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CHAPTER 19

Dopamine/serotonin releasers as medications for stimulant addictions

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Abstract: The use of 'agonist therapy' for cocaine and methamphetamine addiction involves administration of stimulant-like medications (e.g. monoamine releasers) to reduce withdrawal symptoms and prevent relapse. A significant problem with this strategy is that many candidate medications possess abuse liability due to activation of mesolimbic dopamine (DA) neurons in the brain. One way to reduce DA-mediated abuse liability of candidate drugs might be to add in serotonin (5-HT)-releasing properties, since substantial evidence shows that 5-HT neurons provide an inhibitory influence over mesolimbic DA neurons. This chapter addresses several key issues related to the development of dual DA/5-HT releasers for the treatment of substance use disorders. First, we briefly summarize the evidence supporting a dual deficit in DA and 5-HT function during withdrawal from chronic cocaine or alcohol abuse. Second, we discuss data demonstrating that 5-HT release can dampen DA-mediated stimulant effects, and the 'anti-stimulant' role of 5-HT_{2C} receptors is considered. Next, the mechanisms underlying potential adverse effects of 5-HT releasers are described. Finally, we discuss recently published data with PAL-287, a novel non-amphetamine DA/5-HT-releasing agent that suppresses cocaine self-administration but lacks positive reinforcing properties. It is concluded that DA/5-HT releasers could be useful therapeutic adjuncts for the treatment of cocaine and alcohol addictions as well as for obesity, attention deficit disorder and depression.

Keywords: alcohol; amphetamine; cocaine; dopamine; serotonin; treatment; transporter

Introduction

A main goal of this chapter is to review data from our laboratory pertaining to the development of dual dopamine (DA)/serotonin (5-HT) releasers as medications for stimulant addiction and possibly alcohol addiction (Rea et al., 1998; Wojnicki et al., 1999; Baumann et al., 2000, 2001; Rothman and Baumann, 2003; Rothman et al., 2005; Wee et al., 2005). A secondary goal is to integrate our findings with the existing literature to provide a conceptual framework for the design of new medications for addiction disorders. Within the context of this chapter, the term 'stimulant' refers to drugs such as cocaine and amphetamines that produce a spectrum of effects in humans, including cardiovascular stimulation, mood elevation and a decreased need for sleep. At high doses, or after longer periods of use, stimulants can cause a range of adverse effects, such as disordered thoughts and

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psychotic episodes. In laboratory animals, stimulants increase locomotor activity and are readily self-administered due to their powerful reinforcing properties. Figure 1 depicts the chemical structures of drugs mentioned in this chapter. Many of these drugs are useful medications with long histories of efficacy and safety while others are highly addictive substances associated with considerable morbidity and mortality (Musto, 1992; Das, 1993; Anonymous, 1995; Gonzalez Castro et al., 2000). In some cases, as with amphetamine itself, the same drug can be a therapeutic entity or an abused substance, depending upon the context in which the drug is administered (Arnsten, 2006; Greenhill, 2006).

Most stimulant drugs interact with monoamine neurons in the central nervous system (CNS). Neurons that synthesize, store and release monoamine transmitters — norepinephrine (NE), dopamine (DA) and serotonin (5-HT) — are widely distributed in the mammalian CNS. These neurons express specialized plasma membrane proteins that function to transport previously released transmitter molecules from the extracellular space back into the cytoplasm (Amara and Kuhar, 1993; Masson et al., 1999). It is well established that distinct transporter proteins are expressed by each type of monoamine neuron: NE transporters (NET), DA transporters (DAT) and 5-HT transporters (SERT) are associated with NE, DA and 5-HT neurons, respectively. These proteins belong to a superfamily of Na^+/Cl^--dependent transporters that share genetic, morphological and functional homologies (Uhl and Johnson, 1994; Torres and Amara, 2007). Under normal circumstances, the transporter-mediated uptake of monoamine transmitters is the principal mechanism for inactivation of monoamine signalling in the brain. Consequently, drugs that interact with monoamine transporters have profound effects on CNS function, and these effects can be beneficial or detrimental depending upon the dose, route and formulation of the drug administered (Amara and Sonders, 1998; Iversen, 2006).

