

Serotonin releasing agents Neurochemical, therapeutic and adverse effects

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Abstract

This review summarizes the neurochemical, therapeutic and adverse effects of serotonin (5-HT) releasing agents. The 5-HT releaser (\pm)-fenfluramine is composed of two stereoisomers, (+)-fenfluramine and (–)-fenfluramine, which are *N*-de-ethylated to yield the metabolites, (+)-norfenfluramine and (–)-norfenfluramine. Fenfluramines and norfenfluramines are 5-HT transporter substrates and potent 5-HT releasers. Other 5-HT releasing agents include *m*-chlorophenylpiperazine (mCPP), a major metabolite of the antidepressant drug trazodone. Findings from *in vitro* and *in vivo* studies support the hypothesis that fenfluramines and mCPP release neuronal 5-HT via a non-exocytotic carrier-mediated exchange mechanism involving 5-HT transporters. (+)-Norfenfluramine is a potent 5-HT_{2B} and 5-HT_{2C} receptor agonist. The former activity may increase the risk of developing valvular heart disease (VHD), whereas the latter activity is implicated in the anorectic effect of systemic fenfluramine. Anorectic agents that increase the risk of developing primary pulmonary hypertension (PPH) share the common property of being 5-HT transporter substrates. However, these drugs vary considerably in their propensity to increase the risk of PPH. In this regard, neither trazodone nor mCPP is associated with PPH. Similarly, although some 5-HT substrates can deplete brain 5-HT (fenfluramine), others do not (mCPP). In addition to the established indication of obesity, 5-HT releasers may be helpful in treating psychiatric problems such as drug and alcohol dependence, depression and premenstrual syndrome. Viewed collectively, it seems possible to develop new medications that selectively release 5-HT without the adverse effects of PPH, VHD or neurotoxicity. Such agents may have utility in treating a variety of psychiatric disorders. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Monoamine neurons in the brain possess membrane-bound proteins that function to transport neurotransmitter molecules from the extracellular space back into the cytoplasm (Amara and Kuhar, 1993). It is now well established that distinct transporter proteins are expressed on NE neurons (i.e., NE transporters, NET), DA neurons (i.e., DA transporters, DAT) and 5-HT neurons (i.e., 5-HT transporters, SERT). These proteins are members of a superfamily of sodium/chloride-dependent transporters that share genetic, structural and functional homologies (Uhl and Johnson, 1994). Under normal circumstances, the transporter-mediated uptake of monoamine transmitters is the

principal mechanism for inactivation of monoaminergic transmission in the brain. Moreover, monoamine transporters are targets for a variety of therapeutic and abused drugs (Amara and Sonders, 1998).

Drugs that interact with transporters can be divided into two basic classes: reuptake inhibitors and substrate-type releasers. Reuptake inhibitors bind to transporter proteins, but are not transported. These drugs elevate extracellular concentrations of transmitter by blocking transporter-mediated uptake of transmitters from the synapse. Substrate-type releasers also bind to transporter proteins, but these drug molecules are subsequently transported into the cytoplasm of nerve terminals. Releasers elevate extracellular transmitter concentrations by a two-pronged mechanism: (1) they increase cytoplasmic levels of transmitter by disrupting storage of transmitters in vesicles and (2) they promote non-exocytotic release of transmitters by a process of carrier-mediated exchange (Rudnick and Clark, 1993).

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Because substrate-type releasing agents must be transported into the nerve terminal to promote neurotransmitter release, reuptake inhibitors can block the effects of releasers.

Reuptake inhibitors and substrate-type releasers both elevate extracellular concentrations of transmitter via transporter-dependent processes, but there are important differences in their precise mode of action. In particular, the ability of reuptake inhibitors to elevate extracellular neurotransmitter requires that nerve terminals release neurotransmitters via exocytosis. This, in turn, requires electrical depolarization and extracellular calcium. Thus, the ability of reuptake inhibitors to increase extracellular neurotransmitter levels is said to be impulse- and calcium-dependent. Releasing agents, on the other hand, increase synaptic levels of neurotransmitter by a process that is independent of ongoing neuronal firing. Since the action of reuptake inhibitors requires ongoing neuronal firing, autoreceptor-mediated negative feedback mechanisms serve to dampen the ability of 5-HT reuptake inhibitors to elevate synaptic transmitter. Such negative feedback effects exist for 5-HT (Adell and Artigas, 1991; Rutter et al., 1995; Smith and Lakoski, 1997), DA (Hinerth et al., 2000) and NE (Mateo et al., 1998) neuron systems, and these effects do not alter the actions of releasers. Because of negative feedback inhibition, reuptake inhibitors tend to produce small increases in extracellular neurotransmitter whereas releasers tend to produce more robust increases. The *in vivo* microdialysis data in Fig. 1 illustrate the modest and

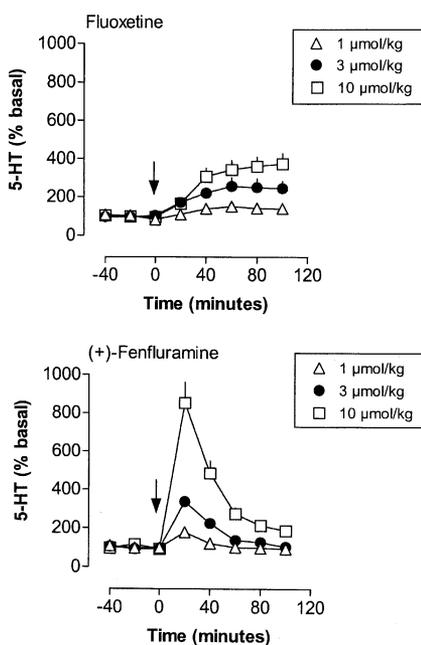


Fig. 1. Effects of fluoxetine (a 5-HT reuptake inhibitor) and (+)-fenfluramine (a 5-HT releaser) on extracellular 5-HT in rat nucleus accumbens. Dialysis methods were carried out as described previously (Baumann et al., 2000). Drugs were administered intravenously at 0 min. Data are expressed as a percentage of the mean of three basal dialysate samples collected prior to drug treatment. Basal dialysate 5-HT level was 0.46 ± 0.17 nM. Values are mean \pm S.E.M. for $N=5$ rats/group.

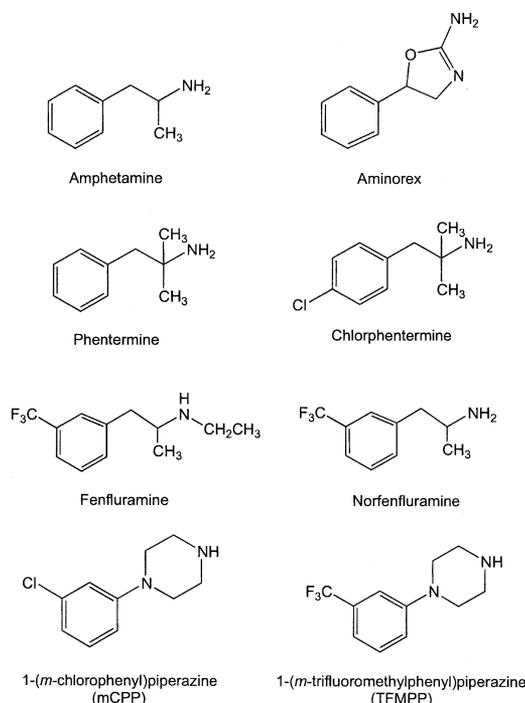


Fig. 2. Chemical structures of representative 5-HT releasing agents.

sustained elevation of extracellular 5-HT evoked by the 5-HT reuptake inhibitor fluoxetine compared to the much larger and transient effect of the 5-HT releaser, (+)-fenfluramine (Berger et al., 1992; Crespi et al., 1997).

