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## Therapeutic and adverse actions of serotonin transporter substrates

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### Abstract

A variety of drugs release serotonin (5-HT, 5-hydroxytryptamine) from neurons by acting as substrates for 5-HT transporter (SERT) proteins. This review summarizes the neurochemical, therapeutic, and adverse actions of substrate-type 5-HT-releasing agents. The appetite suppressant ( $\pm$ )-fenfluramine is composed of (+) and (–) isomers, which are *N*-de-ethylated in the liver to yield the metabolites (+)- and (–)-norfenfluramine. Fenfluramines and norfenfluramines are potent 5-HT releasers. ( $\pm$ )-3,4-Methylenedioxymethamphetamine (( $\pm$ )-MDMA, “ecstasy”) and *m*-chlorophenylpiperazine (mCPP) are substrate-type 5-HT releasers. Fenfluramines, ( $\pm$ )-MDMA, and mCPP release neuronal 5-HT by a common non-exocytotic diffusion-exchange mechanism involving SERTs. (+)-Norfenfluramine is a potent 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptor agonist. The former activity may increase the risk of valvular heart disease, whereas the latter activity is implicated in the anorexic effect of systemic fenfluramine. Appetite suppressants that increase the risk for developing primary pulmonary hypertension (PPH) are all SERT substrates, but these drugs vary considerably in their propensity to increase this risk. For example, fenfluramine and aminorex are clearly linked to the occurrence of PPH, whereas other anorectics are not. Similarly, some SERT substrates deplete brain tissue 5-HT in animals (e.g., fenfluramine), while others do not (e.g., mCPP). In addition to the established indication of obesity, 5-HT releasers may help treat psychiatric disorders, such as drug and alcohol dependence, depression, and premenstrual syndrome. Viewed collectively, we believe new medications can be developed that selectively release 5-HT without increasing the risk for adverse effects of valvular heart disease, PPH, and neurotoxicity. Such agents may be useful for treating a variety of psychiatric disorders.

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*Keywords:* Fenfluramine; Serotonin transporter; Valvular heart disease; Primary pulmonary hypertension; Neurotoxicity

*Abbreviations:* DA, dopamine; 5-HT, serotonin, 5-hydroxytryptamine; mCPP, *m*-chlorophenylpiperazine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxyamphetamine, “ecstasy”; NE, norepinephrine; NET, norepinephrine transporter; PPH, primary pulmonary hypertension; SERT, 5-hydroxytryptamine transporter; SSRI, 5-hydroxytryptamine-selective reuptake inhibitor; TFMPP, *m*-trifluoromethylpiperazine; VHD, valvular heart disease; VMAT, vesicular monoamine transporter.

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## 1. Introduction

Neurons that synthesize, store, and release monoamine transmitters [norepinephrine (NE), dopamine (DA), and serotonin (5-HT, 5-hydroxytryptamine)] are widely distributed in the mammalian CNS. These neurons possess specialized plasma membrane proteins that function to transport previously released transmitter molecules from the extracellular space back into the cytoplasm (Amara & Kuhar, 1993; Masson et al., 1999). It is now well established that there are distinct transporter proteins expressed by NE neurons [i.e., NE transporters (NETs)], DA neurons (i.e., DA transporters), and 5-HT neurons [i.e., 5-HT transporters (SERTs)]. These proteins belong to a superfamily of  $\text{Na}^+/\text{Cl}^-$ -dependent transporters that share genetic, structural, and functional homologies (Blakely et al., 1994; Uhl & Johnson, 1994). Under normal circumstances, the transporter-mediated uptake of monoamine transmitters is the principal mechanism for inactivation of monoaminergic signaling in the brain. Accordingly, a variety of therapeutic and abused drugs interact with monoamine transporter sites (Amara & Sonders, 1998).

Drugs that target transporter proteins can be divided into two basic classes: reuptake inhibitors and substrate-type releasers. Reuptake inhibitors bind to transporter proteins, but are not themselves transported. These drugs elevate extracellular transmitter concentrations by blocking transporter-mediated recapture of transmitter molecules from the synapse. Substrate-type releasers bind to transporter proteins, and these drugs are subsequently transported into the cytoplasm of nerve terminals. Releasers elevate extracellular transmitter concentrations by a two-pronged mechanism: (1) they promote efflux of transmitter by a process of transporter-mediated exchange and (2) they increase cytoplasmic levels of transmitter by disrupting storage of transmitters in vesicles (Rudnick, 1997; Rudnick & Clark, 1993). This latter action increases the pool of neurotransmitter available for release by transporter-mediated exchange. Because substrate-type releasing agents must be transported into nerve terminals to promote transmitter release, reuptake inhibitors block the effects of releasers. Fig. 1 shows a schematic diagram depicting the mechanism of action of the prototypical 5-HT releaser fenfluramine. Fenfluramine acts as a substrate for SERT. SERTs, in turn, mediate the translocation of drug molecules into the cytoplasm in exchange for 5-HT molecules that flow out into the extracellular space (i.e., synapse). Note that fenfluramine is also a substrate for the vesicular monoamine transporter (VMAT) present on intracellular vesicle membranes (Rudnick, 1997; Schuldiner et al., 1993). By disrupting compartmentalization of 5-HT into vesicles, fenfluramine increases the pool of cytoplasmic transmitter available for release.

Although both reuptake inhibitors and releasers elevate synaptic concentrations of transmitter via transporter-dependent processes, there are important differences in their precise modes of action. In particular, the activity of reup-

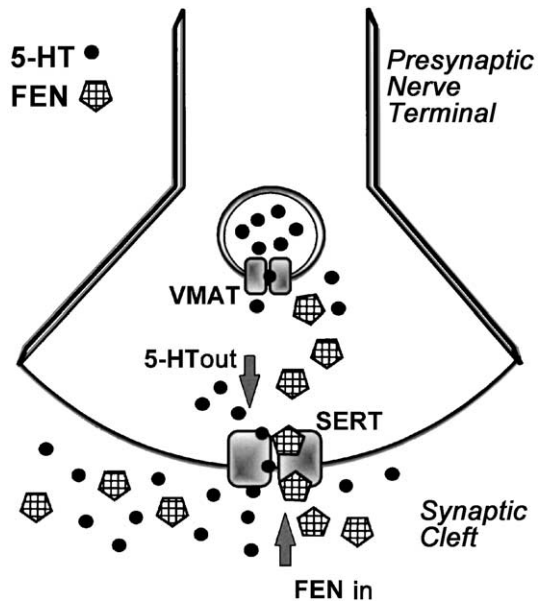


Fig. 1. Mechanism of action of fenfluramine (FEN), a substrate-type 5-HT releasing agent. FEN serves as a substrate for SERT proteins. SERTs, in turn, facilitate the translocation of drug molecules into the presynaptic nerve terminal in exchange for 5-HT molecules that flow out into the synaptic cleft. FEN is also a substrate for VMAT proteins. By disrupting the compartmentalization of 5-HT into vesicles, FEN increases the concentration of cytoplasmic 5-HT available for drug-induced release.

take inhibitors requires ongoing release of transmitters via exocytosis—a process that is dependent upon electrical depolarization and extracellular  $\text{Ca}^{2+}$ . Thus, the capability of reuptake inhibitors to increase synaptic transmitter levels is said to be impulse- and  $\text{Ca}^{2+}$ -dependent. Releasing agents, on the other hand, increase synaptic transmitter levels by a process that is largely independent of ongoing cell firing and exocytotic transmitter release. Cell membrane autoreceptors mediate negative feedback mechanisms that serve to dampen the ability of reuptake inhibitors to elevate synaptic transmitters (reviewed in Pineyro & Blier, 1999). Such negative feedback effects exist for 5-HT (Adell & Artigas, 1991; Rutter et al., 1995; Smith & Lakoski, 1997), DA (Hinerth et al., 2000), and NE (Mateo et al., 1998) neuron systems. While autoreceptor activation can completely abolish the ability of reuptake inhibitors to elevate synaptic transmitter levels, autoreceptor mechanisms have little or no effect on substrate-induced neurotransmitter release (Florin et al., 1994; Gardier et al., 1994; Gundlach et al., 1997; Kamal et al., 1981; Kuczenski et al., 1990). Because of autoreceptor-mediated feedback inhibition, reuptake inhibitors tend to produce small increases in extracellular neurotransmitters, whereas releasers tend to produce more robust increases (Gundlach et al., 1997; Scorza et al., 1999). The *in vivo* microdialysis data in Fig. 2 illustrate the modest and sustained elevation of extracellular 5-HT evoked by the 5-HT reuptake inhibitor fluoxetine compared with the much larger and transient effect of the 5-HT releaser (+)-

