion of the releasable pool combined with inhibition of synthesis which would reduce the replenishment of 5-HT (K. F. Martin, S. Aspley and D. J. Heal, unpublished data).

Fig. 6. Effect of systemic administration of paroxetine, imipramine or nisoxetine combined with fluoxetine on the increase in extracellular 5-HT produced by local infusion of these drugs into the hypothalamus.

Fig. 7. Effect of systemic administration of fenfluramine on the increase in extracellular 5-HT produced by its local infusion into the hypothalamus. Values (mean ± S.E.) are expressed as a percent of the average of four baseline samples. The mean baseline levels for the treatment groups were not significantly different, and the combined value was 2.6 ± 0.2 pg/30 min sample (n = 10). Beginning at time 0, d,l-fenfluramine (100 μM) was infused by reverse dialysis into the hypothalamus (horizontal bars). This treatment caused a significant increase in extracellular 5-HT from baseline levels [F(7,63) = 25.65, P < .0001]. At the time indicated by the arrow, d,l-fenfluramine (10 mg/kg i.p.) or the vehicle was administered systemically. This did not result in a decrease in extracellular 5-HT after systemic fenfluramine [F(1,8) = 0.58, P < .5] (n = 5 for each group).

Values (mean ± S.E.) are expressed as a percent of the average of four baseline samples. The mean baseline levels for the treatment groups were not significantly different, and the combined value was 2.9 ± 0.1 pg/30-min sample (n = 29). Beginning at time 0, paroxetine (A), imipramine (B) or nisoxetine with fluoxetine (C) was infused by reverse dialysis into the hypothalamus (horizontal bars). Infusion of paroxetine (10 μM), imipramine (80 μM) or nisoxetine plus fluoxetine (each 10 μM) caused a significant increase in extracellular 5-HT from baseline levels: paroxetine [F(7,84) = 73.71, P < .0001]; imipramine [F(7,70) = 32.43, P < .0001]; nisoxetine plus fluoxetine [F(7,28) = 26.72, P < .0001]. At the time indicated by the arrow, either the corresponding drug (10 mg/kg i.p.) or the vehicle was administered systemically. This resulted in a significant decrease in extracellular 5-HT after systemic injection of paroxetine [F(6,30) = 33.71, P < .0001] and nisoxetine plus fluoxetine [F(5,20) = 2.33, P < .0001] but not imipramine [F(5,25) = 1.24, P < .319] (n = 5-7 for each group). *Significant decrease from the level before peripheral paroxetine or nisoxetine and fluoxetine administration (Scheffé’s post hoc test, P < .05).
In our experiments, doses of the reuptake inhibitors and fenfluramine were in the range of the ED$_{50}$ for effects on feeding. For example, 1, 3 and 10 mg/kg are the ED$_{50}$ values for the hypophagic effect of d,l-fenfluramine (Francis et al., 1995), sibutramine (Fantino and Souquet, 1995; Jackson et al., 1997) and paroxetine (Caccia et al., 1993), respectively. Thus, at three times the ED$_{50}$ for inhibiting feeding, the effect of the reuptake inhibitors was maximal at 3-fold above baseline 5-HT. In contrast, fenfluramine at three times its ED$_{50}$ produced a 6-fold increase in extracellular 5-HT with increasingly larger effects at higher doses. Together with previously published results, our data confirm that the magnitude of the response to peripheral administration can be used as a distinguishing criterion between reuptake inhibitors and releasing agents.

Dependence on 5-HT neuronal activity. The somatodendritic autoreceptor agonist, 8-OH-DPAT, has previously been shown to reverse the increase in extracellular 5-HT produced by systemic administration of fluoxetine to unanesthetized rats (Perry and Fuller, 1992; Rutter and Auerbach, 1993). This suggests that 5-HT neuronal activity is not comple-

Fig. 8. Effect of systemic administration of sibutramine (A) or BTS 54 505 (B) on the increase in extracellular 5-HT produced by local infusion of these drugs into the hypothalamus. Values (mean ± S.E.) are expressed as a percent of the average of four baseline samples. The mean baseline levels for the treatment groups were not significantly different, and the combined value was 1.7 ± 0.1 pg/30-min sample (n = 25). Beginning at time 0, drug was infused by reverse dialysis into the hypothalamus (horizontal bars). Infusion of sibutramine (2 mM) or BTS 54 505 (10 μM) caused a significant increase in extracellular 5-HT from baseline levels: sibutramine F(8,96) = 17.69 P < .0001; BTS 54 505 F(7,77) = 35.79, P < .0001. At the time indicated by the arrow, either the corresponding drug (10 mg/kg i.p.) or the vehicle was administered systemically. This resulted in a significant decrease in extracellular 5-HT after systemic injection of BTS 54 505 [F(7,35) = 3.89 P < .003] (n = 6 for each group) but not sibutramine [F(6,36) = 1.54, P < .195] (n = 6-7 for each group). *Significant decrease from the level before peripheral BTS 54 505 administration (Scheffé’s post hoc test, P < .05).

