Amphetamine Analogs Increase Plasma Serotonin: Implications for Cardiac and Pulmonary Disease

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ABSTRACT

Elevations in plasma serotonin (5-HT) have been implicated in the pathogenesis of cardiac and pulmonary disease. Normally, plasma 5-HT concentrations are kept low by transporter-mediated uptake of 5-HT into platelets and by metabolism to 5-hydroxyindoleacetic acid (5-HIAA). Many abused drugs (e.g., substituted amphetamines) and prescribed medications (e.g., fluoxetine) target 5-HT transporters and could thereby influence circulating 5-HT. We evaluated the effects of amphetamine analogs [(±)-fenfluramine, (±)-3,4-methylenedioxymethamphetamine, (±)-amphetamine, phentermine] on extracellular levels (i.e., plasma levels) of 5-HT and 5-HIAA in blood from catheterized rats. Effects of the 5-HT uptake blocker fluoxetine were examined for comparison. Drugs were tested in vivo and in vitro; plasma indoles were measured using a novel microdialysis method in whole blood. We found that baseline dialysate levels of 5-HT are ~0.22 nM, and amphetamine analogs evoke large dose-dependent increases in plasma 5-HT ranging from 4 to 20 nM. The ability of drugs to elevate plasma 5-HT is positively correlated with their potency as 5-HT transporter substrates. Fluoxetine produced small, but significant, increases in plasma 5-HT. Although the drug-evoked 5-HT concentrations are below the micromolar levels required for contraction of pulmonary arteries, they approach concentrations reported to stimulate mitogenesis in pulmonary artery smooth muscle cells. Additional studies are needed to determine the effects of chronic administration of amphetamines on circulating 5-HT.

Serotonin (5-hydroxytryptamine, 5-HT) is an endogenous bioactive compound that is widely distributed in neurons, mast cells, enterochromaffin cells, and blood platelets (Cooper et al., 2003; Gershon, 2004). Under normal physiological conditions, plasma 5-HT levels are kept exquisitely low (i.e., <1 nM) due to transporter-mediated uptake of 5-HT into blood platelets and via metabolism of 5-HT to 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase (MAO). Included among the many physiological effects of 5-HT are mitogenesis and vasoconstriction. Accordingly, altered regulation of 5-HT levels in blood has been implicated in the pathogenesis of cardiac valvular heart disease (VHD) (Robio-lio et al., 1995) and primary pulmonary hypertension (PPH) (MacLean et al., 2000). Many substituted amphetamine analogs (e.g., fenfluramine and aminorex) are substrates for 5-HT transporters (SERTs) and release 5-HT from neurons via reversal of SERT (Rothman et al., 1999). One hypothesis to explain the ability of these agents to increase the risk of developing PPH is that they increase plasma 5-HT by stimulating 5-HT release from platelets (i.e., “the 5-HT hypothesis” of PPH) (MacLean et al., 2000). Others have invoked the same mechanism to explain fenfluramine-induced VHD (Fishman, 1999), although a more likely mechanism in this case involves activation of 5-HT_{2B} receptors by the N-deethylated metabolite of fenfluramine, norfenfluramine (Rothman et al., 2000a).

Despite the importance of the 5-HT hypothesis to current dogma regarding the etiology of drug-induced PPH and VHD, the effects of anorectic agents on plasma 5-HT have received little attention. The paucity of data in this regard could be related to the fact that measuring plasma 5-HT is technically challenging. Given that basal 5-HT levels are quite low and that over 99% of blood 5-HT is stored in platelets, even minor disturbance of platelets during sample handling will cause large artificial increases in plasma 5-HT concentrations.