A number of 5-HT selective reuptake inhibitors (SSRIs), such as fluoxetine, sertraline and citalopram, are widely prescribed medications used in the treatment of psychiatric disorders including depression, panic disorder and obsessive-compulsive disorder (for reviews, see Gorman and Kent, 1999; Zohar and Westenberg, 2000). By contrast, there are far fewer 5-HT releasing agents. Because of the withdrawal of the 5-HT releasers, fenfluramine and dexfenfluramine, from the market in September 1997 (Connolly and McGoon, 1999), there are currently no clinically available 5-HT releasing agents. A main goal of this paper is to summarize the potential therapeutic uses and reported adverse effects of 5-HT releasing agents. Furthermore, we hope this review will stimulate continued interest in the development of novel and selective 5-HT releasers that can be used as effective medications.

2. Neurochemical mechanisms of 5-HT releasing agents

(\pm)-Fenfluramine (Pondimin) and its more potent stereoisomer, (+)-fenfluramine (dexfenfluramine, Redux), are substituted amphetamine derivatives. These drugs were used for the treatment of obesity until they were withdrawn from the market in September 1997, due to reports of cardiac valvulopathy (Connolly and McGoon, 1999). (\pm)-Fenfluramine is composed of two stereoisomers, (+)-fenfluramine

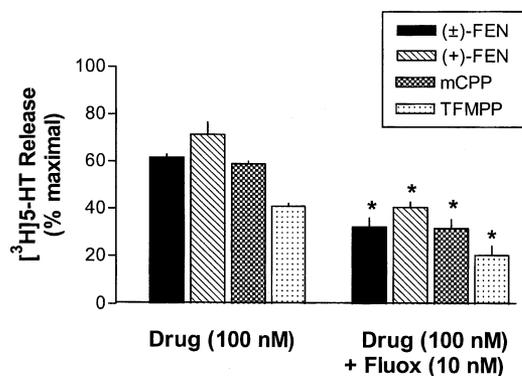


Fig. 3. Effects of fluoxetine on [3 H]5-HT release evoked by (\pm)-fenfluramine, (+)-fenfluramine, mCPP and TFMPP in rat brain synaptosomes. Synaptosomes were preloaded with [3 H]5-HT (5 nM). Test drugs (100 nM) were incubated with preloaded synaptosomes, in the absence or presence of fluoxetine (10 nM). Fluoxetine alone did not release [3 H]5-HT. As described elsewhere (Rothman et al., 2001), [3 H]5-HT release was determined by measuring retained tritium, and maximal release was defined using 100 μ M tyramine. Data are mean \pm S.E.M. for three separate experiments expressed as a percentage of maximal release. * P < .05 when compared to drug alone (Student's t test).

and (–)-fenfluramine, which are N -de-ethylated in the liver to form the metabolites, (+)-norfenfluramine and (–)-norfenfluramine (Pinder et al., 1975; Caccia et al., 1985). Fig. 2 shows the structures of fenfluramine and norfenfluramine. Since all four stereoisomers are biologically active (Garattini et al., 1986), systemic administration of (\pm)-fenfluramine delivers four active pharmacological agents in vivo. Most studies indicate that fenfluramines and norfenfluramines are SERT substrates and potent 5-HT releasing agents (for review, see Garattini, 1995). Other 5-HT releasing agents include the piperazine derivatives, m -chlorophenylpiperazine (mCPP) and m -trifluoromethylpiperazine

(TFMPP) (Pettibone and Williams, 1984; Auerbach et al., 1990; Baumann et al., 1993, 1994; Eriksson et al., 1999). It is noteworthy that mCPP is a major metabolite of the antidepressant trazodone (Otani et al., 1998) and a minor metabolite of nefazodone (Barbhaiya 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, carrier-mediated exchange mechanism involving SERT sites in the brain. As shown in Fig. 3, treatment with the 5-HT uptake inhibitor, fluoxetine, antagonizes the ability of these drugs to release [3 H]5-HT from synaptosomes in vitro.

Historically, it has been difficult to distinguish whether drugs act as reuptake inhibitors or substrate-type releasers using simple test tube assays. With this in mind, we recently developed a high-throughput in vitro method that can be used to discriminate between reuptake inhibitors and releasers (Rothman et al., 2000b, 2001). Using this method, 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. As reported in Table 1, a number of drugs are potent 5-HT releasers (see Fig. 2 for representative chemical 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} = 450 nM (Rothman et al., 2001), indicating about a 10-fold selectivity for SERT. (+)-Fenfluramine and mCPP are potent 5-HT releasers, but in contrast to (+)-fenfluramine, mCPP does not release NE. (–)-Fenfluramine is about three-fold weaker at 5-HT release than the (+)-isomer, and it does not release NE. Amphetamine and phentermine are very weak 5-HT releasers, especially when compared to their potency at NE and DA release.

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

Drug	5-HT release IC_{50} (nM \pm S.D.)	NE release IC_{50} (nM \pm S.D.)	DA release IC_{50} (nM \pm S.D.)
Chlorphentermine	30.9 \pm 5.4	> 10,000	2650 \pm 273
mCPP ^a	38.1 \pm 4.6	> 10,000	> 10,000
5-HT	44.4 \pm 5.3	> 10,000	> 10,000
(+)-Fenfluramine	51.7 \pm 6.1	302 \pm 20	> 10,000
(±)-MDMA	56.6 \pm 2.1	77.4 \pm 3.4	376 \pm 16
(±)-Fenfluramine	79.3 \pm 9.5	739 \pm 57	> 10,000
(–)-Fenfluramine ^a	147 \pm 19	> 10,000	> 10,000
Aminorex	193 \pm 23	26.4 \pm 2.8	49.4 \pm 7.5
(+)-Methamphetamine	736 \pm 45	12.3 \pm 0.7	24.5 \pm 2.1
(+)-Amphetamine	1765 \pm 94	7.07 \pm 0.95	24.8 \pm 3.5
Tyramine	2775 \pm 234	40.6 \pm 3.5	119 \pm 11
Phentermine	3511 \pm 253	39.4 \pm 6.6	262 \pm 21
(–)-Methamphetamine	4640 \pm 243	28.5 \pm 2.5	416 \pm 20
(–)-Ephedrine	> 10,000	72.4 \pm 10.2	1350 \pm 124
Norepinephrine	> 10,000	164 \pm 13	869 \pm 51
Dopamine	> 10,000	66.2 \pm 5.4	86.9 \pm 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 the published methods (Rothman et al., 2001). Each value is the mean \pm S.D. of three experiments. Data are from Rothman et al. (2001).

^a Unpublished data.