Fig. 9. Effect of pretreatment with reuptake inhibitors or sibutramine on fenfluramine-induced increases in hypothalamic 5-HT. Average baseline levels of 5-HT (pg) for the 2-hr pretreatment period were: 1.4 ± 0.13 for paroxetine (n = 5), 0.60 ± 0.05 for fluoxetine (n = 4), 2.4 ± 0.24 for sibutramine (n = 6) and 1.3 ± 0.12 for the vehicle (n = 7). After stable baseline levels were obtained, paroxetine, fluoxetine, sibutramine, d,l-fenfluramine (10 mg/kg i.p.) or the saline vehicle was administered. Values (mean ± S.E.) are expressed as a percent of the level at time 0, the fourth sample (2 hr) after systemic administration of pretreatment compound. Mean absolute values 2 hr after systemic injection were 5.7 ± 1.3 pg for paroxetine, 3.1 ± 0.6 pg for fluoxetine, 4.2 ± 0.4 pg for sibutramine and 1.0 ± 0.2 pg for the vehicle. *Significantly different from pretreatment with paroxetine, fluoxetine or sibutramine (Scheffé’s post hoc test, P < .05).

In our experiments, doses of the reuptake inhibitors and fenfluramine were in the range of the ED$_{50}$ for effects on feeding. For example, ~1, 3 and 10 mg/kg are the ~ED$_{50}$ values for the hypophagic effect of d,l-fenfluramine (Francis et al., 1995), sibutramine (Fantino and Souquet, 1995; Jackson et al., 1997) and paroxetine (Caccia et al., 1993), respectively. Thus, at three times the ED$_{50}$ for inhibiting feeding, the effect of the reuptake inhibitors was maximal at ~3-fold above baseline 5-HT. In contrast, fenfluramine at three times its ED$_{50}$ produced a 6-fold increase in extracellular 5-HT with increasingly larger effects at higher doses. Together with previously published results, our data confirm that the magnitude of the response to peripheral administration can be used as a distinguishing criterion between reuptake inhibitors and releasing agents.
pletely suppressed as might be inferred from the strong inhibitory effect of reuptake inhibitors on single unit activity in the DRN of anesthetized rats (Sheard et al., 1972; Aghajanian, 1972). Our results are consistent with the conclusion that the increase in extracellular 5-HT produced by reuptake inhibitors is dependent on residual activity of 5-HT neurons in freely behaving animals. Thus, we have shown that 8-OH-DPAT reversed the increase produced by systemic paroxetine and fluoxetine. This effect was also demonstrated with the putative reuptake inhibitor, sibutramine. In contrast, the relatively large effect of fenfluramine, MDMA and PCA on extracellular 5-HT may be due to a mechanism of release that is independent of 5-HT neuronal activity (Fuller et al., 1988; Gudelsky and Nash, 1996). Thus, in vitro and in vivo studies indicate that fenfluramine, MDMA- and PCA-induced release of 5-HT is not attenuated by TTX or removal of calcium from the perfusion medium (Kuhn et al., 1985; Sharp et al., 1986; Carboni and DiChiara, 1989; Bonnano et al., 1994). Our results are consistent with this conclusion, as the fenfluramine-induced increase in hypothalamic 5-HT was not attenuated by 8-OH-DPAT. These data, in combination with published results, therefore provide evidence to support the validity of the second criterion to differentiate 5-HT reuptake inhibitors from releasing agents.

**Systemic drug administration during local infusion.** In comparison to the effects of reuptake inhibitors after systemic administration, their perfusion by reverse dialysis into forebrain sites produces larger effects on extracellular 5-HT. For example, fluoxetine infusion into the diencephalon resulted in 6-fold increases in extracellular 5-HT (Rutter and Auerbach, 1993). Local administration into a forebrain terminal site can completely block reuptake while avoiding activation of somatodendritic autoreceptors. Subsequent peripheral injection of these reuptake blockers then produces a net decrease in forebrain extracellular 5-HT as a consequence of inhibition of 5-HT neuronal discharge and release (Rutter et al., 1995).

In our experiments, systemic administration of reuptake blockers during their local infusion had inconsistent effects. Several 5-HT reuptake inhibitors produced the expected response. Thus, during local paroxetine or fluoxetine infusion, extracellular 5-HT was increased to ~600% above baseline levels, and systemic administration of the corresponding high affinity, 5-HT selective drug resulted in a decrease in extracellular 5-HT to ~150-300% above baseline levels.