Studies conducted in the 1990s indicate that acute administration of (±)-fenfluramine does not increase plasma 5-HT concentrations, although this may be due to the short duration of action of fenfluramine. We investigated if drugs that increase plasma 5-HT levels have the potential to increase the risk of drug-induced PPH or VHD by stimulating 5-HT release from platelets (i.e., “the 5-HT hypothesis” of PPH). We have identified a novel class of drugs that act on 5-HT transporters and increase plasma 5-HT levels. These drugs are amphetamine analogs [(±)-fenfluramine, (±)-3,4-methylenedioxymethamphetamine, (±)-amphetamine, phentermine] on extracellular levels (i.e., plasma levels) of 5-HT and 5-HIAA in blood from catheterized rats. Effects of the 5-HT uptake blocker fluoxetine were examined for comparison. Drugs were tested in vivo and in vitro; plasma indoles were measured using a novel microdialysis method in whole blood. We found that baseline dialysate levels of 5-HT are ~0.22 nM, and amphetamine analogs evoke large dose-dependent increases in plasma 5-HT ranging from 4 to 20 nM. The ability of drugs to elevate plasma 5-HT is positively correlated with their potency as 5-HT transporter substrates. Fluoxetine produced small, but significant, increases in plasma 5-HT. Although the drug-evoked 5-HT concentrations are below the micromolar levels required for contraction of pulmonary arteries, they approach concentrations reported to stimulate mitogenesis in pulmonary artery smooth muscle cells. Additional studies are needed to determine the effects of chronic administration of amphetamines on circulating 5-HT.

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine or serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; MAO, monoamine oxidase; VHD, valvular heart disease; PPH, primary pulmonary hypertension; SERT, serotonin transporter; IRP, Intramural Research Program; MDMA, (±)-3,4-methylenedioxymethamphetamine; HPLC, high-performance liquid chromatography; ECD, electrochemical detection; ANOVA, analysis of variance; SSRI, serotonin-selective reuptake inhibitor.
in rats (Martin and Artigas, 1992), and chronic administration of fenfluramine lowers blood 5-HT in humans (see Rothman et al., 2000b and references therein). In the present study, we developed a novel microdialysis method to assess the effects of amphetamine analogs on plasma levels of 5-HT in whole blood samples obtained from conscious catheterized rats. The results show that amphetamine analogs produce significant dose-dependent increases in plasma 5-HT, and this effect is proportional to drug potency as SERT substrates. 5-HT uptake inhibitors, such as fluoxetine, also can increase plasma 5-HT, but to a lesser extent. The physiological significance of these findings is discussed.

Materials and Methods

Animals. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 350 to 450 g were singly housed with food and water freely available. Rats were maintained in facilities accredited by the American Association of the Accreditation of Laboratory Animal Care, and procedures were carried out in accordance with the Animal Care and Use Committee of the National Institute on Drug Abuse Intramural Research Program (IRP).

Drugs and Reagents. (±)-Fenfluramine HCl (fenfluramine, FW 267.7), (±)-3,4-methylenedioxyamphetamine (MDMA, FW 229.7), (±)-methamphetamine HCl (methamphetamine, FW 185.7), (±)-amphetamine sulfate (amphetamine, FW 368.5), phentermine HCl (phentermine, FW 185.7), and pentobarbital sodium were obtained from the National Institute on Drug Abuse, IRP Pharmacy. Fluoxetine HCl (fluoxetine, FW 345.8) was purchased from Spectrum Chemical Manufacturing Company (New Brunswick, NJ). Monocho- roacetic acid was obtained from Mallinckrodt Baker Inc. (Phillipsburg, NJ), and all other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Drug solutions for the in vivo and in vitro studies were prepared in saline immediately before use, and doses are expressed as the salt.

Surgical Procedures. Rats received sodium pentobarbital (60 mg/kg i.p.) for surgical anesthesia. Indwelling jugular catheters, made of Silastic Medical Grade tubing (Dow Corning, Midland, MI), were implanted into the right jugular vein and advanced to the atrium as described previously (Baumann et al., 2001). Rats were allowed to recover for 7 to 10 days postoperatively.