Fenfluramines and mCPP also have direct agonist actions at 5-HT receptors. As noted above, when administered systemically, (\pm)-fenfluramine and (+)-fenfluramine are rapidly metabolized to (\pm)-norfenfluramine and (+)-norfenfluramine, respectively (Caccia et al., 1985; Campbell et al., 1988). These metabolites are pharmacologically active and display long biological half-lives (Caccia et al., 1985). In addition to releasing 5-HT (see above), fenfluramines and their metabolites have direct agonist actions at multiple 5-HT₂ receptor subtypes (Fitzgerald et al., 2000; Rothman et al., 2000a). In fact, the direct activation of 5-HT_{2C} receptors by fenfluramine is thought to contribute directly to the anorectic effect of the drug in rats (Dourish, 1995; Curzon et al., 1997; Vickers et al., 1999). As reported in Table 2, both (+)-norfenfluramine and (–)-norfenfluramine are highly efficacious and potent 5-HT_{2C} receptor agonists ($K_{act} < 20$ nM). By contrast, (+)-fenfluramine and (–)-fenfluramine are about 10-fold less potent at activating human 5-HT_{2C} receptors. Thus, the suspected 5-HT_{2C} receptor actions of systemically administered fenfluramine may be mediated by norfenfluramine. (+)-Norfenfluramine is also a very potent 5-HT_{2B} agonist, which may relate to the valvulopathy side effect (Rothman et al., 2000a). m-CPP is a very potent and efficacious agonist at the human 5-HT_{2C} receptor ($K_{act} = 0.6$ nM) and is also reported to be a potent 5-HT_{1A} agonist (Hoyer et al., 1994).

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 5-HT reuptake blockers like 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 (Gundlach et al., 1997; Baumann et al., 1998). Using this paradigm in human subjects, Pedrinola et al. (1996) showed that (+)-fenfluramine promotes weight loss in patients who receive con-

current 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.

3. Therapeutic applications of 5-HT releasers

As noted above, (\pm)-fenfluramine and (+)-fenfluramine are the only 5-HT releasers ever approved for use in humans, and mCPP has been used clinically as an investigational drug. In fact, all three drugs are “promiscuous” ligands. While these drugs potently release 5-HT, their activation of 5-HT_{2B} and 5-HT_{2C} receptors undoubtedly contributes to their *in vivo* pharmacological effects. 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 clearly established (\pm)-fenfluramine and (+)-fenfluramine as effective weight loss agents (for reviews, see Pinder et al., 1975; McTavish and Heel, 1992; Davis and Faulds, 1996). Other studies show that (\pm)-fenfluramine and (+)-fenfluramine promote weight loss and directly improve insulin sensitivity and diabetic control in Type 2 diabetes (Willey et al., 1992, 1994; Scheen and Lefebvre, 2000). Interestingly, (+)-fenfluramine decreases sympathetic nervous system activity (Hirsch et al., 2000), plasma NE (Andersson et al., 1991; Kolanowski et al., 1992; Flechtner-Mors et al., 1998), plasma renin (Andersson et al., 1991) and blood pressure (Andersson et al., 1991; Kolanowski et al., 1992;

Table 2
Functional activity of test drugs at 5-HT₂ receptor subtypes

Drug	Human 5-HT _{2A} K_{act} (nM \pm S.E.M.) V_{max} (% 5-HT \pm S.E.M.)	Human 5-HT _{2B} K_{act} (nM \pm S.E.M.) V_{max} (% 5-HT \pm S.E.M.)	Human 5-HT _{2C} K_{act} (nM \pm S.E.M.) V_{max} (% 5-HT \pm S.E.M.)
(+)-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
m-CPP	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_{2A} and 5-HT_{2C}) or transiently (5-HT_{2B}) expressed receptors. [³H]inositol phosphate accumulation was determined as previously described (Rothman et al., 2000a). Each value is the mean \pm S.E.M. of three experiments. Data are from Rothman et al. (2000a).

Flehtner-Mors et al., 1998) in humans. Similar effects occur with administration of α_2 adrenergic agonists (Oates et al., 1978; Schoeppe and Brecht, 1980). It is tempting to speculate that NE release evoked by (+)-fenfluramine might contribute to these effects (see Table 1).

3.2. Potential therapeutic indications

Several preclinical studies demonstrate that acute administration of 5-HT releasing agents such as fenfluramine, mCPP and TFMPP decreases ethanol intake by rats (Lu et al., 1993; Buczek et al., 1994; 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). Additionally, the amphetamine/(\pm)-fenfluramine or phentermine/(\pm)-fenfluramine combination eliminates alcohol withdrawal seizures in rats (Mirovsky et al., 1995; Halladay et al., 2000). Case reports also suggest that phentermine plus (\pm)-fenfluramine decreases alcohol intake in humans (Hitzig, 1994; Rothman, 1995). These promising findings await confirmation with controlled clinical trials.

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 fenfluramines could be used, along with phentermine, in the treatment of cocaine dependence (for review, see Rothman and Baumann, 2000). In humans, controlled studies indicate that mCPP (Buydens-Branchey et al., 1997) and fenfluramine (Buydens-Branchey et al., 1998) decrease cocaine craving. The authors were unable to locate any clinical trials that examined the effectiveness of 5-HT releasers as adjuncts in the treatment of stimulant or opioid dependence.

In light of the widespread therapeutic application of SSRIs in treating depression and anxiety disorders, it seems possible that 5-HT releasers might be of therapeutic benefit for these types of illnesses. Indeed, several small-scale studies support this notion. Rickels et al. (1976) reported that (\pm)-fenfluramine reduced 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 symp-

toms (Robinson et al., 1985). Another placebo-controlled study showed that treatment with (+)-fenfluramine helped ameliorate the symptoms of premenstrual depression and the premenstrual rise in calorie, carbohydrate and fat intake (Brzezinski et al., 1990). On the other hand, Price et al. (1990) reported that fenfluramine did not have 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 the Introduction, 5-HT releasers differ from SSRIs in a number of respects. Most importantly, due to the existence of negative feedback loops, 5-HT releasers are able to increase synaptic 5-HT to higher levels when compared to uptake inhibitors. Whether or not this neurochemical effect of releasers will impart enhanced antidepressant efficacy can only be established by controlled clinical studies. Viewed collectively, the aforementioned 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-HT releasing agents

Both (\pm)-fenfluramine and (+)-fenfluramine produce mild and reversible side effects in some patients (Weintraub and Bray, 1989; Weintraub et al., 1984; Hanotin et al., 1998). 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), valvular heart disease (VHD) and perhaps neurotoxicity. In fact, as noted above, a marked increase in the incidence of VHD in patients treated with (\pm)-fenfluramine and (+)-fenfluramine prompted the removal these drugs from the market. The major adverse effects of these medications will be discussed with particular emphasis on possible underlying mechanisms.

4.1. Primary pulmonary hypertension

PPH is a rare and often fatal disease of unknown etiology (Rubin, 1997). Epidemiological data show that fenfluramines and aminorex clearly increase the risk of developing PPH (Gurtner, 1990; Abenhaim et al., 1996). Abenhaim et al. (1996) estimated that taking fenfluramines (either (\pm)-fenfluramine or (+)-fenfluramine) for more than 3 months, increased the risk of developing PPH 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 about seven-fold. Given that PPH is usually an exceedingly rare disorder with an annual incidence of one to two 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.

The mechanism by which fenfluramines might increase the risk of developing PPH is not known. Recent evidence indicates that mutations in the gene encoding bone morphogenetic protein Type II receptor (BMPR-II) may account for most familial forms of PPH and for up to 26% of sporadic PPH cases (Thomson et al., 2000; Machado et al., 2001). At the present time, it is not known whether fenfluramines or other appetite suppressants directly interact with BMPR-II proteins. Future studies should examine the possible relationship between the incidence of fenfluramine-associated PPH and mutations in the BMPR-II gene.