Drugs that target transporter proteins can be divided into two classes based on their precise mechanism of action:reuptake inhibitors and substrate-type releasers (Rothman and Baumann, 2003). Reuptake inhibitors bind to transporter proteins but are not transported. These drugs elevate extracellular transmitter concentrations by blocking transporter-mediated recapture of

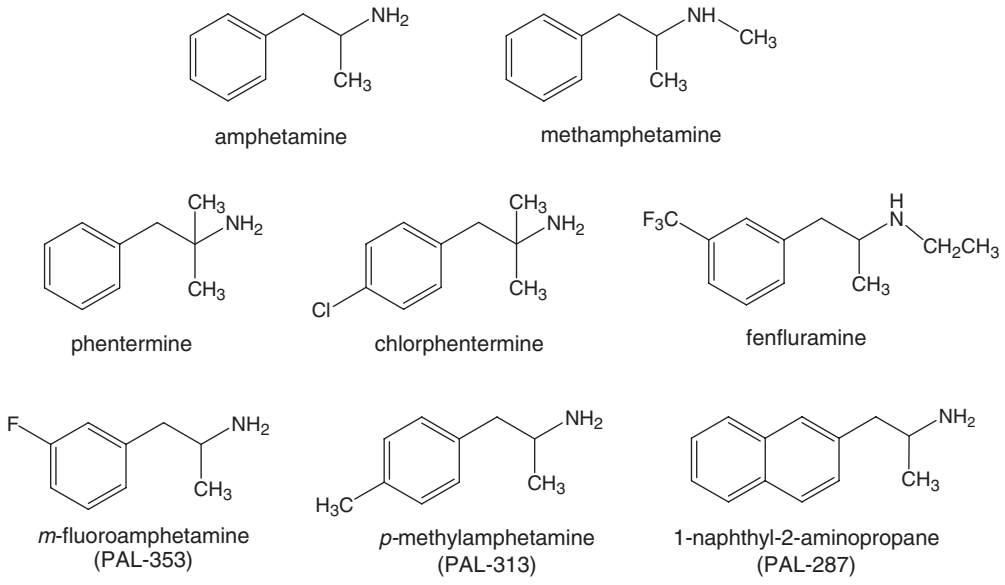

Fig. 1. Chemical structures of stimulants discussed in this chapter.

transmitter molecules from the synapse. Substrate-type releasers bind to transporter proteins and are subsequently transported into the cytoplasm of nerve terminals. Thus, transmitter 'releasers' are often referred to as transporter 'substrates'. 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 via interactions with the vesicular monoamine transporter 2 (VMAT2) (Rudnick, 1997; Fleckenstein et al., 2007). The molecular mechanisms underlying transporter-mediated transmitter release are not completely understood, but ionic currents, oligomerization and reversal of normal transport function appear to be involved (Sitte and Freissmuth, 2003; Blakely et al., 2005; Sulzer et al., 2005). Because substrate-type releasing agents must be transported into nerve terminals to promote transmitter release, reuptake inhibitors can block the effects of releasers.

A dual deficit model of stimulant addiction

The use of stimulants such as cocaine and methamphetamine produces a 'high' or 'rush' that is likely mediated by elevations in extracellular DA levels in mesolimbic circuits (Volkow et al., 2002; Di Chiara et al., 2004), although some evidence indicates that elevations in extracellular NE may also contribute (Rothman et al., 2001; Alexander et al., 2005). Similarly, alcohol-induced increases in extracellular DA are thought to underlie the positive reinforcing effects of this commonly abused substance (Koob et al., 1998; Koob, 2003). Repeated misuse of stimulants, especially when they are self-administered via the smoked or intravenous routes, can lead to serious addiction in susceptible individuals. The chronic abuse of stimulants and alcohol, despite negative consequences, causes long-term changes in neurochemistry and brain circuitry via processes of synaptic plasticity (Volkow and Li, 2004; Hyman, 2005; Kalivas and O'Brien, 2008).