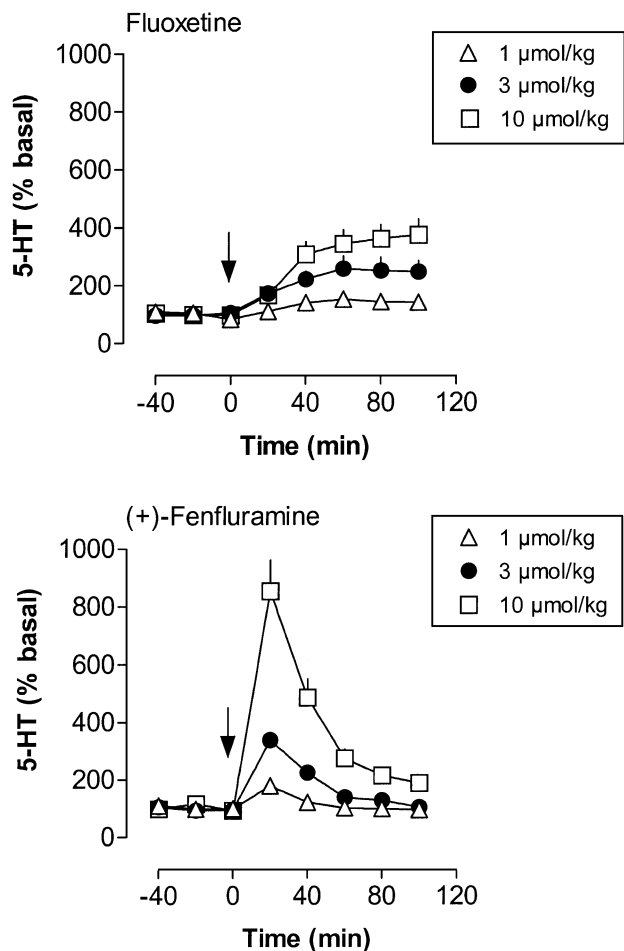


Fig. 2. Effects of fluoxetine (a 5-HT reuptake inhibitor) and (+)-fenfluramine (a 5-HT releaser) on extracellular 5-HT in the rat nucleus accumbens. Dialysis methods were carried out as described previously (Baumann et al., 2000). Drugs were administered i.v. at time 0 min. Data are expressed as a percentage of the mean of three basal dialysate samples collected prior to drug treatment. The basal dialysate 5-HT level was  $0.46 \pm 0.17$  nM. Values are mean  $\pm$  SEM for  $n = 5$  rats per group.

fenfluramine (Berger et al., 1992; Crespi et al., 1997; Gundlach et al., 1997).

A variety of 5-HT-selective reuptake inhibitors (SSRIs), such as fluoxetine, sertraline, and citalopram, are medications used for the treatment of psychiatric disorders, including depression, panic disorder, and obsessive-compulsive disorder (for reviews, see Gorman & Kent, 1999; Zohar & Westenberg, 2000). In contrast, there are far fewer 5-HT releasing agents that have been used in human patients. Because of the withdrawal of the appetite suppressants fenfluramine and (+)-fenfluramine from the market in September 1997 (Connolly & McGoon, 1999), there are currently no clinically available 5-HT releasing agents. A main goal of this review is to summarize the potential therapeutic uses and reported adverse effects of substrate-type 5-HT releasing agents, including the illicit drug 3,4-methylenedioxymethamphetamine (( $\pm$ )-MDMA or “ecstasy”) (Fitzgerald & Reid, 1990; Gudelsky & Nash, 1996; Johnson

et al., 1991a; Rothman et al., 2001). Furthermore, we hope this review will foster continued interest in the development of novel and selective 5-HT releasers that might be used as effective medications.

## 2. Neurochemical mechanisms of substrate-type 5-hydroxytryptamine releasing agents

Some of the earliest work with 5-HT releasers was done by Fuller and colleagues, who examined the pharmacology of substituted amphetamine derivatives such as *p*-chloroamphetamine (Fuller, 1976, 1978). These investigators showed that addition of halogen-containing groups to the phenyl ring of amphetamine generates compounds that potently release neuronal 5-HT rather than NE or DA. The toxicity of *p*-chloroamphetamine clearly precluded its clinical use (Fuller et al., 1975). ( $\pm$ )-Fenfluramine (Pondimin<sup>™</sup>) and the more potent stereoisomer (+)-fenfluramine (dexfenfluramine, Redux<sup>™</sup>) are also substituted amphetamines. These drugs were widely prescribed for the treatment of obesity until they were withdrawn from the market in September 1997 due to reports of cardiac valvulopathy (Connolly et al., 1997; Connolly & McGoon, 1999). ( $\pm$ )-Fenfluramine is composed of two stereoisomers, (+)- and (–)-fenfluramine, which are *N*-de-ethylated in the liver to form the metabolites (+)- and (–)-norfenfluramine (Caccia et al., 1985; Pinder et al., 1975). Fig. 3 shows the chemical structures of fenfluramine, norfenfluramine, a number of other 5-HT releasers, and structurally related agents. Both stereoisomers of fenfluramine and norfenfluramine are biologically active (Garattini et al., 1986), so systemic administration of ( $\pm$ )-fenfluramine actually delivers four active pharmacological agents in vivo. Most studies agree that fenfluramines and norfenfluramines are SERT substrates and potent 5-HT releasing agents (reviewed in Garattini, 1995; Garattini et al., 1986).

Other 5-HT releasers include the non-amphetamine piperazine derivatives *m*-chlorophenylpiperazine (mCPP) and *m*-trifluoromethylpiperazine (TFMPP) (Auerbach et al., 1990; Baumann et al., 1993; Eriksson et al., 1999; Pettibone & Williams, 1984). It is noteworthy and clinically significant that mCPP is a major metabolite of the antidepressant trazodone (Otani et al., 1998) and a minor metabolite of nefazodone (Barbhuiya et al., 1996). In vitro release data and in vivo microdialysis data support the hypothesis that ( $\pm$ )-fenfluramine, (+)-fenfluramine, mCPP, and TFMPP release neuronal 5-HT via a non-exocytotic diffusion-exchange mechanism involving SERT sites in the brain. For example, the 5-HT releasing capability of all of these agents is reversed by pretreatment with the SSRI fluoxetine confirming a role for SERTs in the mechanism of drug action.

Historically, it has been difficult to distinguish whether drugs act as reuptake inhibitors or substrate-type releasers by using simple test tube assays. To address this issue, we

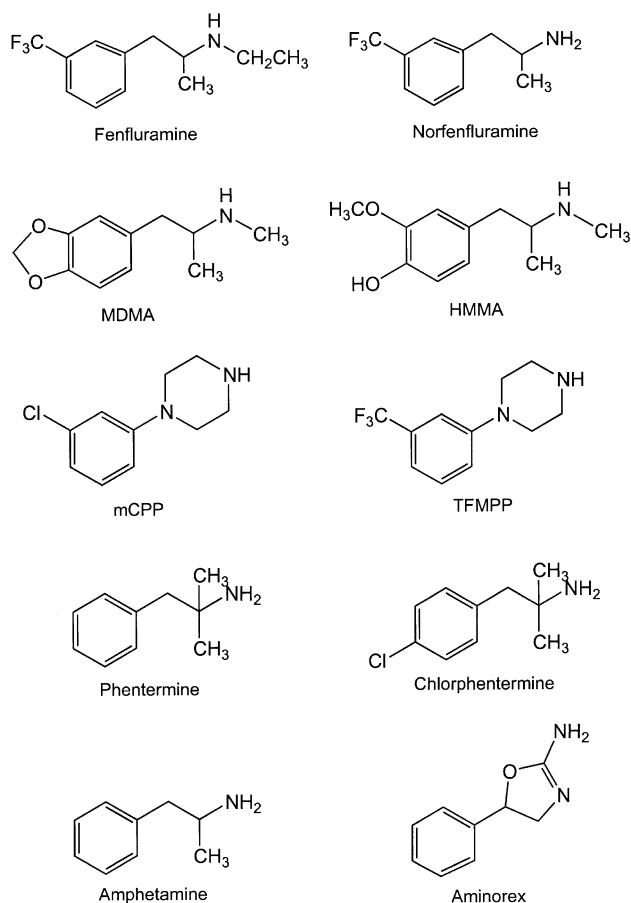


Fig. 3. Chemical structures of substrate-type 5-HT releasing agents and related drugs. HMMA, 4-hydroxy-3-methoxymethamphetamine.