Some investigators have hypothesized that fenfluramine and other anorectics elevate plasma 5-HT by releasing 5-HT normally stored in platelets. According to the “5-HT hypothesis” of PPH, drug-induced elevations in circulating 5-HT cause chronic increases in pulmonary blood pressure and growth of arterial smooth muscle thereby producing PPH in susceptible individuals (Herve et al., 1995; Fishman, 1999a). A major flaw in the 5-HT hypothesis is that there is little evidence to support it. In fact, substantial data shows 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 (Raleigh et al., 1986; Stubbs et al., 1986; Sherman et al., 1989; Martin and Artigas, 1992; Celada et al., 1994; Redmon et al., 1997). Despite the overwhelming evidence that fenfluramines do not increase plasma 5-HT, the 5-HT hypothesis continues to gain wide acceptance (Fishman, 1999a; MacLean, 1999; Stahl, 1997). These considerations prompted Rothman et al. (2000c) to measure plasma 5-HT levels in human 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 must 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 anorectic medications 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 (Ramamoorthy et al., 1993; Chang et al., 1996). In addition, the 5-HT transport mechanism in the brain and lungs is similar (Paczkowski et al., 1996; James and Bryan-Lluka, 1997). 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 data from Table 1 demonstrate that drugs known or sus-

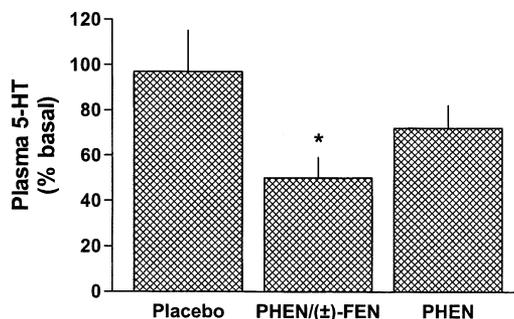


Fig. 4. Effects of placebo, phentermine/(\pm)-fenfluramine and phentermine treatment on plasma 5-HT levels in human subjects. As reported elsewhere (Rothman et al., 2000c), 44 patients with Type 2 diabetes enrolled in a randomized double-blind, placebo-controlled, clinical trial to determine the effect of the phentermine/fenfluramine combination [phentermine (37.5 mg po/day)+fenfluramine (20 mg po tid)] 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 seven patients were treated with phentermine alone (37.5 mg po/day) after 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 phentermine/fenfluramine group showed a significant decrease in plasma 5-HT. There was a nonsignificant trend towards a decrease in plasma 5-HT in the phentermine group. * $P < .05$ when compared to placebo.

pected to increase the risk of PPH (i.e., (\pm)-fenfluramine, (+)-fenfluramine, aminorex and chlorphentermine) are potent SERT substrates, whereas drugs not associated with PPH (i.e., amphetamine and phentermine) are less potent in this regard. The intracranial microdialysis data depicted in Fig. 5 show that all of the drugs associated with PPH are powerful 5-HT releasers in vivo. These findings led us to propose a “gateway hypothesis” of PPH. According to this hypothesis, anorectic medications that are SERT substrates get translocated into pulmonary cells where PPH could develop as a response to high levels of these drugs or their metabolites. 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 (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.

It appears that being a SERT substrate may be a necessary, but not sufficient, criterion to increase the risk of PPH. For example, there are potent SERT substrates that are not associated with PPH. As mentioned previously,

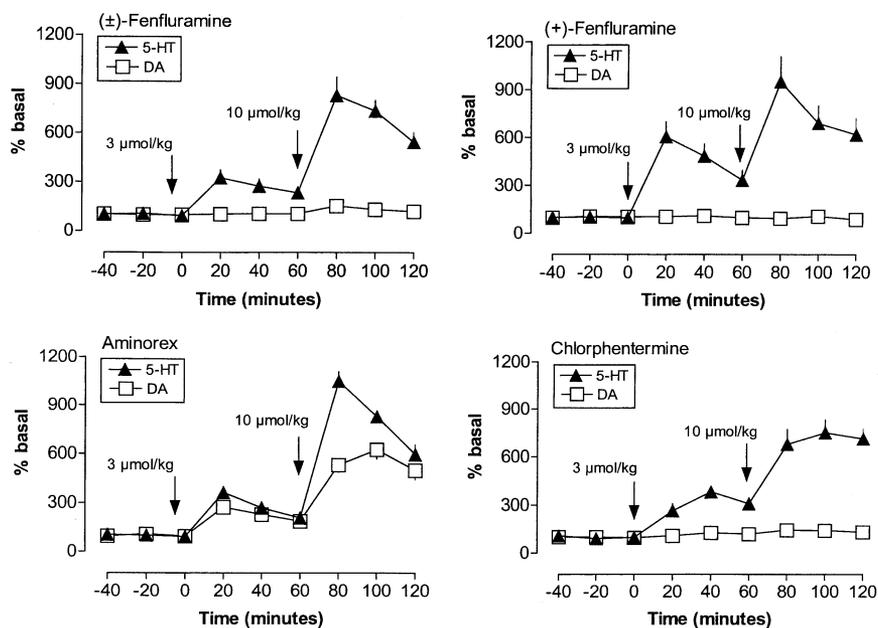


Fig. 5. Effects of (±)-fenfluramine, (+)-fenfluramine, aminorex and chlorphentermine on extracellular 5-HT and DA in rat nucleus accumbens. Dialysis methods were carried out as described previously (Baumann et al., 2000). Drugs were administered at 0 min (3 $\mu\text{mol/kg}$) and 60 min (10 $\mu\text{mol/kg}$).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

mCPP is a SERT substrate that increases extracellular 5-HT in rat brain (Baumann et al., 1993, 1994). Moreover, this drug binds to SERT sites in 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.

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 million patients (Rich et al., 2000). It is possible that the toxic potential of aminorex, (±)-fenfluramine and (+)-fenfluramine is related to the amphetamine-like chemical structure of these drugs. In this regard, mCPP has a non-amphetamine structure and is not known to increase the risk of PPH. 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 was recently reviewed (Connolly and McGoon, 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 FDA-defined VHD in patients treated with anorectic medications (Weiss-

man et al., 1998), while other studies report that about 12% of patients treated with (±)-fenfluramine or (+)-fenfluramine develop mild asymptomatic VHD (Gardin et al., 2000). There has been much speculation as to the mechanism of fenfluramine-associated VHD (Fishman, 1999b). 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 anorectic medications produce VHD via elevations in circulating 5-HT (for review, see Fishman, 1999b). However, as noted above, 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 and 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 that the 5-HT_{2B} receptor subtype (Rothman et al., 2000a; Fitzgerald et al., 2000). Importantly, medications not associated with increased risk of VHD (phentermine, fluoxetine and its major metabolite, norfluoxetine) lack agonist activity at the 5-HT_{2B} receptor (Rothman et al., 2000a). Given that 5-HT_{2B} receptors are expressed on heart valves (Fitzgerald et al., 2000), it seems possible that 5-HT_{2B} receptor activation is involved in etiology of fenfluramine-associated VHD. Consistent with this notion, serotonergic medications that are devoid of agonist activity at 5-HT_{2B} receptors should not produce VHD. Further evidence is needed to definitively establish a link between 5-HT_{2B} receptors and VHD.

4.3. Fenfluramine neurotoxicity

It is well established that administration of high-dose (\pm)-fenfluramine or (+)-fenfluramine causes long-term depletion of forebrain 5-HT in laboratory animals (Kleven and Seiden, 1989; McCann et al., 1997). The fenfluramine-induced loss of brain 5-HT is accompanied by parallel reductions in presynaptic 5-HT markers, such as tryptophan hydroxylase and SERT sites. We have recently shown that 5-HT depletion produced by (\pm)-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 are neurotoxic and produce lesions in central 5-HT nerve terminals (Molliver et al., 1990; McCann et al., 1997). Depending on experimental conditions, the reductions in 5-HT markers can return to normal levels with time (Molliver et al., 1990; Sotelo, 1991). Whether or not such fenfluramine-induced deficits in 5-HT systems are indicative of “true” neurotoxicity is still a matter of debate (O’Callaghan and Miller, 1994; Baumann and Rothman, 1998).