In Vivo Drug Administration. Between 8:00 and 9:00 AM, rats were moved into the testing room and allowed to acclimate to the surroundings for 1 h. Extension tubes were attached to catheters, and 0.5 ml of heparin flush (48 IU/ml in saline) was injected as noted above. In these experiments, serial blood samples were withdrawn from untreated donor rats and transferred into 300-μl polypropylene tubes that were kept at 25°C. These tubes were prefilled with 20 μl of heparin (1000 IU/ml). Test drugs or vehicle were added directly to blood samples in 10-μl volumes to yield final concentrations of 0.3, 1, 3, or 33 μM. Microdialysis probes were placed into the blood samples, and dialysate efflux was collected for 15 min and assayed for 5-HT and 5-HIAA using HPLC-ECD. Each blood sample was dialyzed for 15 min to generate a single dialysate sample. Two baseline samples were collected before addition of test drugs to subsequent samples. Probe recoveries were performed before and after blood sampling using a 10-μg 5-HT standard.

HPLC-ECD Analysis of 5-HT and 5-HIAA. Aliquots of the dialysate (5 μl) were injected onto a microbore HPLC column (Unijet, 100 × 1 mm, 5 μM octadecylsilane; Bioanalytical Systems, Inc., West Lafayette, IN) that was coupled to an amperometric detector (Model LC-4C, Bioanalytical Systems, Inc.). A glassy carbon electrode was set at a potential of +650 mV relative to Ag/AgCl reference. Mobile phase consisted of 180 μM Na2EDTA, 150 mM monochloroacetic acid, 125 mM NaOH, and 690 μM sodium octanesulfonic acid, with 7.5% MeOH and 7.5% CH3CN/water (final pH 3.15). Mobile phase was pumped through the column at 60 μl/min (260D, syringe pump; Teledyne ISCO, Lincoln, NE). Chromatographic data were acquired on-line and exported to a Millennium software system (Waters Associates, Milford, MA) for peak amplification, integration, and analysis. The concentration of 5-HT and 5-HIAA in dialysate samples was compared with known standards, and the lower limit of detection was --0.05 pg/μl (0.047 nM) for both indoles.

Statistical Analyses. In all studies, the first two dialysate samples collected before any treatment were considered baseline samples. 5-HT and 5-HIAA measures are mean ± S.E.M. expressed as picograms per 5-μl sample. For in vivo experiments, data were evaluated by two-way ANOVA (drug treatment × time) and one-way ANOVA (at each time point). For in vitro experiments, data were evaluated by one-way ANOVA (drug dose). When significant F values were obtained, Newman-Keuls post hoc tests were performed to compare group means. For data correlations, peak drug effects measured in vivo after 1.0 mg/kg were compared with peak drug effects measured in vitro after 3 μM and with drug EC50 values for [H]5-HT release from synaptosomes (Rothman et al., 2001). A value of p < 0.05 was considered as the minimum criterion for statistical significance.

Results

Baseline 5-HT Levels in Plasma. For all animals used in this study (n = 172 rats), the mean basal concentration of dialysate 5-HT in blood was 0.25 ± 0.01 pg/5 μl (i.e., 0.22 ± 0.01 nM). Baseline dialysate 5-HT levels differed slightly depending upon experimental conditions. In the in vivo drug administration experiments (n = 120 rats), blood samples were maintained on ice to reduce SERT-mediated “leak” of 5-HT from platelets, and basal plasma 5-HT was 0.20 ± 0.01 pg/5 μl. In the in vitro drug experiments (n = 52 rats), blood samples were kept at room temperature to optimize SERT function, and this resulted in a slightly higher basal level of plasma 5-HT, 0.25 ± 0.02 pg/5 μl. Baseline 5-HT did not differ significantly between the various treatment groups within the in vivo and in vitro conditions. It should be noted that microdialysis probes had in vitro recovery rates of approximately 25% when tested in a physiological salt solution, and this value did not vary significantly before, during or
amphetamine significantly increased plasma 5-HT to 21-fold elevations at the 0.3 and 1.0 mg/kg doses. Methamphetamine in the saline-injected control group. The 5-HT reuptake cant 5-fold rise. Phentermine had a very weak effect on 5-HT,