recently developed a high-throughput *in vitro* method that discriminates between reuptake inhibitors and substrate-type releasers (Rothman et al., 2000b, 2001). Using this approach, it is possible to determine the ability of test drugs to release [ $^3$ H]NE, [ $^3$ H]DA, and [ $^3$ H]5-HT from rat brain synaptosomes under similar assay conditions. The data summarized in Table 1 show that a number of drugs are potent 5-HT releasers; most of these drugs are phenethylamine derivatives, structurally related to amphetamine (see Fig. 3 for representative structures). The appetite suppressant chlorphentermine is the most potent 5-HT releaser tested. Although this agent does not release NE, it blocks NE uptake with an  $IC_{50}$  of 450 nM (Rothman et al., 2001), indicating about a 10-fold selectivity for SERT over NET. mCPP and (+)-fenfluramine are potent and selective 5-HT releasers, comparable in potency to 5-HT itself—the endogenous substrate for SERT. In contrast to (+)-fenfluramine, mCPP does not release NE at any dose tested. (–)-Fenfluramine is ~3-fold weaker at 5-HT release than the (+)-isomer, and it does not release NE. (±)-MDMA is a potent releaser of both 5-HT and NE, with less potent stimulation of DA release. Methamphetamine, amphetamine, and phentermine are all relatively weak 5-HT releasers, especially when compared with their potency at NE and DA release.

(±)-MDMA, like (±)-fenfluramine, is a mixture of two stereoisomers, and systemic administration of (±)-MDMA gives rise to a number of potentially bioactive metabolites (de la Torre et al., 2000; Mas et al., 1999). In human subjects, the plasma levels of some (±)-MDMA metabolites such as 4-hydroxy-3-methoxymethamphetamine can equal or exceed plasma levels of (±)-MDMA itself (de la Torre et al., 2000). There is also evidence for preferential stereoselective conversion of (+)-MDMA to (+)-3,4-methylenedioxyamphetamine ((+)-MDA) via *N*-demethylation (Fitzgerald et al., 1989). As reported in Table 1, (±)-MDMA is a potent substrate of SERT and NET, and is ~10-fold weaker at DA transporters. Consistent with these data, fluoxetine attenuates (±)-MDMA-induced 5-HT release in rat nucleus accumbens, as measured by *in vivo* microdialysis sampling (Gudelsky, 1996). Interestingly, the NET inhibitor desipramine blocks (±)-MDMA-induced DA release in the rat hippocampus, suggesting that NE nerve terminals are a source of released DA in this brain region (Shankaran & Gudelsky, 1998). Currently, there is very little information regarding the pharmacological activity of (±)-MDMA metabolites, and future studies should assess the transporter and receptor activities of these metabolites. It is reasonable to assume that low doses of (±)-MDMA act to preferentially release 5-HT and NE. As the dose of (±)-MDMA is increased, the DA-releasing ability of the drug will be recruited as well. The collective findings clearly show that (±)-MDMA is not a selective SERT substrate. Attempting to make conclusions about the mechanism of (±)-MDMA action based on whole animal studies is

Table 1  
Effects of test drugs on release of [ $^3$ H]5-HT, [ $^3$ H]NE, and [ $^3$ H]DA from synaptosomes

Drug	EC <sub>50</sub> (nM ± SD)		
	5-HT release	NE release	DA release
Chlorphentermine	30.9 ± 5.4	> 10,000	2650 ± 273
mCPP <sup>1</sup>	38.1 ± 4.6	> 10,000	> 10,000
5-HT	44.4 ± 5.3	> 10,000	> 10,000
(+)-Fenfluramine	51.7 ± 6.1	302 ± 20	> 10,000
(±)-MDMA	56.6 ± 2.1	77.4 ± 3.4	376 ± 16
(±)-Fenfluramine	79.3 ± 9.5	739 ± 57	> 10,000
(–)-Fenfluramine <sup>1</sup>	147 ± 19	> 10,000	> 10,000
Aminorex	193 ± 23	26.4 ± 2.8	49.4 ± 7.5
(+)-Methamphetamine	736 ± 45	12.3 ± 0.7	24.5 ± 2.1
(+)-Amphetamine	1765 ± 94	7.07 ± 0.95	24.8 ± 3.5
Tyramine	2775 ± 234	40.6 ± 3.5	119 ± 11
Phentermine	3511 ± 253	39.4 ± 6.6	262 ± 21
(–)-Methamphetamine	4640 ± 243	28.5 ± 2.5	416 ± 20
(–)-Ephedrine	> 10,000	72.4 ± 10.2	1350 ± 124
NE	> 10,000	164 ± 13	869 ± 51
DA	> 10,000	66.2 ± 5.4	86.9 ± 9.7

Rat brain synaptosomes were preloaded with [ $^3$ H]neurotransmitter. Test drugs (1–10,000 nM) were incubated with preloaded synaptosomes, and [ $^3$ H]neurotransmitter release was determined according to published methods (Rothman et al., 2001). Each value is the mean ± SD of 3 experiments. Data from Rothman et al. (2001).

<sup>1</sup> Unpublished data.

complicated by the presence of stereoisomers, multiple metabolites with unknown biological activity, and the non-selective nature of MDMA itself.

The most selective 5-HT releasers available, fenfluramines and mCPP, also have direct agonist actions at 5-HT receptor subtypes. When administered systemically, ( $\pm$ )-fenfluramine and (+)-fenfluramine are rapidly metabolized to ( $\pm$ )-norfenfluramine and (+)-norfenfluramine, respectively (Campbell et al., 1988). These pharmacologically active metabolites display long biological half-lives (Caccia et al., 1985). In addition to releasing 5-HT, fenfluramines and their metabolites have direct agonist actions at multiple 5-HT<sub>2</sub> receptor subtypes (Fitzgerald et al., 2000; Rothman et al., 2000a). The direct activation of 5-HT<sub>2C</sub> receptors by fenfluramine is thought to contribute to the anorectic effect of the drug in rats (Curzon et al., 1997; Dourish, 1995; Vickers et al., 1999). The data summarized in Table 2 show that both (+)-norfenfluramine and (–)-norfenfluramine are potent and highly efficacious 5-HT<sub>2C</sub> receptor agonists ( $K_{act} < 20$  nM). (+)-Fenfluramine and (–)-fenfluramine are  $\sim 20$ -fold less potent at activating human 5-HT<sub>2C</sub> receptor sites. It seems feasible, therefore, that the reported 5-HT<sub>2C</sub> receptor actions of systemically administered fenfluramine may be mediated in part by the metabolite norfenfluramine. (+)-Norfenfluramine is also a very potent 5-HT<sub>2B</sub> agonist, which may relate to the valvular heart disease (VHD) side effect observed in some patients who have taken fenfluramine as an appetite suppressant (Rothman et al., 2000a).

mCPP has been widely touted as a selective 5-HT<sub>2C</sub> agonist, but the drug exhibits direct agonist activity at multiple 5-HT receptors, including 5-HT<sub>1A,1B,1D</sub>, 5-HT<sub>2A,2B,2C</sub>, and 5-HT<sub>3</sub> sites (Hoyer et al., 1994; Owens et al., 1997; Porter et al., 1999; Schoeffter & Hoyer, 1989).

It is noteworthy that the EC<sub>50</sub> of mCPP for releasing [<sup>3</sup>H]5-HT in vitro (38 nM; see Table 1) is similar to the binding K<sub>i</sub> of the drug at human 5-HT<sub>2C</sub> receptors (59 nM) (Rothman et al., 2000a). Moreover, these potency estimates are close to the EC<sub>50</sub> of mCPP for activating 5-HT<sub>2C</sub> receptors in rat brain (Fiorella et al., 1995) and in Chinese hamster ovary-K1 cells expressing the cloned human 5-HT<sub>2C</sub> receptor (Porter et al., 1999). Anorectic doses of mCPP release 5-HT in vivo in rats (Baumann et al., 2001; De Vry et al., 2000), suggesting mCPP activates 5-HT<sub>2C</sub> receptors and releases neuronal 5-HT with similar potency. The data in Table 2 confirm that mCPP is a very potent and efficacious agonist at human 5-HT<sub>2C</sub> receptors, with considerably less potent actions at 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> sites. The more potent actions of mCPP at 5-HT<sub>2C</sub> receptors observed in our experiments ( $K_{act} = 0.6$  nM) (Rothman et al., 2000a), when compared with the work of Porter et al. (1999), likely arises from different expression levels of the 5-HT<sub>2C</sub> receptor between the studies. Thus, mCPP is a SERT substrate that displays substantial 5-HT<sub>2</sub> receptor agonist actions.