An important question is whether or not therapeutic doses of fenfluramine deplete 5-HT in human brain (McCann et al., 1997). Preclinical studies indicate that fenfluramines and norfenfluramines must achieve brain concentrations of about 50 μ M in order to produce neurotoxicity (Zaczek et al., 1990; Mennini et al., 1996), 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 patients, some investigators used methods of “interspecies scaling” to extrapolate drug doses in animals to equivalent doses in humans (McCann et al., 1997). 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 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 (MRS). 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 po b.i.d.) for weight loss. These investigators demonstrated that patients achieve stable (+)-fenfluramine plus (+)-norfenfluramine levels in brain of about 4 μ M. 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 involved in the long-term 5-HT depletion caused by amphetamine-type drugs (Berger et al., 1992; Seiden and 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, which cause cellular damage (Wrona and Dryhurst, 1998). On the other hand, a number of potent 5-HT releasing agents have been identified that are devoid of neurotoxic properties. The Nichols group (Nichols et al., 1990; Johnson et al., 1991) synthesized “non-neurotoxic” analogs of methylenedioxymphetamine (MDA) and methylenedioxymphetamine (MDMA). These analogs are substrate-type 5-HT releasers in vitro yet they do not deplete forebrain 5-HT in vivo. We have shown that mCPP releases 5-HT by a SERT-dependent mechanism (Baumann et al., 1993), and high-dose administration of mCPP does not deplete 5-HT in rat brain tissue (Baumann et al., 2001). These data demonstrate that drug-induced 5-HT release is not necessarily coupled to long-term 5-HT depletion.

5. Summary

The pharmacology of 5-HT releasing agents is relatively unexplored. This situation is likely due to the limited number of drugs that selectively release 5-HT relative to DA and NE. Additionally, all of the available 5-HT releasers possess significant 5-HT receptor affinities. 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_{2B} and 5-HT_{2C} 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 terminate investigation of 5-HT releasing agents as potential therapeutic agents. As reviewed elsewhere (Rothman and Baumann, 2000), we believe it may be possible to develop 5-HT releasers devoid of serious adverse effects. For example, the Nichols group identified a number of potent 5-HT releasing agents that lack neurotoxic properties (Nichols et al., 1990; Johnson et al., 1991). We have shown that mCPP releases 5-HT by a SERT-dependent mechanism analogous to (\pm)-fenfluramine and (+)-fenfluramine (Baumann et al., 1993), yet high-dose administration of mCPP does not deplete 5-HT in rat brain tissue (Baumann et al., 2001). Similar to the way that research advances improved the side-effects profile of 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 application in the treatment of obesity, substance dependence and other psychiatric disorders.

References

- Abenham L, Moride Y, Brenot F, Rich S, Benichou J, Kurz X, Higebottam T, Oakley C, Wouters E, Aubier M, Simonneau G, Begaud B, International Primary Pulmonary Hypertension Study Group. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. *N Engl J Med* 1996;335:609–16.
- Adell A, Artigas F. Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. An in vivo brain microdialysis study. *Naunyn-Schmiedeberg's Arch Pharmacol* 1991;343:237–44.
- Amara SG, Kuhar MJ. Neurotransmitter transporters: recent progress. *Ann Rev Neurosci* 1993;16:73–93.
- Amara SG, Sonders MS. Neurotransmitter transporters as molecular targets for addictive drugs. *Drug Alcohol Depend* 1998;51:87–96.
- Andersson B, Zimmermann ME, Hedner T, Bjorntorp P. Haemodynamic, metabolic, and endocrine effects of short-term dexfenfluramine treatment in young, obese women. *Eur J Clin Pharmacol* 1991;40:249–54.
- Auerbach SB, Kamalakannan N, Rutter JJ. TFMPP and RU24969 enhance serotonin release from rat hippocampus. *Eur J Pharmacol* 1990;190:51–7.
- Backmann R, Dengler H, Gahl K, Greiser E, Jesdinsky HJ, Loogen F. Primary pulmonary hypertension. Report of the Commission of the German Society for research on blood circulation. *Verh Dtsch Ges Kreislaufforsch* 1972;38:134–41.
- Barbey JT, Anderson M, Ciprandi G, Frew AJ, Morad M, Priori SG, Ongini E, Afrime MB. Cardiovascular safety of second-generation antihistamines. *Am J Rhinol* 1999;13:235–43.
- Barbhaiya RH, Buch AB, Greene DS. Single and multiple dose pharmacokinetics of nefazodone in subjects classified as extensive and poor metabolizers of dextromethorphan. *Br J Clin Pharmacol* 1996;42:573–81.
- Baumann MH, Rothman RB. Combined phentermine/fenfluramine administration and central serotonin neurons (letter). *Synapse* 1998;28:339–42.
- Baumann MH, Rutter JJ, Auerbach SB. Intravenous administration of the serotonin agonist *m*-chlorophenylpiperazine (mCPP) increases extracellular serotonin in the diencephalon of awake rats. *Neuropharmacology* 1993;32:1381–6.
- Baumann MH, Ayestas MA, Rothman RB. Effects of *d*-fenfluramine and *m*-chlorophenylpiperazine on acute 5-HT release and long-term 5-HT depletion in rat brain. *Soc Neurosci Abstr* 1994;24:1028.
- Baumann MH, Mash DC, Staley JK. The serotonin agonist *m*-chlorophenylpiperazine (mCPP) binds to serotonin transporter sites in human brain. *NeuroReport* 1995;6:2150–2.
- Baumann MH, Ayestas MA, Rothman RB. Functional consequences of central serotonin depletion produced by repeated fenfluramine administration in rats. *J Neurosci* 1998;18:9069–77.
- Baumann MH, Ayestas MA, Dersch CM, Brockington A, Rice KC, Rothman RB. Effects of phentermine and fenfluramine on extracellular dopamine and serotonin in rat nucleus accumbens: therapeutic implications. *Synapse* 2000;36:102–13.
- Baumann MH, Ayestas MA, Dersch CM, Rothman RB. 1-(*m*-Chlorophenyl)piperazine (mCPP) dissociates in vivo serotonin release from long-term serotonin depletion in rat brain. *Neuropsychopharmacology* 2001;24:492–501.
- Berger UV, Gu XF, Azmitia EC. The substituted amphetamines 3,4-methylenedioxy-methamphetamine, methamphetamine, *p*-chloroamphetamine, and fenfluramine induce 5-hydroxytryptamine release via a common mechanism blocked by fluoxetine and cocaine. *Eur J Pharmacol* 1992;215:153–60.
- Blouin AG, Blouin JH, Perez EL, Bushnik T, Zuro C, Mulder E. Treatment of bulimia with fenfluramine and desipramine. *J Clin Psychopharmacol* 1988;8:261–9.
- Brzezinski AA, Wurtman JJ, Wurtman RJ, Gleason R, Greenfield J, Nader T. *d*-Fenfluramine suppresses the increased calorie and carbohydrate intakes and improves the mood of women with premenstrual depression. *Obstet Gynecol* 1990;76:296–301.
- Buczek Y, Tomkins DM, Higgins GA, Sellers EM. Dissociation of serotonergic regulation of anxiety and ethanol self-administration: a study with mCPP. *Behav Pharmacol* 1994;5:470–84.
- Buydens-Branchey L, Branchey M, Ferguson P, Hudson J, McKernin C. Craving for cocaine in addicted users. Role of serotonergic mechanisms. *Am J Addict* 1997;6:665–73.
- Buydens-Branchey L, Branchey M, Hudson J, Rothman M, Ferguson P, McKernin C. Effect of fenfluramine challenge on cocaine craving in addicted male users. *Am J Addict* 1998;7:142–55.
- Caccia S, Conforti I, Duchier J, Garattini S. Pharmacokinetics of fenfluramine and norfenfluramine in volunteers given D- and DL-fenfluramine for 15 days. *Eur J Clin Pharmacol* 1985;29:221–4.
- Campbell DB, Gordon BH, Ings RM, Richards R, Taylor DW. Factors that may affect the reduction of hunger and body weight following *d*-fenfluramine administration. *Clin Neuropharmacol* 1988;11(Suppl. 1):S160–72.
- Celada P, Martin F, Artigas F. Effects of chronic treatment with dexfenfluramine on serotonin in rat blood, brain, and lung tissue. *Life Sci* 1994;55:1237–43.
- Chang AS, Chang SM, Starnes DM, Schroeter S, Bauman AL, Blakely RD. Cloning and expression of the mouse serotonin transporter. *Brain Res Mol Brain Res* 1996;43:185–92.
- Christensen JD, Babb SM, Cohen BM, Renshaw PF. Quantitation of dexfenfluramine/*d*-norfenfluramine concentration in primate brain using F-19 NMR-spectroscopy. *Magn Reson Med* 1998;39:149–54.
- Christensen JD, Yurgelun-Todd DA, Babb SM, Gruber SA, Cohen BM, Renshaw PF. Measurement of human brain dexfenfluramine concentration by 19F magnetic resonance spectroscopy. *Brain Res* 1999;834:1–5.
- Connolly HM, McGoon MD. Obesity drugs and the heart. *Curr Probl Cardiol* 1999;24:745–92.
- Connolly HM, Crary JL, McGoon MD, Hensrud DD, Edwards BS, Schaff HV. Valvular heart disease associated with fenfluramine–phentermine. *N Engl J Med* 1997;337:581–8.
- Crespi D, Mennini T, Gobbi M. Carrier-dependent and Ca(2+)-dependent 5-HT and dopamine release induced by (+)-amphetamine, 3,4-methylenedioxymethamphetamine, *p*-chloroamphetamine and (+)-fenfluramine. *Br J Pharmacol* 1997;121:143–1735.
- Curzon G, Gibson EL, Oluoyomi AO. Appetite suppression by commonly