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with 7- and 12-fold elevations at 0.3 and 1.0 mg/kg doses. Methamphetamine significantly increased plasma 5-HT [$F(2,120) = 29.75; p < 0.0001$] but to a lesser extent than fenfluramine, with 7- and 12-fold elevations at 0.3 and 1.0 mg/kg doses. Amphetamine had weak effects on 5-HT levels [$F(2,120) = 3.64; p = 0.03$], and only the 1 mg/kg dose caused a significant 5-fold rise. Phentermine had a very weak effect on 5-HT, and post hoc tests failed to demonstrate any significant effects of the 1.0 and 3.0 mg/kg doses, possibly due to variability in the saline-injected control group. The 5-HT reuptake inhibitor, fluoxetine, produced a modest increase in plasma 5-HT [$F(2,120) = 49.16; p < 0.0001$] with 0.3 and 1.0 mg/kg doses increasing 5-HT levels 4- and 7-fold. Interestingly, none of the in vivo treatments affected plasma 5-HIAA levels (data not shown). Fenfluramine, MDMA, and methamphetamine produced transient increases in plasma 5-HT that had mostly returned to baseline values after 90 min.

Some investigators (Ulus et al., 2000) have proposed that pharmacological doses of phentermine and other amphetamine analogs will block MAO activity in vivo, an effect that can be detected as decreased 5-HIAA levels. Others have suggested that coadministration of phentermine with fenfluramine would enhance fenfluramine-induced increases in plasma 5-HT (Fishman, 1999; Ulus et al., 2000). To address these hypotheses, we compared the effects of phentermine and fenfluramine, alone and in combination, at 1.0 mg/kg doses. As shown in Fig. 2, no treatment altered plasma 5-HIAA, and coadministration of phentermine plus fenfluramine did not enhance plasma 5-HT higher than fenfluramine alone.

**In Vivo Drug Administration Experiments.** Figure 1 depicts the effects of fenfluramine, MDMA, methamphetamine, amphetamine, phentermine, and fluoxetine on dialysate 5-HT levels in whole blood, when drugs were administered via i.v. catheters in vivo. Fenfluramine significantly increased dialysate 5-HT [$F(2,120) = 56.55; p < 0.0001$], and this stimulatory action was dose-dependent. Fenfluramine elevated extracellular 5-HT to 15- and 19-fold after 0.3 and 1.0 mg/kg doses. MDMA also significantly increased 5-HT levels [$F(2,120) = 64.22; p < 0.0001$], with 12- and 21-fold elevations at the 0.3 and 1.0 mg/kg doses. Methamphetamine significantly increased plasma 5-HT [$F(2,120) = 29.75; p < 0.0001$] but to a lesser extent than fenfluramine, with 7- and 12-fold elevations at 0.3 and 1.0 mg/kg doses. Amphetamine had weak effects on 5-HT levels [$F(2,120) = 3.64; p = 0.03$], and only the 1 mg/kg dose caused a significant 5-fold rise. Phentermine had a very weak effect on 5-HT, and post hoc tests failed to demonstrate any significant effects of the 1.0 and 3.0 mg/kg doses, possibly due to variability in the saline-injected control group. The 5-HT reuptake inhibitor, fluoxetine, produced a modest increase in plasma 5-HT [$F(2,120) = 49.16; p < 0.0001$] with 0.3 and 1.0 mg/kg doses increasing 5-HT levels 4- and 7-fold. Interestingly, none of the in vivo treatments affected plasma 5-HIAA levels (data not shown). Fenfluramine, MDMA, and methamphetamine produced transient increases in plasma 5-HT that had mostly returned to baseline values after 90 min.