Preclinical and human research findings demonstrate that withdrawal from stimulant and alcohol abuse is associated with deficits in DA and 5-HT function. For example, rats withdrawn from chronic cocaine or alcohol administration display decreased levels of extracellular DA and 5-HT in the nucleus accumbens (Parsons et al., 1991, 1995; Rossetti et al., 1992; Weiss et al., 1996). Human brain imaging studies show that cocaine addicts have reductions in evoked DA release and a loss of DA D_2 receptors in the striatum (Volkow et al., 1997, 2002, Martinez et al., 2007). Neuroendocrine responsiveness to 5-HT releasers is diminished in rats withdrawn from repeated cocaine injections (Levy et al., 1994; Baumann et al., 1995a), and similar findings have been reported in abstinent human cocaine addicts (Haney et al., 2001; Ghitza et al., 2007). Taken together, these data suggest that a cardinal feature of withdrawal from chronic cocaine, and possibly alcohol, is decreased synaptic levels of DA and 5-HT in critical brain circuits.

Additional clinical support for the existence of 5-HT deficits in cocaine addicts is the occurrence of symptoms resembling major depression during abstinence (Dackis and Gold, 1985; Gawin and Kleber, 1986), coupled with an increased prevalence of suicidal ideation and suicide attempts (Garlow et al., 2003). The well-accepted importance of 5-HT dysfunction in mediating depression and suicide (for review, see Mann, 2003) suggests a parallel role for decreased synaptic 5-HT in cocaine and alcohol withdrawal states (Lesch, 2005). Indeed, the spectrum of symptoms often reported by patients withdrawing from stimulant or alcohol use — depressed mood, suicidal ideations, obsessive thoughts, intense craving, anhedonia, increased impulsivity and susceptibility to drug-related cues — presumably reflects long-term changes in brain function and structure. We speculate that deficits in monoamine systems underlie at least some of the symptoms experienced during withdrawal (for review, see Baumann and Rothman, 1998b).

In particular, we have proposed a dual deficit model of stimulant addiction in which drug-induced DA and 5-HT dysfunction contributes to withdrawal symptoms, drug craving and relapse (Baumann and Rothman, 1998a, b; Rothman

et al., 1998; Baumann et al., 2000). Depicted diagrammatically in Fig. 2, the dual deficit model postulates that decreased synaptic DA during stimulant withdrawal underlies anhedonia and psychomotor retardation, whereas decreased synaptic 5-HT gives rise to depressed mood, obsessive thoughts and lack of impulse control. Consistent with this model, rats receiving repeated injections of abused stimulants exhibit neurobiological changes similar to those observed in human patients with major depression (Markou and Koob, 1991; Baumann et al., 1995a; Baumann and Rothman, 1998a; Lin et al., 1999). If abstinent stimulant addicts exhibit DA and 5-HT deficits, medications capable of correcting abnormalities in DA and 5-HT function might be effective in treating stimulant and alcohol dependence.

In agreement with the dual deficit hypothesis, drugs that release DA (phentermine, amphetamine) or 5-HT (fenfluramine) display properties consistent with the effective treatment of substance use disorders (Rothman et al., 1994, 1998; Yu et al., 1997; Halladay et al., 1999). For instance, acute or chronic administration of low doses of DA releasers, such as D-amphetamine, decreases cocaine self-administration behaviour in rhesus monkeys (Glowa et al., 1995; Negus and Mello, 2003a, b). The data in Fig. 3 demonstrate that the DA-releasing agent phentermine suppresses responding for cocaine injections without affecting food-reinforced behaviour, and this effect is maintained by daily administration of phentermine (Wojnicki et al., 1999). Such preclinical results provide a rationale for using DA releasers as medications for treating cocaine addiction (Rothman et al., 2002; Grabowski et al., 2004b; Lile, 2006).