One approach for discriminating between drug-induced presynaptic (i.e., 5-HT release) versus postsynaptic (i.e., 5-HT receptor agonism) serotonergic actions is to pretreat with SSRIs such as fluoxetine (Berger et al., 1992). Because fluoxetine will selectively antagonize SERT-mediated phenomena, fluoxetine-reversibility can be used as a criterion to identify effects of fenfluramine that involve presynaptic mechanisms (Baumann et al., 1998; Gundlach et al., 1997). Using this paradigm in human subjects, Pedrinola et al. (1996) showed that (+)-fenfluramine promotes weight loss in patients who receive concurrent fluoxetine treatment. Thus, in both animals and humans, it appears that fenfluramine anorexia is mediated, at least in part, by postsynaptic actions of fenfluramine or its principal metabolite.

Table 2  
Functional activity of test drugs at 5-HT<sub>2</sub> receptor subtypes

Drug	$K_{act}$ (nM $\pm$ SEM) [ $V_{max}$ (% 5-HT $\pm$ SEM)]		
	Human 5-HT <sub>2A</sub>	Human 5-HT <sub>2B</sub>	Human 5-HT <sub>2C</sub>
(+)-Fenfluramine	> 10,000	379 $\pm$ 70 [38 $\pm$ 8.2]	362 $\pm$ 64 [80 $\pm$ 5.9]
(–)-Fenfluramine	5279 $\pm$ 587 [43 $\pm$ 4.2]	1248 $\pm$ 252 [47 $\pm$ 2.9]	360 $\pm$ 91 [84 $\pm$ 7.4]
(+)-Norfenfluramine	630 $\pm$ 141 [88 $\pm$ 5.3]	18.4 $\pm$ 5.3 [73 $\pm$ 3.5]	13 $\pm$ 2.4 [100 $\pm$ 6.5]
(–)-Norfenfluramine	1565 $\pm$ 190 [93 $\pm$ 5.3]	357 $\pm$ 105 [71 $\pm$ 8.8]	18 $\pm$ 5.3 [80 $\pm$ 10]
mCPP	65 $\pm$ 10 [55 $\pm$ 6.5]	64 $\pm$ 15 [43 $\pm$ 8.2]	0.64 $\pm$ 0.17 [79 $\pm$ 8.8]
5-HT	66 $\pm$ 15 [100]	2.4 $\pm$ 0.9 [100]	0.6 $\pm$ 0.1 [100]

Phosphoinositide hydrolysis assays were performed in stably (5-HT<sub>2A</sub> and 5-HT<sub>2C</sub>) or transiently (5-HT<sub>2B</sub>) expressed receptors. [<sup>3</sup>H]inositol phosphate accumulation was determined as previously described (Rothman et al., 2000a). Each value is the mean  $\pm$  SEM of three experiments. Data from Rothman et al. (2000a).

### 3. Therapeutic applications of 5-hydroxytryptamine releasers

( $\pm$ )-Fenfluramine and (+)-fenfluramine are the only 5-HT releasers ever approved for use in humans. mCPP has been used clinically as an investigational drug and ( $\pm$ )-MDMA, although subjected to limited clinical studies in humans (Mas et al., 1999), is a popular drug of abuse. While the fenfluramines and mCPP potently release 5-HT, these drugs are “promiscuous” ligands, and their direct activation of postsynaptic 5-HT receptors undoubtedly contributes to their pharmacological activity in vivo. Our inferences concerning potential therapeutic uses of 5-HT releasing agents necessarily derive from studies of fenfluramine and mCPP. Until such time as these inferences can be tested with truly selective 5-HT releasers, any hypothesis developed on the basis of studies with fenfluramine and mCPP must be considered somewhat speculative.

### 3.1. Established therapeutic indications

Numerous double-blind, placebo-controlled studies have established ( $\pm$ )-fenfluramine and (+)-fenfluramine as effective weight-loss agents, and this topic will not be discussed further here (for reviews, see Davis & Faulds, 1996; McTavish & Heel, 1992; Pinder et al., 1975). Other studies show that ( $\pm$ )-fenfluramine and (+)-fenfluramine promote weight loss and directly improve insulin sensitivity and diabetic control in Type 2 diabetes (Scheen & Lefebvre, 2000; Willey et al., 1992, 1994). Interestingly, (+)-fenfluramine decreases sympathetic nervous system activity, plasma NE levels, plasma renin activity, and blood pressure in humans (Andersson et al., 1991; Flechtner-Mors et al., 1998; Hirsch et al., 2000; Kolanowski et al., 1992). Similar effects occur upon administration of  $\alpha_2$ -adrenergic agonists (Oates et al., 1978; Schoeppe & Brecht, 1980). It is tempting to speculate that central NE release evoked by (+)-fenfluramine or its metabolite might contribute to these effects (see Table 1). Further studies are needed to clarify the possible utility of 5-HT releasers in cardiovascular medicine.

### 3.2. Potential therapeutic indications

Acute administration of 5-HT releasing agents, such as fenfluramine, mCPP, and TFMPP, decreases ethanol intake by rats (Buczek et al., 1994; Lu et al., 1993; Wilson et al., 1998). The ability of fenfluramine to decrease alcohol intake is enhanced by concurrent administration of amphetamine (Mirovsky et al., 1995; Yu et al., 1997) or phentermine (Halladay et al., 1999, 2000). The amphetamine/( $\pm$ )-fenfluramine or phentermine/( $\pm$ )-fenfluramine combination eliminates alcohol withdrawal seizures as well (Halladay et al., 2000; Mirovsky et al., 1995). Consistent with these preclinical findings, case reports suggest that phentermine plus ( $\pm$ )-fenfluramine decreases alcohol intake in humans (Hitzig, 1994; Rothman, 1995). These findings await confirmation with controlled trials using co-administration of clinically approved releasing agents.

A growing body of literature suggests that 5-HT releasing agents may be helpful in treating substance use disorders in general. In rats, ( $\pm$ )-fenfluramine decreases the self-administration of methamphetamine (Munzar et al., 1999), while (+)-fenfluramine suppresses heroin intake (Higgins et al., 1994; Wang et al., 1995). A number of studies indicate that fenfluramine could be used, along with phentermine, in the treatment of cocaine dependence (for a review, see Rothman & Baumann, 2000). In humans, controlled studies show that mCPP and fenfluramine decrease cocaine craving (Buydens-Branchey et al., 1997, 1998). Furthermore, it has been reported that neuroendocrine responses to ( $\pm$ )-fenfluramine are blunted in human cocaine addicts admitted to inpatient research settings, suggesting 5-HT dysfunction during cocaine withdrawal (Baumann, 1997; Haney et al., 2001). At this point in time, no clinical trials have examined

the effectiveness of 5-HT releasers as adjuncts in the treatment of stimulant or opioid dependence.

The widespread therapeutic application of SSRIs in treating depression and anxiety disorders suggests that 5-HT releasers might also be of therapeutic benefit for these types of illnesses. Some preclinical evidence supports this hypothesis (Fuller, 1987; Marona-Lewicka & Nichols, 1997; Scorza et al., 1999). Moreover, several small-scale clinical studies also support this notion. Rickels et al. (1976) reported, for example, that ( $\pm$ )-fenfluramine reduced the emotional symptoms in obese patients, while Ward et al. (1985) suggested that acute administration of (+)-fenfluramine to depressed patients produced antidepressant-like effects. Similarly, a small double-blind, placebo-controlled crossover clinical trial (18 patients) indicated that (+)-fenfluramine effectively treated seasonal affective disorder (O'Rourke et al., 1989). Blouin et al. (1988) conducted a small (22 patients) double-blind, placebo-controlled crossover clinical trial comparing desipramine and ( $\pm$ )-fenfluramine in the treatment of bulimia. The results indicated that ( $\pm$ )-fenfluramine had a beneficial effect. An earlier study reported that acute administration of ( $\pm$ )-fenfluramine reduced bulimic symptoms (Robinson et al., 1985). Another small placebo-controlled study showed that treatment with (+)-fenfluramine helped reduce the symptoms of premenstrual depression and the premenstrual rise in calorie, carbohydrate, and fat intake (Brzezinski et al., 1990).