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**In Vitro Drug Administration Experiments.** Figure 3 shows the effects of fenfluramine, MDMA, methamphetamine, amphetamine, phentermine, and fluoxetine on dialysate 5-HT levels in blood, when drugs were administered directly into blood samples in vitro. Fenfluramine significantly increased plasma 5-HT in a dose-dependent manner [$F(3,23) = 64.54; p < 0.0001$], producing 16- and 91-fold elevations when administered at concentrations of 3 and 33 

$$\mu$$M, respectively. MDMA had similar effects [$F(3,23) = 16.15; p < 0.0001$] and increased dialysate 5-HT to 8- and 42-fold above baseline at 3 and 33 

$$\mu$$M doses. Methamphetamine [$F(3,23) = 96.19; p < 0.0001$] and amphetamine [$F(3,23) = 50.03; p < 0.0001$] were somewhat less efficacious than MDMA, significantly increasing 5-HT by 4- and 44-fold at the 3 and 33 

$$\mu$$M doses, respectively. Phentermine significantly increased 5-HT levels only at the 33 

$$\mu$$M dose [$F(3,23) = 9.71; p < 0.0004$]. Fluoxetine dose dependently increased plasma 5-HT [$F(3,23) = 17.76; p < 0.0001$] to 3-, 14-, and 22-fold above baseline at 0.3, 3, and 33 

$$\mu$$M, respectively.

The data in the top panel of Fig. 4 show that the ability of amphetamine analogs to increase plasma 5-HT in vitro at a 3 

$$\mu$$M dose is significantly correlated with the increase in 5-HT levels produced in vivo administration of 1.0 mg/kg ($p <
This finding suggests that these drugs are increasing extracellular 5-HT in blood by a similar mechanism under in vitro and in vivo conditions. We previously reported the EC_{50} values for drugs releasing [3H]5-HT from synaptosomes, which is a measure of the potency of these compounds as substrates for SERT (Rothman et al., 2001). The bottom panel of Fig. 4 demonstrates that the calculated EC_{50} values for test drugs to release [3H]5-HT from synaptosomes is highly correlated with the ability of the same agents to increase plasma 5-HT in vivo (p < 0.01). Taken together, these correlative relationships suggest that amphetamine analogs increase plasma 5-HT in whole blood by a process involving SERT proteins, possibly those found on platelets.

Studies in nervous tissue have shown that uptake inhibitors can attenuate the ability of transporter substrates to release 5-HT via carrier-mediated exchange (Baumann et al., 2001; Rothman et al., 2001). To test whether uptake blockers could affect drug-induced 5-HT efflux in blood samples, we coadministered fluoxetine and MDMA in blood samples in vitro and performed microdialysis. The top panel of Fig. 5 demonstrates that low-dose fluoxetine (i.e., 0.3 μM) did not block the rise in plasma 5-HT produced by low-dose MDMA (i.e., 1.0 μM). This type of experiment is complicated by the fact that fluoxetine pretreatment alone causes increases in plasma 5-HT that are comparable with the effects of low-dose MDMA. However, as reported in the bottom panel of Fig. 5, a higher dose of fluoxetine (i.e., 3 μM) significantly reduced the large increase in plasma 5-HT produced by 33 μM MDMA by 50% [F(3,39) = 70.61; p < 0.0001].