Under certain conditions, the 5-HT releaser fenfluramine decreases responding for cocaine in rhesus monkeys as well (Negus et al., 2007). Combined administration of phentermine plus fenfluramine produces a 75% decrease in cocaine self-administration in monkeys (Glowa et al., 1997). The mixture of phentermine and

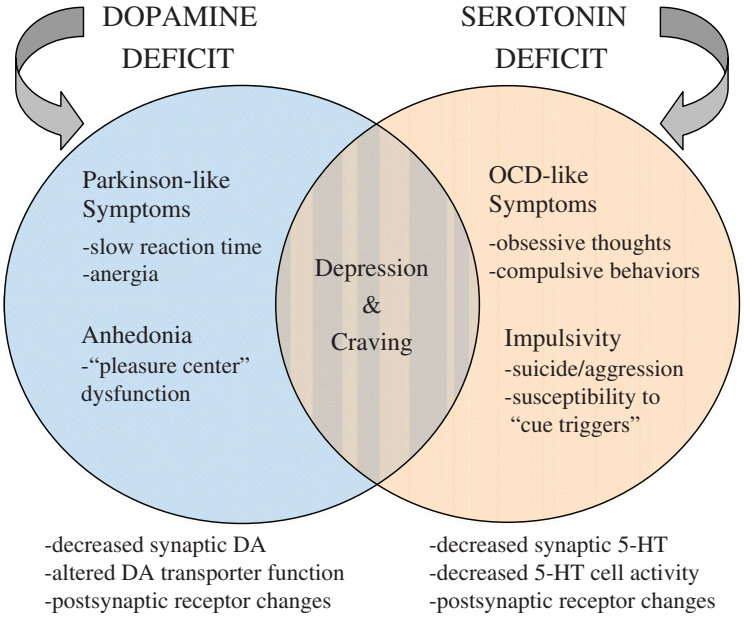

Fig. 2. The dual deficit model of stimulant addiction. According to the model, withdrawal from chronic stimulant use leads to decreased synaptic availability of DA and 5-HT. This dual deficit contributes to withdrawal symptoms, drug craving and relapse. DA dysfunction underlies anhedonia and psychomotor disturbances, whereas 5-HT dysfunction causes depressed mood, obsessive thoughts and lack of impulse control. Protracted withdrawal phenomena are postulated to contribute significantly to relapse. Adapted with permission from Rothman and Baumann (2003).

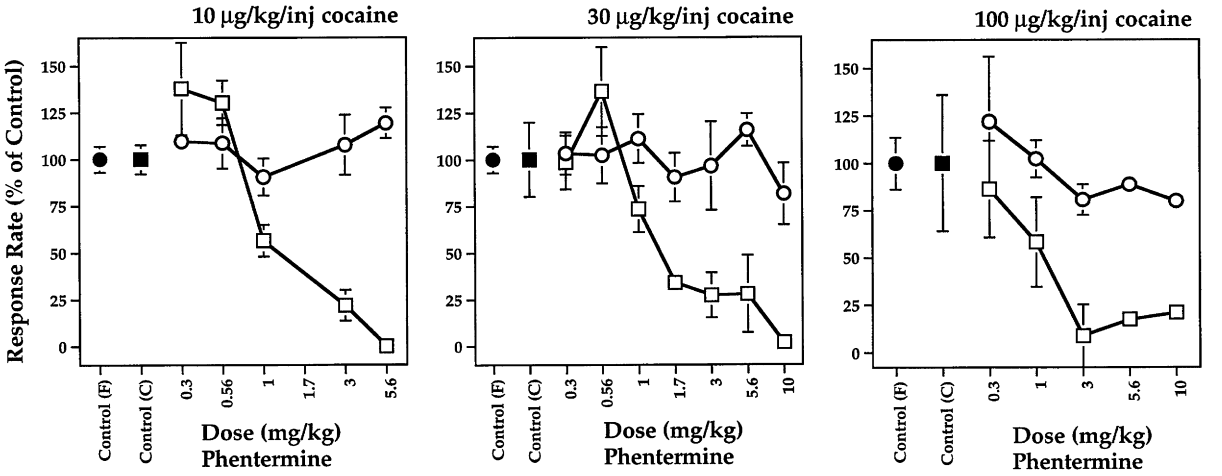

Fig. 3. Acute effects of phentermine on rates of responding maintained under a fixed ratio (FR) 30 schedule of food (○) or cocaine (□) reinforcement. Different unit doses of intravenous cocaine, 10–100 μg/kg/injection, are indicated. Phentermine was administered intravenously. Effects on responding are mean ± SEM, expressed as percentage of the individual control rates of responding for $N = 3$–4 monkeys. Control variability (filled symbols) is expressed as the average of individual coefficients of variation. Data taken with permission from Wojnicki et al. (1999).