Some studies report negative findings, however. In a small pilot study, Price et al. (1990) reported that fenfluramine lacked antidepressant effects in patients refractory to, and concurrently treated with, desipramine. Donnelly et al. (1989) reported that ( $\pm$ )-fenfluramine lacked efficacy in the treatment of attention deficit disorder. As noted in Section 1, 5-HT releasers differ from SSRIs in a number of respects. For example, due to the existence of negative-feedback mechanisms, 5-HT releasers can increase synaptic 5-HT levels more rapidly and to a greater extent when compared with the effects of SSRIs. Whether or not the unique neurochemical profile of 5-HT releasers would impact antidepressant efficacy can only be established by further clinical investigations.

( $\pm$ )-MDMA is a popular drug of abuse that elicits positive subjective effects, such as feelings of emotional closeness, warmth, and empathy. These behavioral effects of ( $\pm$ )-MDMA led some investigators to propose its adjunctive use in psychotherapy (for a review, see Vollenweider et al., 1998), a use contraindicated by the possibility of serious adverse effects. Nonetheless, if the precise neurochemical mechanisms underlying these "positive" MDMA effects could be determined, then it might be possible to develop new medications with potential psychiatric applications without significant MDMA-like adverse effects. At the present time, there is no simple neurochemical explanation for the unique profile of subjective effects produced by ( $\pm$ )-MDMA (Bankson & Cunningham, 2001), although the ability of MDMA to activate 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors

may contribute to its subjective effects in humans (Nash et al., 1994). Taken together, these above-mentioned considerations suggest that additional investigations should be undertaken to determine the efficacy of 5-HT releasers in the treatment of a variety of psychiatric disorders.

#### 4. Adverse effects of 5-hydroxytryptamine releasing agents

Both ( $\pm$ )-fenfluramine and (+)-fenfluramine produce mild and reversible side effects in some patients (Hanotin et al., 1998; Weintraub & Bray, 1989; Weintraub et al., 1984). Of greater concern to the risk-benefit ratio of these medications is the increased risk of developing serious side effects, such as primary pulmonary hypertension (PPH), VHD, and perhaps neurotoxicity. In fact, an increase in the incidence of VHD in patients treated with ( $\pm$ )-fenfluramine and (+)-fenfluramine prompted the removal of these drugs from the market. The major adverse effects of these medications will be considered, with particular emphasis on possible underlying mechanisms. Adverse effects of ( $\pm$ )-MDMA have been reviewed recently (Burgess et al., 2000), and will not be discussed in detail here.

##### 4.1. Primary pulmonary hypertension

PPH is a rare and often fatal disease of unknown etiology (Rubin, 1997). Epidemiological data clearly show that fenfluramines and aminorex increase the risk of developing PPH (Abenhaim et al., 1996; Gurtner, 1990). Abenhaim et al. (1996) estimated that taking fenfluramines (either ( $\pm$ )-fenfluramine or (+)-fenfluramine) for more than 3 months increased the risk of developing PPH by 23-fold. Results from a more recent study (Rich et al., 2000) conducted in the United States demonstrate that a history of ( $\pm$ )-fenfluramine or (+)-fenfluramine exposure, but not phentermine exposure, increases the risk of PPH by  $\sim$ 7-fold. Given that PPH is usually an exceedingly rare disorder with an annual incidence of 1–2 cases per million, several large studies will be needed to accurately determine the risk posed by fenfluramines. It is noteworthy that Rich et al. (2000) found no link between phentermine and PPH. With the exception of a few isolated case reports (Backmann et al., 1972; Schnabel et al., 1976), there is currently no systematic scientific evidence for an increased risk of PPH in patients receiving phentermine alone.

Recent genetic studies promise new insights into the pathogenesis of PPH. Mutations in a gene encoding the bone morphogenetic protein Type II receptor accounts for familial forms of PPH and for up to 26% of sporadic cases of PPH (Machado et al., 2001; Thomson et al., 2000). At the present time, it is not known if appetite suppressants such as fenfluramine directly interact with bone morphogenetic protein Type II receptor or similar receptor proteins.

Eddahibi et al. (2001) reported that patients with PPH overexpress SERT proteins in their platelets and pulmonary smooth muscle cells. Additionally, 5-HT-induced proliferation of pulmonary smooth muscle cells occurs at a faster rate in PPH patients when compared with control patients. Since increased expression of SERT would enhance the entry of 5-HT into pulmonary smooth muscle cells, Eddahibi et al. (2001) suggested that increased SERT expression may predispose patients to develop PPH. The finding that PPH patients more frequently carry the LL variant of the SERT gene, which produces 2–3 times higher levels of SERT (Heils et al., 1997), supports this hypothesis. The applicability of this hypothesis to fenfluramine-associated PPH is not clear, since such patients carry the LL variant at the same frequency as controls (Rabinovitch, 2001).

The mechanism by which fenfluramines might increase the risk of developing PPH is not understood. Investigators have hypothesized that these drugs elevate plasma 5-HT by releasing 5-HT normally stored in platelets (MacLean, 1999). According to the “5-HT hypothesis of PPH,” elevations in circulating 5-HT cause chronic increases in pulmonary blood pressure and growth of arterial smooth muscle, thereby producing PPH in susceptible individuals (Fishman, 1999; Herve et al., 1995). A major shortcoming of the 5-HT hypothesis is that there is little evidence to support it. In fact, substantial data show that administration of ( $\pm$ )-fenfluramine or (+)-fenfluramine in animals and humans actually *lowers* blood levels of 5-HT and *does not increase* plasma levels of 5-HT (Celada et al., 1994; Martin & Artigas, 1992; Raleigh et al., 1986; Redmon et al., 1997; Sherman et al., 1989; Stubbs et al., 1986). Despite the overwhelming evidence that fenfluramines do not increase plasma 5-HT, the 5-HT hypothesis continues to gain wide acceptance (Fishman, 1999; MacLean, 1999; Stahl, 1997). These considerations prompted Rothman et al. (2000c) to measure plasma 5-HT levels in patients who had taken phentermine alone or in combination with ( $\pm$ )-fenfluramine. The results illustrated in Fig. 4 show that treatment with phentermine/( $\pm$ )-fenfluramine lowers plasma 5-HT, whereas treatment with phentermine alone has no significant effect. The collective data indicate that mechanisms other than increased plasma 5-HT should be considered to explain how certain anorectic medications increase the risk for development of PPH.

In our laboratory, we recently tested the hypothesis that the fenfluramines and other appetite suppressants might increase the risk of PPH via interactions with SERT sites in the lung (Rothman et al., 1999). It is well established that SERT proteins expressed in brain and lung tissue are identical (Chang et al., 1996; Ramamoorthy et al., 1993). In addition, the 5-HT transport mechanism in the brain and lungs is similar (James & Bryan-Lluka, 1997; Paczkowski et al., 1996). In order to determine the SERT substrate activity of various anorectic medications, we examined the effects of these drugs on [ $^3$ H]5-HT release from synaptosomes in vitro and 5-HT efflux from rat brain in vivo. The

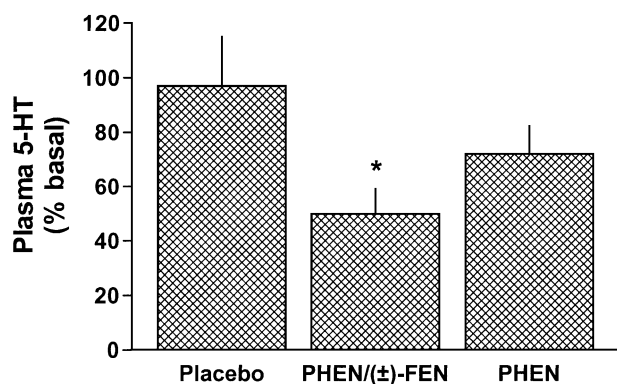


Fig. 4. Effects of placebo, phentermine/(±)-fenfluramine (PHEN/(±)-FEN), and phentermine (PHEN) treatment on plasma 5-HT levels in human subjects. As reported elsewhere (Rothman et al., 2000c), 44 patients with Type 2 diabetes enrolled in a randomized double-blind, placebo-controlled clinical trial to determine the effect of the PHEN/(±)-FEN combination [PHEN (37.5 mg p.o. per day) plus (±)-FEN [20 mg p.o. t.i.d.]] on the disease process (Redmon et al., 1999). Of the 44 patients enrolled, 37 (16 placebo-treated and 21 drug-treated) patients had both a baseline and a 2-month plasma sample available for analysis. The remaining 7 patients were treated with PHEN alone [37.5 mg p.o. per day] after a 1-year treatment with placebo. Using a within-subjects analysis, the plasma 5-HT at 2 months was divided by the plasma 5-HT at baseline and multiplied by 100 for each subject. Only the PHEN/(±)-FEN group showed a significant decrease in plasma 5-HT. There was a nonsignificant trend towards a decrease in plasma 5-HT in the PHEN group. \*,  $P < 0.05$  when compared with placebo.