- used drugs depends on 5-HT receptors but not on 5-HT availability. *Trends Pharmacol Sci* 1997;18:21–5.
- Davis R, Faulds D. Dexfenfluramine. An updated review of its therapeutic use in the management of obesity. *Drugs* 1996;52:696–724.
- Donnelly M, Rapoport JL, Potter WZ, Oliver J, Keysor CS, Murphy DL. Fenfluramine and dextroamphetamine treatment of childhood hyperactivity. Clinical and biochemical findings. *Arch Gen Psychiatry* 1989;46:205–12.
- Dourish CT. Multiple serotonin receptors: opportunities for new treatments for obesity? *Obes Res* 1995;3(Suppl. 4):449S–62S.
- Eriksson E, Engberg G, Bing O, Nissbrandt H. Effects of mCPP on the extracellular concentrations of serotonin and dopamine in rat brain. *Neuropsychopharmacology* 1999;20:287–96.
- Fishman AP. Aminorex to Fen/Phen: an epidemic foretold (responses to letters). *Circulation* 1999a;100:e147.
- Fishman AP. Aminorex to fen/phen: an epidemic foretold. *Circulation* 1999b;99:156–61.
- Fitzgerald LW, Burn TC, Brown BS, Patterson JP, Corjay MH, Valentine, PA, Sun JH, Link JR, Abbaszade I, Hollis JM, Largent BL, Hartig PR, Hollis GF, Meunier PC, Robichaud AJ, Robertson DW. Possible role of valvular serotonin 5-HT_{2B} receptors in the cardiopathy associated with fenfluramine. *Mol Pharmacol* 2000;57:75–81.
- Flechtner-Mors M, Ditschuneit HH, Yip I, Adler G. Blood pressure and plasma norepinephrine responses to dexfenfluramine in obese postmenopausal women. *Am J Clin Nutr* 1998;67:611–5.
- Garattini S. Biological actions of drugs affecting serotonin and eating. *Obes Res* 1995;3(Suppl. 4):463S–70S.
- Garattini S, Mennini T, Bendotti C, Invernizzi R, Samanin R. Neurochemical mechanism of action of drugs which modify feeding via the serotonergic system. *Appetite* 1986;7:15–38 (Suppl.).
- Gardin JM, Schumacher D, Constantine G, Davis KD, Leung C, Reid CL. Valvular abnormalities and cardiovascular status following exposure to dexfenfluramine or phentermine/fenfluramine (see comments). *JAMA, J Am Med Assoc* 2000;283:1703–9.
- Gorman JM, Kent JM. SSRIs and SMRIs: broad spectrum of efficacy beyond major depression. *J Clin Psychiatry* 1999;60(Suppl. 4):33–8.
- Gundlach C, Martin KF, Heal DJ, Auerbach SB. In vivo criteria to differentiate monoamine reuptake inhibitors from releasing agents: sibutramine is a reuptake inhibitor. *J Pharmacol Exp Ther* 1997;283:581–91.
- Gurtner HP. Aminorex and pulmonary hypertension. *Cor Vasa* 1985;27:160–71.
- Gurtner HP. Aminorex pulmonary hypertension. In: Fishman AP, editor. *The pulmonary circulation: normal and abnormal*. Philadelphia: University of Pennsylvania Press, 1990. pp. 397–411.
- Halladay AK, Wagner GC, Hsu T, Sekowski A, Fisher H. Differential effects of monoaminergic agonists on alcohol intake in rats fed a tryptophan-enhanced diet. *Alcohol* 1999;18:55–64.
- Halladay AK, Fisher H, Wagner GC. Effects of phentermine and fenfluramine on alcohol consumption and alcohol withdrawal seizures in rats. *Alcohol* 2000;20:19–29.
- Hanotin C, Thomas F, Jones SP, Leutenegger E, Drouin P. A comparison of sibutramine and dexfenfluramine in the treatment of obesity. *Obes Res* 1998;6:285–91.
- Herve P, Launay J-M, Scrobahaci M-L, Brenot F, Simonneau G, Petitpretz P, Poubreau P, Cerrina J, Duroux P, Drouet L. Increased plasma serotonin in primary pulmonary hypertension. *Am J Med* 1995;99:249–54.
- Higgins GA, Wang Y, Corrigan WA, Sellers EM. Influence of 5-HT₃ receptor antagonists and the indirect 5-HT agonist, dexfenfluramine, on heroin self-administration in rats. *Psychopharmacology (Berlin)* 1994;114:611–9.
- Hinerth MA, Collins HA, Baniecki M, Hanson RN, Waszczak BL. Novel in vivo electrophysiological assay for the effects of cocaine and putative “cocaine antagonists” on dopamine transporter activity of substantia nigra and ventral tegmental area dopamine neurons. *Synapse* 2000;38:305–12.
- Hirsch J, Mackintosh RM, Aronne LJ. The effects of drugs used to treat obesity on the autonomic nervous system. *Obes Res* 2000;8:227–33.
- Hitzig P. Combined serotonin and dopamine indirect agonists correct alcohol craving and alcohol-associated neurosis. *J Subst Abuse Treat* 1994;11:489–90.
- Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* 1994;46:157–203.
- James KM, Bryan-Lluka LJ. Efflux studies allow further characterisation of the noradrenaline and 5-hydroxytryptamine transporters in rat lungs. *Naunyn-Schmiedeberg's Arch Pharmacol* 1997;356:126–33.
- Jick H, Vasilakis C, Weinrauch LA, Meier CR, Jick SS, Derby LE. A population-based study of appetite-suppressant drugs and the risk of cardiac-valve regurgitation. *N Engl J Med* 1998;339:719–24.
- Johnson MP, Frescas SP, Oberlender R, Nichols DE. Synthesis and pharmacological examination of 1-(3-methoxy-4-methylphenyl)-2-aminopropane and 5-methoxy-6-methyl-2-aminoindan: similarities to 3,4-(methylenedioxy)methamphetamine (MDMA). *J Med Chem* 1991;34:1662–8.
- Kleven MS, Seiden LS. D-, L- and DL-Fenfluramine cause long-lasting depletions of serotonin in rat brain. *Brain Res* 1989;505:351–3.
- Kolanowski J, Younis LT, Vanbutsele R, Detry JM. Effect of dexfenfluramine treatment on body weight, blood pressure, and noradrenergic activity in obese hypertensive patients. *Eur J Clin Pharmacol* 1992;42:599–605.
- Lu MR, Wagner GC, Fisher H. Ethanol consumption following acute fenfluramine, fluoxetine, and dietary tryptophan. *Pharmacol Biochem Behav* 1993;44:931–7.
- Machado RD, Pauciulo MW, Thomson JR, Lane KB, Morgan NV, Wheeler JA, Phillips JA, Newman J, Williams D, Galie N, Manes A, McNeil K, Yacoub M, Mikhail G, Rogers P, Corris P, Humbert M, Donnai D, Martensson G, Tranebjaerg L, Loyd JE, Trembath RC, Nichols WC. BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. *Am J Hum Genet* 2001;68:92–102.
- MacLean MR. Pulmonary hypertension, anorexigens, and 5-HT: pharmacological synergism in action? *Trends Pharmacol Sci* 1999;20:490–5.
- Mahmood I. Allometric issues in drug development. *J Pharm Sci* 1999;88:1101–6.
- Martin F, Artigas F. Simultaneous effects of *p*-chloroamphetamine, *d*-fenfluramine, and reserpine on free and stored 5-hydroxytryptamine in brain and blood. *J Neurochem* 1992;59:1138–44.
- Mateo Y, Pineda J, Meana JJ. Somatodendritic alpha2-adrenoreceptors in the locus coeruleus are involved in the in vivo modulation of cortical noradrenaline release by the antidepressant desipramine. *J Neurochem* 1998;71:790–8.
- McCann UD, Seiden LS, Rubin LJ, Ricaurte GA. Brain serotonin neurotoxicity and primary pulmonary hypertension from fenfluramine and dexfenfluramine. A systematic review of the literature. *JAMA, J Am Med Assoc* 1997;278:666–72.
- McTavish D, Heel RC. Dexfenfluramine. A review of its pharmacological properties and therapeutic potential in obesity (published erratum appears in *Drugs* 1992 Jul.;44(1):8). *Drugs* 1992;43:713–33.
- Mennini T, Fracasso C, Cagnotto A, Bergami A, Frittoli E, Gobbi M, Caccia S, Garattini S. In vitro and in vivo effects of the anorectic agent dexfenfluramine on the central serotonergic neuronal systems of non-human primates. A comparison with the rat. *Naunyn-Schmiedeberg's Arch Pharmacol* 1996;353:641–7.
- Mirovsky Y, Yu Y-L, Wagner GC, Sekowski A, Goldberg M, Fisher H. Novel synergistic treatment of ethanol withdrawal seizures in rats with dopamine and serotonin agonists. *Alcohol Clin Exp Res* 1995;19:160–3.
- Molliver ME, Berger UV, Mamounas LA, Molliver DC, O'Hearn E, Wilson MA. Neurotoxicity of MDMA and related compounds: anatomic studies. *Ann NY Acad Sci* 1990;600:649–61.
- Munzar P, Baumann MH, Shoab M, Goldberg SR. Effects of dopamine and serotonin-releasing agents on methamphetamine discrimination and self-administration in rats. *Psychopharmacology (Berlin)* 1999;141:287–96.
- Nichols DE, Brewster WK, Johnson MP, Oberlender R, Riggs RM.