Discussion

Fenfluramine, MDMA, methamphetamine, and amphetamine are known to be substrates for SERT proteins and to release 5-HT from neurons in the brain (Berger et al., 1992; Crespi et al., 1997; Rothman et al., 2001). The anorectic
medication, fenfluramine, is associated with the occurrence of PPH and VHD and was withdrawn from the marketplace in 1997. Determining the mechanism(s) whereby fenfluramine increases the risk of developing VHD and PPH is important to understand, so that newly discovered serotonergic medications will not produce these serious adverse effects (Rothman and Baumann, 2003). Many investigators have theorized that fenfluramine increases the risk of PPH and VHD by elevating plasma 5-HT levels in blood, consistent with its known 5-HT-releasing properties in nervous tissue (Connolly et al., 1997; Fishman, 1999; MacLean et al., 2000). Studies conducted in the 1990s provide little support for this hypothesis. For example, Martin and Artigas (1992) reported that acute administration of (+)-fenfluramine does not increase plasma 5-HT in rats. Moreover, chronic administration of fenfluramine lowers blood 5-HT in humans (see Rothman et al., 2000b and references therein). Given the importance of the 5-HT hypothesis to current dogma regarding fenfluramine-associated PPH and VHD, we decided to directly investigate this issue by using a novel microdialysis method to assess the effects of amphetamine analogs on plasma levels of 5-HT in conscious rats. In addition to fenfluramine, we assessed the actions of other substituted amphetamines on plasma 5-HT, both therapeutic agents such as amphetamine and drugs of abuse such as MDMA and methamphetamine.

Measuring plasma 5-HT is technically challenging. Given that over 99% of blood 5-HT is stored in platelets, and platelets are very fragile, even minor disturbance or damage to platelets during sample handling will cause large artificial increases in plasma 5-HT. The method we developed to measure dialysate 5-HT levels ex vivo minimizes trauma to platelets and permits an accurate determination of plasma 5-HT. The mean baseline level of dialysate 5-HT in blood collected from rats in these studies (n = 172) was 0.22 ± 0.01 nM. Because in vitro probe recoveries averaged approximately 25%, the “actual” corrected baseline level of plasma 5-HT in our experiments is likely in the range of 0.88 nM. This level is comparable with plasma 5-HT levels in humans, which are reportedly in the subnanomolar range (Herve et al., 1995).

With the exception of phentermine, all of the test drugs produced dose-dependent increases in plasma 5-HT when administered in vivo (see Fig. 1). These findings differ from those of Martin and Artigas (1992), who did not observe elevations in plasma 5-HT after administration of (+)-fenfluramine (2.5 mg/kg) to rats. The reason for the discrepancy between our results and those of others is not known but could be related to differences in blood sampling procedures. Specifically, we obtained blood samples within 15 min of fenfluramine administration, whereas Martin and Artigas sampled 60 min after injection, possibly missing the peak effect of the drug on circulating 5-HT.

The ability of amphetamine analogs to increase plasma 5-HT in vivo is correlated with their capacity to increase plasma 5-HT in vitro and with their potency at releasing [3H]5-HT from synaptosomes (see Fig. 4). Because platelets and neurons express the same SERT protein (Lesch et al., 1993), the potency of agents at releasing [3H]5-HT from platelets and from synaptosomes should be similar. Indeed, the EC50 value reported by Schuldiner et al. (1993) for fenfluramine-induced release of [3H]5-HT from platelets (170 nM) is similar to the value we reported for fenfluramine in rat brain synaptosomes (79 nM) (Rothman et al., 2001). These observations support the notion that amphetamine analogs act as substrates for platelet SERT proteins, thereby explaining their ability to increase plasma 5-HT. This interpretation is further supported by the finding that fluoxetine could partially reverse the ability of high-dose MDMA to release 5-HT in vitro (see Fig. 5). In this experiment, we believe that fluoxetine prevents MDMA from entering platelets by blocking SERT sites, thus preventing massive release of 5-HT via disruption of storage vesicles (Schuldiner et al., 1993). Both methamphetamine and amphetamine release 5-HT from synaptosomes with low potency (~1 µM), but methamphetamine is much more potent at increasing extracellular 5-HT in brain via a SERT-related mechanism (Rothman and Baumann, 2003; Rothman et al., 2005). Although we have no explanation for this peculiar phenomenon, it also occurs in blood since methamphetamine, but not amphetamine, increases plasma 5-HT at the 0.3 mg/kg dose. As noted above, the 40-fold increase in plasma 5-HT produced by 33 µM amphetamine in vitro likely results from disruption of 5-HT storage vesicles in the platelets.