data in Table 1 show that drugs known or suspected to increase the risk of PPH (i.e., (±)-fenfluramine, (+)-fenfluramine, aminorex, chlorphentermine) are potent SERT substrates, whereas drugs not associated with PPH (i.e., amphetamine, phentermine) are less potent in this regard. The intracranial microdialysis data depicted in Fig. 5 confirm that all of the drugs associated with PPH are powerful 5-HT releasers in vivo. These findings led us to propose the “gateway hypothesis of PPH.” According to this hypothesis, anorectic medications that are SERT substrates get translocated into pulmonary cells, where PPH develops as a response to high levels of these drugs or their metabolites. SERT sites play a crucial role in the process since these proteins are required for the accumulation of drug molecules inside of cells. The development of PPH would depend upon the degree of drug retention, the intrinsic toxicity of the drug, and individual variations in susceptibility.

Hyperplasia of pulmonary artery smooth muscle is a hallmark pathological feature of PPH (Rubin, 1997). The gateway hypothesis does not clarify how SERT substrate activity might be involved in causing PPH. One possibility is that the accumulation of medications in arterial smooth muscle cells could trigger mitogenesis via inhibition of  $K^+$  channels (Belohlavkova et al., 2001; Weir et al., 1996). This effect requires drug concentrations at least 10-fold greater than the drug concentrations expected after therapeutic doses of fenfluramines. Thus, the role of SERT sites could be to translocate drug molecules into pulmonary cells, providing a mechanism to concentrate drugs to a level

where  $K^+$ -channel blockade might occur. Alternatively, since SERT-mediated uptake of 5-HT causes proliferation of pulmonary smooth muscle cells (Eddahibi et al., 2001), it is possible that certain anorectics activate intracellular 5-HT receptors that mediate proliferation.

It appears that being a SERT substrate may be a necessary, but not sufficient, criterion to increase the risk of PPH. For example, potent SERT substrates such as mCPP (Baumann et al., 1993, 2001) are not associated with PPH. Moreover, this drug binds to SERT sites in the human brain with a greater potency than the prototypical 5-HT releaser (±)-fenfluramine (Baumann et al., 1995). As reported in Table 1, mCPP releases 5-HT in vitro more potently than (+)-fenfluramine. Neither trazodone nor mCPP has been associated with PPH. Thus, mCPP represents a SERT substrate that does not increase the risk of PPH. Additionally, despite its widespread use as a recreational drug, the SERT substrate (±)-MDMA (see Table 1) has not been reported to cause PPH.

Anorectic drugs vary considerably in their propensity to increase the risk of PPH. For instance, aminorex causes PPH in 2 of every 100 patients (Gurtner, 1985). Fenfluramines, on the other hand, are estimated to cause PPH in 7 of every 1,000,000 patients (Rich et al., 2000). It is possible that the amphetamine-like chemical structure of aminorex, (±)-fenfluramine, and (+)-fenfluramine activates a pharmacological mechanism that ultimately increases the risk of PPH. In this regard, mCPP has a non-amphetamine structure, is not known to increase the risk of PPH, and probably does not activate the toxic pharmacological mechanism. Thus, it seems feasible that SERT substrates with non-amphetamine chemical structures may not increase the risk of PPH.

#### 4.2. Valvular heart disease

The history of fenfluramine-associated VHD has been reviewed recently (Connolly & McGoan, 1999). Current findings indicate that severe VHD in phentermine/(±)-fenfluramine users, as initially reported by Connolly et al. (1997), is a rare occurrence (Jick et al., 1998). Some studies report no statistically significant increase in the prevalence of the United States Food and Drug Administration-defined VHD in patients treated with anorectic medications (Burger et al., 2001; Weissman et al., 1998), while other studies report that ~12% of patients treated with (±)-fenfluramine or (+)-fenfluramine develop mild asymptomatic VHD (Gardin et al., 2000; Weissman, 2001). There has been much speculation as to the mechanism of fenfluramine-associated VHD (Fishman, 1999; Michelakis & Weir, 2001). The lack of any VHD cases associated with the use of phentermine alone, along with the fact that VHD occurs in users of phentermine/(±)-fenfluramine and (+)-fenfluramine, strongly implicates fenfluramines as the likely causative agents of VHD.

Perhaps because (±)-fenfluramine and (+)-fenfluramine increase synaptic levels of 5-HT (Rothman et al., 1999; Baumann et al., 2000), investigators have proposed that

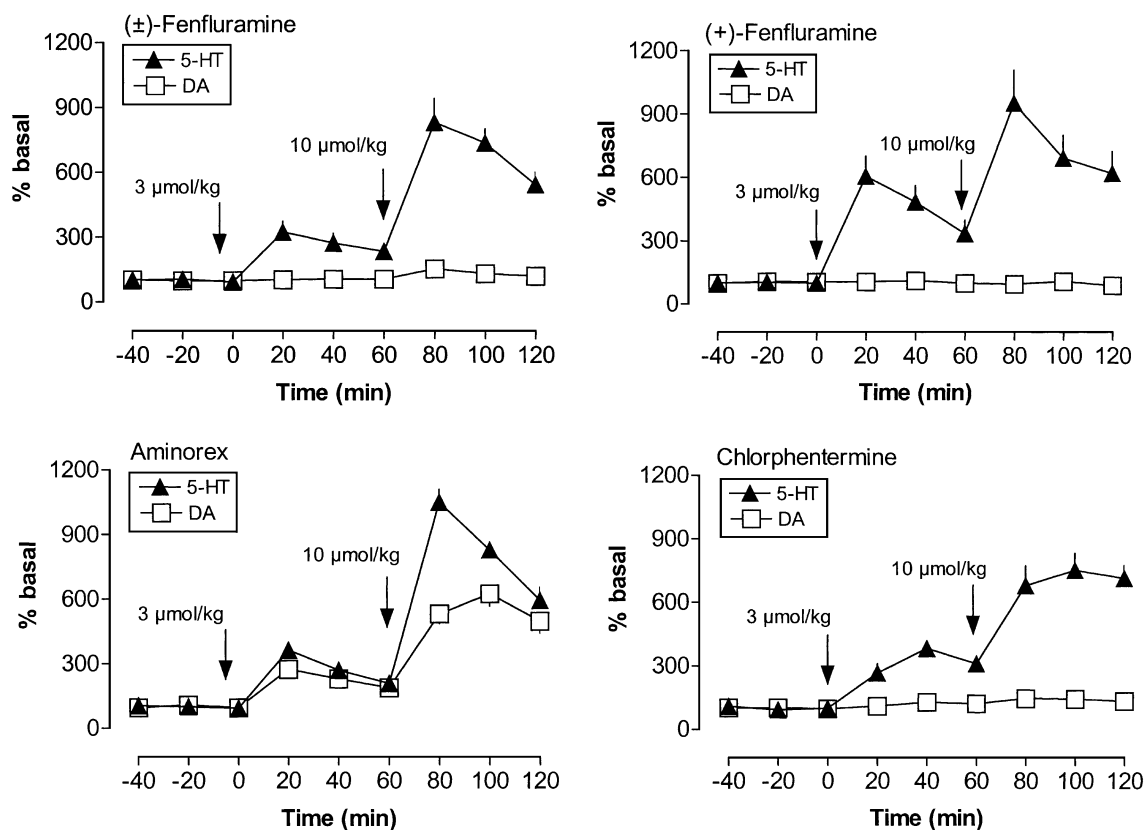


Fig. 5. Effects of (±)-fenfluramine, (+)-fenfluramine, aminorex, and chlorphentermine on extracellular 5-HT and DA in the rat nucleus accumbens. Dialysis methods were carried out as described previously (Baumann et al., 2000). Drugs were administered at time 0 min (3 μmol/kg) and 60 min (10 μmol/kg). Data are expressed as a percentage of the mean of three baseline dialysate samples collected prior to drug treatment. Baseline dialysate 5-HT and DA levels were  $0.53 \pm 0.11$  and  $1.96 \pm 0.34$  nM, respectively. Values are mean  $\pm$  SEM for  $n = 5$  rats per group. Data from Rothman et al. (1999).

anorectic medications produce VHD via elevations in circulating 5-HT (for a review, see Fishman, 1999). However, systemic administration of fenfluramines does not increase blood 5-HT levels in animals or humans, and phentermine/(±)-fenfluramine treatment lowers plasma 5-HT in human patients (Rothman et al., 2000c). Therefore, some explanation other than drug-induced elevations in plasma 5-HT must be put forth to explain how fenfluramines could cause VHD.