D-fenfluramine reduces cocaine self-administration by 80% in rats, yet this mixture is not self-administered (Glatz et al., 2002). Importantly, 5-HT-releasing agents suppress cue-elicited cocaine-seeking behaviour in rats (Burmeister et al., 2003) and decrease cocaine craving in cocaine-dependent human patients (Buydens-Branchey et al., 1998). The collective findings suggest that combined treatment with DA and 5-HT releasers may have a greater therapeutic value than treatment with either drug alone, in terms of decreasing stimulant self-administration and reducing cue-induced relapse. Moreover, a growing body of evidence shows that DA/5-HT-releasing agents may provide similar therapeutic benefits for alcohol dependence (Yu et al., 1997; Halladay et al., 1999, 2006).

5-HT release counteracts stimulant effects of DA release

The use of stimulant-like medications to treat stimulant addictions is an approach known as 'agonist' therapy. This strategy involves administering medications that are less potent and less addictive than cocaine or methamphetamine, but that decrease stimulant abuse because of shared neurochemical properties with the abused drugs (Gorelick, 1998). Accordingly, we have described agonist therapy as neurochemical 'normalization' therapy — that is, the stimulant medication serves to normalize neurochemical deficits caused by chronic exposure to the abused stimulant (Rothman et al., 2002; Rothman and Baumann, 2003). Neurochemical normalization therapy has generated effective treatments for nicotine dependence (Henningfield, 1995; Rollema et al., 2007) and opioid dependence (Ling et al., 1994; White and Lopatko, 2007). This approach has been explored for the treatment of cocaine dependence as well (Alim et al., 1995; Grabowski et al., 1997; Kampman et al., 2000; Walsh et al., 2000), and a number of placebo-controlled trials have shown promising results (Grabowski et al., 2001, 2004a; Shearer et al., 2003). A significant limitation of this strategy, however, is that candidate medications often exhibit inherent abuse liability due to activation of mesolimbic DA neurons in the brain (for review, see Grabowski et al., 2004b).

One feasible means to decrease the abuse liability of candidate medications is to add 5-HT-releasing properties to these drugs. Several lines of evidence support the hypothesis that elevations in

synaptic 5-HT counteract the stimulant and reinforcing effects mediated by elevations in synaptic DA (Czoty et al., 2002; Daw et al., 2002; Higgins and Fletcher, 2003; Burmeister et al., 2004). Administration of the 5-HT precursor L-tryptophan, which increases 5-HT synthesis and release in the CNS, decreases self-administration of cocaine and amphetamine in rats (Smith et al., 1986; McGregor et al., 1993). Likewise, pretreatment with 5-HT reuptake inhibitors can reduce intravenous cocaine self-administration in rats and squirrel monkeys (Carroll et al., 1990; Howell and Byrd, 1995). Cocaine analogues that have potent affinity at SERT support less self-administration behaviour than analogues with weak affinity for SERT (Roberts et al., 1999; Howell et al., 2007). Consistent with these findings, agents that broadly activate brain 5-HT systems can reduce self-administration of stimulants and other drugs of abuse (Higgins and Fletcher, 2003). The 'anti-stimulant' effects of increasing extracellular 5-HT are readily observed after combined administration of 5-HT and DA releasers, or after administration of single agents that release both neurotransmitters.