Our results are also in agreement with previous observations that some NE and 5-HT monoamine reuptake inhibitors including imipramine and amitriptyline have low efficacy in local-peripheral tests (Auerbach et al., 1995). In contrast, duloxetine, a NE and 5-HT monoamine reuptake inhibitor with high affinity for the 5-HT transporter produced a large attenuation in 5-HT when tested in the local peripheral experimental paradigm (S. B. Auerbach and S. Hjorth, unpublished observations). In addition, our results indicate that nisoxetine, a high affinity NE selective reuptake inhibitor (Tejani-Butt et al., 1990), in combination with fluoxetine, produced a decrease in 5-HT similar to that seen with fluoxetine alone. Systemic administration of sibutramine during its local infusion did not produce a decrease in hypothalamic 5-HT. In contrast, systemic BTS 54 505, as with duloxetine, and the combination of fluoxetine with nisoxetine, did attenuate its locally induced increase in 5-HT.

Efficacy in the local-peripheral experimental paradigm was significantly correlated with selectivity for blocking 5-HT reuptake (Auerbach et al., 1995). This suggested that enhancement of extracellular NE might attenuate autoreceptor-mediated inhibition of 5-HT release. However, results indicate that efficacy in this paradigm is also correlated with affinity (fig. 10) and suggest that blocking NE reuptake is not the factor responsible for the weak effect of some NE and 5-HT reuptake blockers. Paroxetine and fluoxetine, drugs with high affinity and selectivity for the 5-HT transporter had high efficacy. Combining the NE reuptake inhibitor nisoxetine with fluoxetine did not alter the ability of peripheral fluoxetine to modulate the effect of local infusion. Duloxetine and BTS 54 505 have similar profiles to the combination of nisoxetine and fluoxetine, being high affinity, NE and 5-HT reuptake inhibitors, and were similarly efficacious. Thus, the drugs that displayed good efficacy all have high affinity for the 5-HT reuptake site, but several show little selectivity between 5-HT and NE (fig. 10). The drugs with the lowest affinity for the 5-HT reuptake site, imipramine and sibutramine, had the lowest efficacy. In summary, our results suggest that low affinity for the 5-HT reuptake carrier, not ability to block NE reuptake, is the factor more likely responsible for low efficacy in these “local-peripheral” experiments. However, because most nonselective monoamine reuptake inhibitors also have relatively low affinity for the 5-HT reuptake site, both factors appear to be correlated with efficacy, and further experiments will be necessary to determine the explanation for these variable results.

Although extracellular levels were increased 7-fold during local infusion of fenfluramine, 5-HT release was not decreased but was slightly increased, by subsequent systemic administration of fenfluramine. This further supports the conclusion that the enhancement in extracellular 5-HT after administration of fenfluramine is not dependent on 5-HT neuronal discharge. In summary this criterion can differentiate high affinity reuptake inhibitors from 5-HT releasing agents.

**Ability to attenuate fenfluramine-induced 5-HT release.** The releasing effect of fenfluramine involves its binding to the 5-HT reuptake carrier. Thus, because reuptake inhibitors block the binding of fenfluramine to the transporter (Garattini et al., 1989), they attenuate fenfluramine-induced 5-HT release in vitro and in vivo (Raiteri et al., 1995; Sabol et al., 1992a; Gobbi et al., 1992; Kreiss et al., 1993). Furthermore, fluoxetine markedly attenuates the 5-HT releasing effects of MDMA (Bel and Artigas, 1992). Consistent with these data, pretreatment with fluoxetine or paroxetine greatly reduced the large increase in extracellular 5-HT produced by fenfluramine. This confirms the validity of this criterion, i.e., reuptake inhibitors attenuate the releasing effect of fenfluramine.

Similar to the reuptake inhibitors paroxetine and fluoxetine, sibutramine also attenuated the large increase in 5-HT produced by fenfluramine. Sibutramine is a drug with low affinity for the 5-HT reuptake site, and produced a smaller attenuation than the higher affinity drug, fluoxetine. Paroxetine has even higher affinity than fluoxetine, and produced the greatest attenuation. Thus, affinity for the reuptake transporter was correlated with the efficacy of these drugs in preventing fenfluramine-induced 5-HT release.

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In conclusion, we have identified and tested four criteria, which, when applied together, differentiated 5-HT reuptake inhibitors from 5-HT releasing agents in vivo. Using these criteria, we have also determined that the novel antiobesity drug sibutramine acts as a 5-HT reuptake inhibitor in vivo, and not as a 5-HT releasing agent. This is consistent with in vitro data indicating that sibutramine is a 5-HT and NE reuptake inhibitor (Buckett et al., 1988; Cheetham et al., 1993, Cheetham et al., 1996).

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References


Uptake Inhibitors vs. Releasers