The principal pathological feature of fenfluramine-associated VHD is stimulated growth of fibroblasts located on heart valves (Connolly & McGoon, 1999), a process termed mitogenesis. One possible mechanism to explain drug-induced VHD is that fenfluramines or their major metabolites may directly activate a mitogenic 5-HT receptor. According to this proposal, any drug known to produce VHD similar to that produced by (±)-fenfluramine and (+)-fenfluramine (i.e., methysergide and ergotamine) would be expected to have agonist activity at the putative mitogenic 5-HT receptor. Two recent studies have reported that (+)-norfenfluramine, methylergonovine (the major metabolite of methysergide), and ergotamine are potent and efficacious agonists at the 5-HT<sub>2B</sub> receptor subtype (Fitzgerald et al., 2000; Rothman et al., 2000a). Importantly, medications that are not associated with increased risk of VHD (phentermine,

fluoxetine, and its major metabolite, norfluoxetine) lack agonist activity at the 5-HT<sub>2B</sub> receptor (Rothman et al., 2000a). Given that 5-HT<sub>2B</sub> receptors are expressed on heart valves (Fitzgerald et al., 2000), it seems possible that 5-HT<sub>2B</sub> receptor activation is involved in the etiology of fenfluramine-associated VHD. Consistent with this notion, serotonergic medications that are devoid of agonist activity at 5-HT<sub>2B</sub> receptors should not produce VHD. Further evidence is needed to definitively establish a link between 5-HT<sub>2B</sub> receptors and VHD.

#### 4.3. Serotonergic neurotoxicity

It is well established that administration of high-dose (±)-fenfluramine, (+)-fenfluramine, (±)-norfenfluramine, (+)-norfenfluramine, or (±)-MDMA causes long-term depletion of forebrain 5-HT in laboratory animals (Battaglia et al., 1987; Cozzi et al., 1998; Johnson & Nichols, 1990; Kleven & Seiden, 1989; McCann et al., 1997b; Sprague et al., 1998). The fenfluramine- and (±)-MDMA-induced loss of brain 5-HT is accompanied by parallel reductions in presynaptic 5-HT markers, such as tryptophan hydroxylase and SERTs. We have shown that 5-HT depletion produced by (±)-fenfluramine in rats is associated with adverse

functional consequences *in vivo* (Baumann et al., 1998). The collective findings have led some investigators to conclude that fenfluramines and ( $\pm$ )-MDMA are neurotoxic and that they produce lesions in central 5-HT nerve terminals (McCann et al., 1997a; Molliver et al., 1990). Depending on experimental conditions, the reductions in 5-HT markers can return to normal levels with time (Battaglia et al., 1988; Molliver et al., 1990; Sotelo, 1991). Whether or not such fenfluramine-induced deficits in 5-HT systems are indicative of “true” neurotoxicity is still debated (Baumann & Rothman, 1998; O’Callaghan & Miller, 1994).

An important question is whether or not therapeutic doses of fenfluramine deplete 5-HT in the human brain (McCann et al., 1997a). There is also concern that ( $\pm$ )-MDMA use leads to serotonergic neurotoxicity in humans (for reviews, see McCann et al., 2000; Scheffel et al., 1998). More research will be needed to clarify this important clinical issue. Preclinical studies indicate that fenfluramines and norfenfluramines must achieve brain concentrations of  $\sim 50 \mu\text{M}$  in order to produce neurotoxicity (Mennini et al., 1996; Zaczek et al., 1990), and this relationship is conserved across species. In an attempt to determine if clinical doses of ( $\pm$ )-fenfluramine or (+)-fenfluramine might lead to neurotoxic levels of the drug in human patients, some investigators used methods of “interspecies scaling” to extrapolate drug doses in animals to equivalent doses in humans (McCann et al., 1997a). The scaling approach is based on the assumption that there are physiological and biochemical similarities between diverse animal species (Mahmood, 1999). For example, at the cellular level, all eukaryotes metabolize simple sugars via the same aerobic mechanisms. Unfortunately, scaling methods cannot account for unique species-specific differences in pharmacokinetic parameters such as brain-to-plasma ratios of fenfluramine and its metabolites. In brief, interspecies scaling is an indirect method that is subject to numerous and often erroneous assumptions.

A more direct way of measuring fenfluramine concentrations in the brain is to use magnetic resonance spectroscopy. This method actually measures fluorine atoms that are present in the chemical structure of fenfluramine and its metabolites. Christensen et al. (1999) used this method to measure brain levels of (+)-fenfluramine and (+)-norfenfluramine in 12 obese women who were taking (+)-fenfluramine (15 mg p.o. b.i.d.) for weight loss. These investigators demonstrated that patients achieve stable (+)-fenfluramine plus (+)-norfenfluramine levels of  $\sim 4 \mu\text{M}$  in the brain. Thus, using a direct and validated measurement method (Christensen et al., 1998), these investigators have shown that humans taking the recommended dose of (+)-fenfluramine achieve drug concentrations in the brain 10 times lower than those needed to produce neurotoxicity in animals.

The mechanism underlying fenfluramine-induced depletion of brain 5-HT in animals is not known. Some investigators have speculated that acute 5-HT release is somehow involved in the long-term 5-HT depletion caused by

amphetamine-type drugs (Berger et al., 1992; Seiden & Sabol, 1996). There is evidence, for instance, that drug-induced elevations in extracellular 5-HT can lead to the formation of toxic 5-HT metabolites that cause cellular damage (Wrona & Dryhurst, 1998). On the other hand, a number of potent 5-HT releasing agents have been identified that are devoid of neurotoxic properties. Nichols and co-workers (Johnson et al., 1991b; Nichols et al., 1990) have synthesized several “non-neurotoxic” analogs of MDA and MDMA. These analogs are substrate-type 5-HT releasers *in vitro*, yet they do not deplete forebrain 5-HT *in vivo*.

Compelling evidence indicates that mCPP and (+)-fenfluramine release 5-HT from neurons by an analogous SERT-

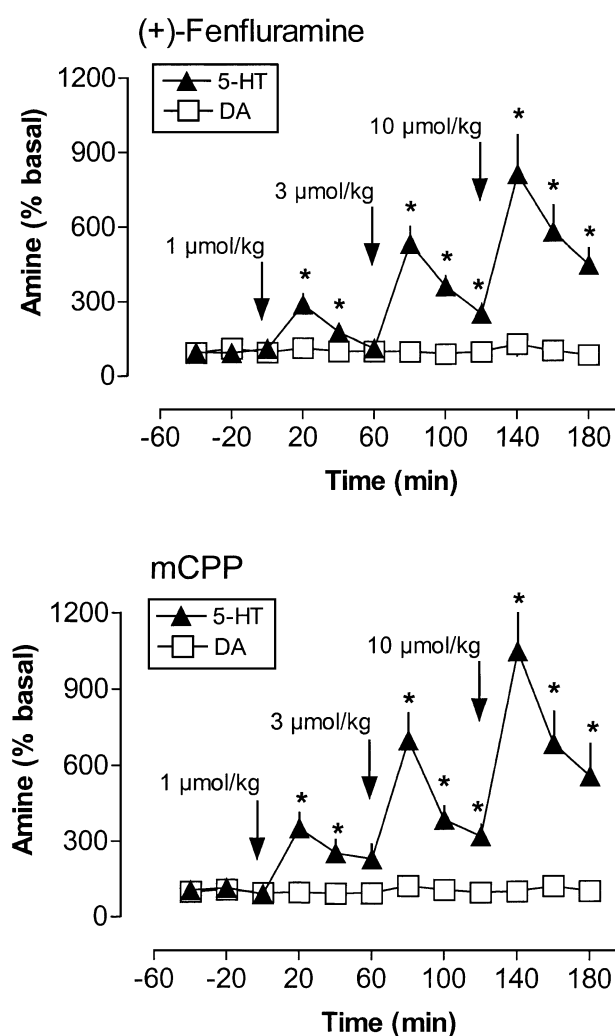


Fig. 6. Effects of i.v. injection of (+)-fenfluramine or mCPP on extracellular 5-HT and DA in the rat nucleus accumbens. Drugs were injected via jugular catheter on an ascending dose schedule, with 1, 3, and 10  $\mu\text{mol/kg}$  administered at time 0, 60, and 120 min, respectively. Data are expressed as a percentage of the mean of three baseline dialysate samples collected prior to treatment. Baseline values for 5-HT and DA were  $0.37 \pm 0.05$  and  $2.43 \pm 0.39 \text{ nM}$ , respectively. Values are mean  $\pm$  SEM for  $n = 5$  rats per group. \*,  $P < 0.05$  with respect to preinjection control. Data from Baumann et al. (2001).