As summarized in Table 1, drugs that release [^{3}H]DA more potently than [^{3}H]5-HT in vitro (e.g. amphetamine and phentermine) increase endogenous extracellular DA more than extracellular 5-HT in vivo. Such indirect DA agonists are strong locomotor stimulants and support self-administration behaviour. Drugs that release [^{3}H]5-HT more potently than [^{3}H]DA in vitro (e.g. fenfluramine and chlorphentermine) increase endogenous extracellular 5-HT more than extracellular DA. Such indirect 5-HT agonists produce minimal motor activity and do not support self-administration behaviour. The anti-stimulant effect of 5-HT releasers is also seen in the conditioned place preference (CPP) assay as shown in Fig. 4, where a low dose of fenfluramine greatly reduces the positive CPP induced by phentermine.

The precise mechanisms responsible for anti-stimulant effects of 5-HT releasers have not been characterized, but increases in extracellular 5-HT would be expected to activate multiple 5-HT receptor subtypes known to modulate DA function (Muller and Huston, 2006; Alex and Pehek, 2007). As mentioned already, stimulant and reinforcing properties of drugs such as cocaine and methamphetamine are mediated via the activation of mesolimbic DA neurons. Cell bodies of mesolimbic DA neurons reside in the midbrain ventral tegmental area (VTA) and send axonal projections to many regions of the forebrain, most notably the nucleus accumbens (Ungerstedt, 1971; Moore and Bloom, 1978). The nucleus accumbens is a critical limbic–motor interface receiving

Table 1. Summary of serotonergic and dopaminergic effects of selected releasing agents

Drug	[^{3}H]5-HT release EC_{50} (nM)	[^{3}H]DA release EC_{50} (nM)	Peak % increase in dialysate 5-HT (dose, mg/kg)	Peak % increase in dialysate DA (dose, mg/kg)	Self-administered	Locomotor activation
Amphetamine[a]	1756	8.0	45 (0.3 mg/kg i.p.)	224 (0.3 mg/kg i.p.)	Yes	Strong
Phentermine[a]	3511	262	32 (1.0 mg/kg i.p.)	156 (1.0 mg/kg i.p.)	Yes	Strong
PAL-353[c]	1937	24.2	170 (1.0 mg/kg i.v.)	432 (1.0 mg/kg i.v.)	Yes	Strong
Fenfluramine[a]	79.3	>10,000	215 (1.0 mg/kg i.p.)	20 (1.0 mg/kg i.p.)	No	None
Chlorphentermine[a]	30.9	2650	228 (1.0 mg/kg i.p.)	86 (1.0 mg/kg i.p.)	No	None
Phentermine + fenfluramine[a]	N/A	N/A	222 (1.0 + 1.0 mg/kg i.p.)	144 (1.0 + 1.0 mg/kg i.p.)	No	Weak
PAL-313[c]	53.4	44.1	544 (1.0 mg/kg i.v.)	130 (1.0 mg/kg i.v.)	Weak	Weak
PAL-287[b]	3.4	12.6	464 (1.0 mg/kg i.v.)	133 (1.0 mg/kg i.v.)	No	Weak

A summary of data illustrating the tendency for increasing extracellular 5-HT to reduce reinforcing and locomotor effects mediated by increases in extracellular DA. Microdialysis data unpublished. This table originally appeared in Rothman et al. (2007).
[a]From Baumann et al. (2000) and Rothman et al. (2001).
[b]From Rothman et al. (2005).
[c]From Wee et al. (2005).