- Nonneurotoxic tetralin and indan analogues of 3,4-(methylenedioxy) amphetamine (MDA). *J Med Chem* 1990;33:703–10.
- O'Callaghan JP, Miller DB. Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mouse. *J Pharmacol Exp Ther* 1994;270:741–51.
- O'Rourke D, Wurtman JJ, Wurtman RJ, Chebli R, Gleason R. Treatment of seasonal depression with *d*-fenfluramine. *J Clin Psychiatry* 1989;50:343–7.
- Oates HF, Stoker LM, MacCarthy EP, Monaghan JC, Stokes GS. Comparative haemodynamic effects of clonidine and guanfacine. *Arch Int Pharmacodyn Ther* 1978;231:148–56.
- Otani K, Tybring G, Mihara K, Yasui N, Kaneko S, Ohkubo T, Nagasaki T, Sugawara K. Correlation between steady-state plasma concentrations of mianserin and trazodone in depressed patients. *Eur J Clin Pharmacol* 1998;53:347–9.
- Paczkowski NJ, Vuocolo HE, Bryan-Lluka LJ. Conclusive evidence for distinct transporters for 5-hydroxytryptamine and noradrenaline in pulmonary endothelial cells of the rat. *Naunyn-Schmiedeberg's Arch Pharmacol* 1996;353:423–30.
- Pedrinola F, Szejnszajd C, Lima N, Halpern A, Medeiros-Neto G. The addition of dexfenfluramine to fluoxetine in the treatment of obesity: a randomized clinical trial. *Obes Res* 1996;4:549–54.
- Pettibone DJ, Williams M. Serotonin-releasing effects of substituted piperazines in vitro. *Biochem Pharmacol* 1984;33:1531–5.
- Pinder RM, Brogden RN, Sawyer PR, Speight TM, Avery GS. Fenfluramine: a review of its pharmacological properties and therapeutic efficacy in obesity. *Drugs* 1975;10:241–323.
- Price LH, Charney DS, Delgado PL, Heninger GR. Fenfluramine augmentation in tricyclic-refractory depression. *J Clin Psychopharmacol* 1990;10:312–7.
- Raleigh MJ, Brammer GL, Ritvo ER, Geller E, McGuire MT, Yuwiler A. Effects of chronic fenfluramine on blood serotonin, cerebrospinal fluid metabolites, and behavior in monkeys. *Psychopharmacology* 1986;90:503–8.
- Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, Ganapathy V, Blakely RD. Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. *Proc Natl Acad Sci USA* 1993;90:2542–6.
- Redmon JB, Raatz S, Bantle JP. Valvular heart disease associated with fenfluramine-phentermine. *N Engl J Med* 1997;337:1772–5.
- Redmon JB, Raatz SK, Kwong CA, Swanson JE, Thomas W, Bantle JP. Pharmacologic induction of weight loss to treat Type 2 diabetes. *Diabetes Care* 1999;22:896–903.
- Rich S, Rubin L, Walker AM, Schneeweiss S, Abenham L. Anorexigens and pulmonary hypertension in the United States: results from the Surveillance of North American Pulmonary Hypertension. *Chest* 2000;117:870–4.
- Rickels K, Hesbacher P, Fisher E, Perloff MM, Rosenfeld H. Emotional symptomatology in obese patients treated with fenfluramine and dextroamphetamine. *Psychol Med* 1976;6:623–30.
- Robinson PH, Checkley SA, Russell GF. Suppression of eating by fenfluramine in patients with bulimia nervosa. *Br J Psychiatry* 1985;146:169–76.
- Rothman RB. Treatment of alcohol and cocaine addiction by the combination of pemoline and fenfluramine: a preliminary case series. *J Subst Abuse Treat* 1995;12:449–53.
- Rothman RB, Baumann MH. Neurochemical mechanisms of phentermine and fenfluramine: therapeutic and adverse effects. *Drug Dev Res* 2000;51:52–65.
- Rothman RB, Ayestas MA, Dersch CM, Baumann MH. Aminorex, fenfluramine, and chlorphentermine are serotonin transporter substrates: implications for primary pulmonary hypertension. *Circulation* 1999;100:869–75.
- Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufisein S, Roth BL. Evidence for possible involvement of 5-HT_{2B} receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation* 2000a;102:2836–41.
- Rothman RB, Partilla JS, Baumann MH, Dersch CM, Carroll FI, Rice KC. Neurochemical neutralization of methamphetamine with high affinity non-selective inhibitors of biogenic amine transporters: a pharmacological strategy for treating stimulant abuse. *Synapse* 2000b;35:222–7.
- Rothman RB, Redmon JB, Raatz SK, Kwong CA, Swanson JE, Bantle JP. Chronic treatment with phentermine combined with fenfluramine lowers plasma serotonin. *Am J Cardiol* 2000c;85:913–5.
- Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI, Partilla JS. Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse* 2001;39:32–41.
- Rubin LJ. Primary pulmonary hypertension. *N Engl J Med* 1997;336:111–7.
- Rudnick G, Clark J. From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. *Biochim Biophys Acta* 1993;1144:249–63.
- Rutter JJ, Gundlach C, Auerbach SB. Systemic uptake inhibition decreases serotonin release via somatodendritic autoreceptor activation. *Synapse* 1995;20:225–33.
- Scheen AJ, Lefebvre PJ. Antiobesity pharmacotherapy in the management of Type 2 diabetes. *Diabetes Metab Res Rev* 2000;16:114–24.
- Schnabel KF, Schulz V, Busch S, Just H. Drug-induced primary vascular pulmonary hypertension. *Med Welt* 1976;27:1300–3.
- Schoeppe W, Brecht HM. Guanfacine in essential hypertension: effect on blood pressure, plasma noradrenaline concentration, and plasma renin activity. *Br J Clin Pharmacol* 1980;10(Suppl. 1):97S–101S.
- Seiden LS, Sabol KE. Methamphetamine and methylenedioxymethamphetamine neurotoxicity: possible mechanisms of cell destruction. *NIDA Res Monogr* 1996;163:251–76.
- Sherman J, Factor DC, Swinson R, Darjes RW. The effects of fenfluramine (hydrochloride) on the behaviors of fifteen autistic children. *J Autism Dev Disord* 1989;19:533–43.
- Smith JE, Lakoski JM. Electrophysiological effects of fluoxetine and duloxetine in the dorsal raphe nucleus and hippocampus. *Eur J Pharmacol* 1997;323:69–73.
- Sotelo C. Immunohistochemical study of short- and long-term effects of DL-fenfluramine on the serotonergic innervation of the rat hippocampal formation. *Brain Res* 1991;541:309–26.
- Stahl SM. Serotonin: it's possible to have too much of a good thing. *J Clin Psychiatry* 1997;58:520–1.
- Stubbs EG, Budden SS, Jackson RH, Terdal LG, Ritvo ER. Effects of fenfluramine on eight outpatients with the syndrome of autism. *Dev Med Child Neurol* 1986;28:229–35.
- Thomson JR, Machado RD, Pauciulo MW, Morgan NV, Humbert M, Elliott GC, Ward K, Yacoub M, Mikhail G, Rogers P, Newman J, Wheeler L, Higenbottam T, Gibbs JS, Egan J, Crozier A, Peacock A, Allcock R, Corris P, Loyd JE, Trembach RC, Nichols WC. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family. *J Med Genet* 2000;37:741–5.
- Uhl GR, Johnson PS. Neurotransmitter transporters: three important gene families for neuronal function. *J Exp Biol* 1994;196:229–36.
- Vickers SP, Clifton PG, Dourish CT, Tecott LH. Reduced satiating effect of *d*-fenfluramine in serotonin 5-HT(2C) receptor mutant mice. *Psychopharmacology (Berlin)* 1999;143:309–14.
- Wang Y, Joharchi N, Fletcher PJ, Sellers EM, Higgins GA. Further studies to examine the nature of dexfenfluramine-induced suppression of heroin self-administration. *Psychopharmacology (Berlin)* 1995;120:134–41.
- Ward NG, Ang J, Pavinich G. A comparison of the acute effects of dextroamphetamine and fenfluramine in depression. *Biol Psychiatry* 1985;20:1090–7.
- Weintraub M, Bray GA. Drug treatment of obesity. *Med Clin North Am* 1989;73:237–49.
- Weintraub M, Hasday JD, Mushlin AI, Lockwood DH. A double-blind clinical trial in weight control. Use of fenfluramine and phentermine alone and in combination. *Arch Intern Med* 1984;144:1143–8.
- Weir EK, Reeve HL, Huang JM, Michelakis E, Nelson DP, Hampl V, Archer SL. Anorexic agents aminorex, fenfluramine, and dexfenflu-