Steady-state plasma 5-HT levels are maintained in the subnanomolar range in part by SERT-mediated uptake of 5-HT into platelets. Thus, it is not surprising that the 5-HT uptake inhibitor fluoxetine also increases plasma 5-HT in vivo and in vitro. The stimulatory effect of fluoxetine is much smaller in magnitude than the effects of amphetamines, suggesting that uptake blockers produce minor changes in plasma 5-HT compared with SERT substrates. It should be noted that fluoxetine, unlike SERT substrates, cannot be removed from the plasma by translocation into cells that express SERT, and this could prolong the actions of fluoxetine and its bioactive metabolite, norfluoxetine. Because the ability of fluoxetine to increase plasma 5-HT arises from its inhibition of SERT, other serotonin-selective reuptake inhibitors (SSRIs) might cause small and transient increases in plasma 5-HT. It is unlikely that SSRI-induced increases in plasma 5-HT contribute to the antidepressant effects of this class of medication. However, it is tempting to speculate that SSRI-induced increases in plasma 5-HT contribute to the ability of SSRIs to increase ejaculatory threshold (de Jong et al., 2006).

Our findings provide at least partial confirmation of the 5-HT hypothesis that fenfluramine can increase plasma 5-HT. However, it is possible that the ability of fenfluramine and other SERT substrates to increase plasma 5-HT might be different after chronic administration. In this instance, platelet 5-HT is markedly reduced (Raleigh et al., 1986; Celada et al., 1994), and it is not yet known if plasma 5-HT is reduced under these circumstances or if the ability of fenfluramine to increase plasma 5-HT will be attenuated. In the case of chronic treatment with SERT inhibitors, both platelet and plasma 5-HT are markedly reduced (Celada et al., 1992a). Regarding PPH, another factor to consider is whether the maximal drug-induced plasma 5-HT concentrations approach those needed to contract pulmonary arteries or to stimulate mitogenesis in pulmonary artery endothelium or smooth muscles. The maximal concentration of drug-induced dialysate 5-HT was ~5 nM when high i.v. drug doses were administered; correcting for probe recovery estimates, this value is increased to ~20 nM. Serotonin contracts human pulmonary arteries with EC50 values that range from ap-
proximately 100 nM (Morecroft et al., 1999) to the micromolar range (Cortijo et al., 1997). Thus, it would seem unlikely that fenfluramine and other SERT substrates could increase plasma 5-HT to a concentration that would directly contract pulmonary arteries.

Recent studies indicate that SERT plays a key role in mitogenic effect of 5-HT on pulmonary artery smooth muscle cells, and this effect can be prevented by SERT inhibitors fluoxetine and paroxetine but not by the 5-HT$_{2A}$ receptor antagonist ketanserin (Eddahibi et al., 2002). The threshold concentration for 5-HT-stimulated mitogenic responses in cultured human pulmonary artery smooth muscle cells is approximately 10 nM (Eddahibi et al., 2001; Marcos et al., 2003), although higher 5-HT (~100 nM) concentrations are needed to stimulate mitogenic responses in rat pulmonary artery smooth muscle cells (Pitt et al., 1994; Eddahibi et al., 1999). It seems possible that high doses of fenfluramine, MDMA, and methamphetamine could transiently produce plasma 5-HT concentrations sufficient to stimulate mitogenic responses in pulmonary smooth muscle cells. Consequently, these drugs could increase the risk of developing PPH in susceptible individuals, should the exposure to higher than normal plasma 5-HT continue long enough.