dependent mechanism (Baumann et al., 1993, 2001; Eriksson et al., 1999). The *in vivo* microdialysis data depicted in Fig. 6 illustrate that mCPP and (+)-fenfluramine are essentially equipotent in their ability to elevate extracellular 5-HT levels in rat nucleus accumbens. Additionally, the transmitter-releasing action elicited by these agents is selective for 5-HT, since DA levels are not affected. Our *in vivo* data agree completely with the *in vitro* findings shown in Table 1. More importantly, as shown in Fig. 7, repeated high-dose administration of (+)-fenfluramine causes long-term depletion of brain tissue levels of 5-HT, whereas identical doses of mCPP do not have this effect (Baumann et al., 1994, 2001). Taken together, the findings suggest that drug-induced 5-HT release is not necessarily coupled to long-term 5-HT depletion. Since fenfluramine causes hypothermia (Cryan et al., 2000) and mCPP causes hyperthermia (Quested et al., 1997), it appears that changes in body temperature are not required to produce 5-HT depletion.

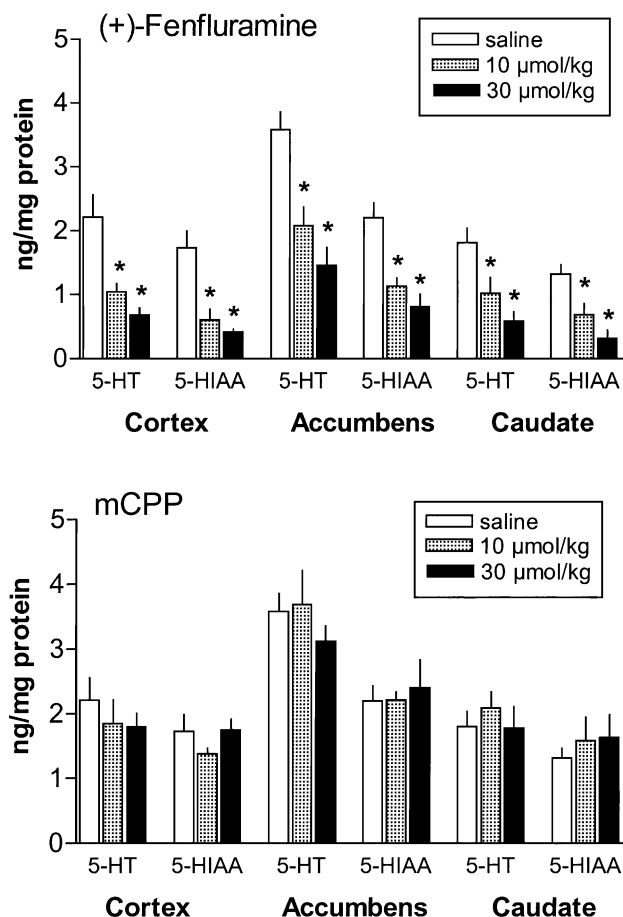


Fig. 7. Effects of repeated high-dose administration of (+)-fenfluramine or mCPP on postmortem tissue levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the cingulate cortex, nucleus accumbens, and caudate nucleus. Drugs were administered *i.p.* at 10 or 30  $\mu\text{mol/kg}$ , every 2 hr, for 4 doses. Rats were killed 2 weeks after the dosing regimen. Data are mean  $\pm$  SEM expressed as nanogram/milligram protein for  $n = 4-6$  rats per group. \*,  $P < 0.05$  compared with saline-treated group. Data from Baumann et al. (2001).

The mechanism underlying ( $\pm$ )-MDMA-induced 5-HT depletion is an area of active investigation (Gudelsky, 1996; Sanchez et al., 2001; Shankaran et al., 1999, 2001; Sprague et al., 1998). ( $\pm$ )-MDMA-induced hyperthermia clearly plays a crucial role in mediating 5-HT depletion produced by this drug (Malberg & Seiden, 1998). Furthermore, accumulating evidence suggests that ( $\pm$ )-MDMA-associated 5-HT neurotoxicity may be due to a reactive metabolite and not ( $\pm$ )-MDMA itself (Sanchez et al., 2001). For example, direct injection of ( $\pm$ )-MDMA into the brain fails to deplete 5-HT (Esteban et al., 2001). The first step in the pathogenic pathway leading to 5-HT depletion is likely to be the SERT-mediated transport of an unidentified reactive metabolite of ( $\pm$ )-MDMA into the 5-HT nerve terminal, since SSRIs can completely prevent ( $\pm$ )-MDMA-induced neurotoxicity without altering the accompanying hyperthermia (Sanchez et al., 2001). Early studies suggested a major role for DA in ( $\pm$ )-MDMA-induced 5-HT depletion (Sprague et al., 1998), but subsequent investigations suggest that manipulations of DA transmission influence 5-HT depletion secondary to altering body temperature (for a review, see Sanchez et al., 2001). More recent studies implicate oxidative stress in the pathogenesis of ( $\pm$ )-MDMA-induced 5-HT depletion (Cadet et al., 2001; Esteban et al., 2001). Clearly more work in this area of research is warranted.

As mentioned in Section 4.1, SERT substrates differ considerably in their apparent ability to increase the risk of PPH. The same principle appears to apply to the 5-HT neurotoxicity produced by various SERT substrates—some SERT substrates, such as fenfluramines, norfenfluramines, and neurotoxic metabolites of ( $\pm$ )-MDMA, deplete 5-HT (Cozzi et al., 1998; Johnson & Nichols, 1990), whereas other SERT substrates, such as mCPP, ( $\pm$ )-MDMA, and aminorex, do not (Baumann et al., 2001; Esteban et al., 2001; Zheng et al., 1997).

## 5. Summary

The pharmacology of substrate-type 5-HT releasing agents is an area of active exploration. A major difficulty with studying 5-HT releasers is the lack of agents that are selective for 5-HT neurons relative to DA and NE neurons. Furthermore, all of the available 5-HT releasers interact directly with at least some 5-HT receptor sites. When administered systemically, ( $\pm$ )-fenfluramine generates a total of four active drugs. These drugs not only release endogenous 5-HT, but also activate multiple 5-HT receptors, including 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> subtypes. Only two 5-HT releasing agents were ever approved for use in humans, and these were withdrawn due to serious adverse effects. Despite the unfortunate clinical experience with 5-HT releasing agents, it may be premature to abandon investigation of 5-HT releasing agents as potential therapeutic entities. As reviewed elsewhere (Rothman & Baumann, 2000), we

believe it may be possible to develop 5-HT releasers devoid of serious adverse effects. For instance, Nichols and co-workers (Johnson et al., 1991b; Nichols et al., 1990) have identified a number of potent 5-HT releasing agents that lack neurotoxic properties. We have shown that mCPP releases 5-HT by a SERT-dependent mechanism analogous to fenfluramines, yet high-dose administration of mCPP does not deplete 5-HT in rat brain tissue (Baumann et al., 1994, 2001). Similar to the manner in which research advances improved the histamine receptor antagonist terfenadine (Barbey et al., 1999), we believe that it will be possible to develop new medications that selectively release 5-HT without the adverse effects of PPH, VHD, or neurotoxicity. Such agents would have potential therapeutic application in obesity, substance dependence, and other psychiatric disorders.

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