- ramine inhibit potassium current in rat pulmonary vascular smooth muscle and cause pulmonary vasoconstriction. *Circulation* 1996;94: 2216–20.
- Weissman NJ, Tighe JFJ, Gottdeiner JS, Gwynne JT. An assessment of heart-valve abnormalities in obese patients taking dexfenfluramine, sustained-release dexfenfluramine, or placebo. *N Engl J Med* 1998;339: 725–32.
- Willey KA, Molyneaux LM, Overland JE, Yue DK. The effects of dexfenfluramine on blood glucose control in patients with Type 2 diabetes. *Diabetes Med* 1992;9:341–3.
- Willey KA, Molyneaux LM, Yue DK. Obese patients with Type 2 diabetes poorly controlled by insulin and metformin: effects of adjunctive dexfenfluramine therapy on glycaemic control. *Diabetes Med* 1994;11: 701–4.
- Wilson AW, Neill JC, Costall B. An investigation into the effects of 5-HT agonists and receptor antagonists on ethanol self-administration in the rat. *Alcohol* 1998;16:249–70.
- Wrona MZ, Dryhurst G. Oxidation of serotonin by superoxide radical: implications to neurodegenerative brain disorders. *Chem Res Toxicol* 1998;11:639–50.
- Yu Y-L, Fisher H, Sekowski A, Wagner GC. Amphetamine and fenfluramine suppress ethanol intake in ethanol-dependent rats. *Alcohol* 1997;14:45–8.
- Zaczek R, Battaglia G, Culp S, Appel NM, Contrera JF, De Souza EB. Effects of repeated fenfluramine administration on indices of monoamine function in rat brain: pharmacokinetic, dose response, regional specificity, and time course data. *J Pharmacol Exp Ther* 1990;253:104–12.
- Zohar J, Westenberg HG. Anxiety disorders: a review of tricyclic antidepressants and selective serotonin reuptake inhibitors. *Acta Psychiatr Scand, Suppl* 2000;403:39–49.