Regarding VHD, it is well established that 5-HT can induce mitogenic responses in human and animal heart interstitial cells in vitro (Rajamannan et al., 2001; Jian et al., 2002; Setola et al., 2003). Although mitogenic responses have been observed with 5-HT concentrations as low as 10 nM (Hafizi et al., 2000; Rajamannan et al., 2001), other studies report that 5-HT concentrations in the micromolar range are required to stimulate mitogenic responses (Jian et al., 2002; Setola et al., 2003). Importantly, cells must be exposed to 5-HT for 24 to 48 h to produce the mitogenic response, a period of time far greater than the 60 to 90 min of elevated plasma 5-HT produced by administration of fenfluramine and the other substituted amphetamines. Thus, it seems unlikely that a single i.v. dose of fenfluramine, MDMA, or methamphetamine could transiently produce high enough plasma 5-HT concentrations to stimulate mitogenic responses in cardiac valvular cells. This interpretation of our data is supported by the fact that high 5-HT concentrations (>500 nM) are required to produce valvulopathy in carcinoid syndrome (Robiolio et al., 1995). Modest 2- to 3-fold elevations of plasma 5-HT, such as those occurring with the preadministration of lithium (Artigas et al., 1989) and MAO inhibitors (Celada et al., 1992b) are not associated with VHD. Our previous findings indicated that medications known to cause VHD (fenfluramine, ergotamine, methysergide) produce metabolites that are 5-HT$_{2B}$/ receptor agonists (Rothman et al., 2000a; Setola et al., 2003). More recently, two medications used to treat Parkinson’s disease, pergolide and cabergoline, were identified as 5-HT$_{2B}$/ agonists that also cause VHD (for review, see Setola and Roth, 2005). Collectively the data indicate that fenfluramine produces VHD through the activation of cardiac valvular 5-HT$_{2B}$/ receptors by its metabolite, norfenfluramine, and not from elevation of plasma 5-HT. On the other hand, the translocation of 5-HT transporter substrates into cells in exchange for endogenous 5-HT and the subsequent “trapping” of these agents inside of cells could contribute to the mechanism of toxicity (Rothman et al., 1999).

Our experiments provided the unique opportunity to examine other hypotheses concerning the pathogenesis of fenfluramine-associated VHD. Ulus et al. (2000) suggested that phentermine and other amphetamines block MAO activity in vivo, and this action could contribute to the development of VHD when phentermine and fenfluramine are administered together. MAO is an enzyme that metabolizes 5-HT to 5-HIAA. Inhibition of MAO produces measurable decreases in plasma 5-HIAA. Several types of evidence obtained in rat nervous tissue suggest that phentermine and fenfluramine do not affect MAO (Baumann et al., 2000; Kilpatrick et al., 2001), and the present data confirm that high i.v. doses of these drugs do not affect plasma 5-HIAA (see Fig. 2), consistent with data from nonhuman primates (Alexander et al., 2005). Other investigators proposed that phentermine would enhance fenfluramine-induced increases in plasma 5-HT (Fishman, 1999; Ulus et al., 2000). Our data clearly show that phentermine does not enhance the rise in plasma 5-HT produced by fenfluramine.

In summary, contrary to previously published data (Martin and Artigas, 1992), we demonstrate that fenfluramine and other SERT substrates produce significant dose-dependent increases in plasma 5-HT when administered under in vivo and in vitro conditions. This effect most likely involves SERT-mediated exchange of drug molecules for platelet 5-HT. Agents with less potent SERT substrate activity, such as amphetamine and phentermine, are considerably less effective in elevating plasma 5-HT compared with fenfluramine, MDMA, and methamphetamine. The fact that MDMA and methamphetamine increase plasma 5-HT to concentrations that stimulate mitogenic responses in pulmonary artery smooth muscle cells suggests that this effect may contribute to the cardiovascular toxicities associated with illicit use of these drugs. Further research will be needed to elucidate the physiological significance of our findings.

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References