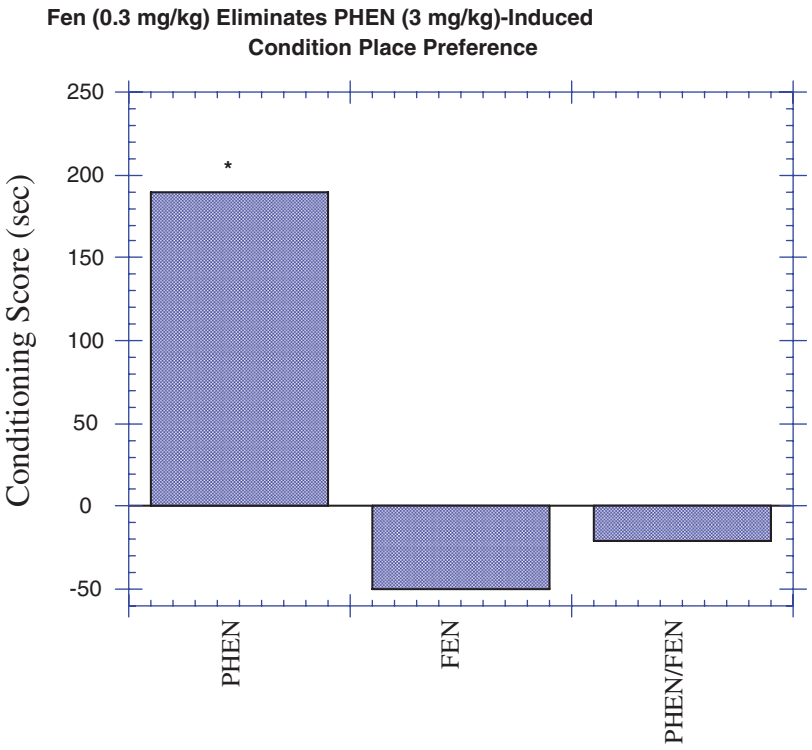

Fig. 4. Effects of phentermine (3 mg/kg) and fenfluramine (0.3 mg/kg), given alone or in combination, on conditioned place preference. Conditioning score represents the mean difference between time (s) spent in the drug vs. vehicle-paired side of the test chamber. All drugs were administered i.p. Each column represents the mean of $N = 9$–10 rats. * = Significant place preference (Wilcoxon test, $P < 0.05$). Data taken with permission from Rea et al. (1998).

afferent inputs from the prefrontal cortex, hippocampus and amygdala, while sending efferent outputs to the ventral pallidum and other regions known to modulate locomotor centres in the brainstem (Mogenson et al., 1980; Pennartz et al., 1994). 5-HT neurons in the midbrain raphe nuclei send axonal projections that densely innervate the mesolimbic system at the level of the VTA and the nucleus accumbens (Steinbusch, 1981; Molliver, 1987). Furthermore, 5-HT neurons innervate various regions providing excitatory afferents to the accumbens (e.g. prefrontal cortex). In this manner, 5-HT nerve terminals are uniquely positioned to influence the activity of mesolimbic DA neurons at multiple levels.

The serotonergic modulation of DA function is inherently complex due to the presence of at least 14 different 5-HT receptor subtypes in the CNS (Barnes and Sharp, 1999; Hoyer et al., 2002). While most 5-HT receptor subtypes enhance DA transmission (Muller and Huston, 2006; Alex and Pehek, 2007), 5-HT_{2C} receptors provide a strong inhibitory influence on mesolimbic DA neurons (Di Matteo et al., 2001; Bubar and Cunningham, 2006). For instance, systemic administration of the 5-HT_{2C} agonist Ro 60-0175 markedly inhibits DA cell firing in the VTA and decreases extracellular levels of DA in forebrain projection areas (Di Matteo et al., 1999, 2000; Gobert et al., 2000). Pretreatment with Ro 60-0175 reduces locomotor activity and self-administration behaviour produced by cocaine, whereas pretreatment with the 5-HT_{2C} antagonist SB 242084 has the opposite effect (Grottick et al., 2000; Fletcher et al., 2002). In fact, SB 242084 when given alone increases burst firing of DA cells in the VTA, suggesting that 5-HT_{2C} receptors provide tonic inhibition of mesolimbic DA activity. Collectively, these

findings indicate a potential role for 5-HT_{2C} receptors in mediating the anti-stimulant effects of 5-HT releasers (Higgins and Fletcher, 2003).

Recent data show that anti-stimulant effects of 5-HT_{2C} receptor activation involve at least two separate mechanisms — one mechanism in the VTA and another in the prefrontal cortex. Microinjection of Ro 60-0175 into the VTA blocks behavioural effects of cocaine (Fletcher et al., 2004), perhaps reflecting inhibition of DA cell firing and release as noted above (Di Matteo et al., 2001). Microinjection of Ro 60-0175 into the prefrontal cortex also markedly reduces cocaine-induced locomotor activity (Filip and Cunningham, 2003), and this action may involve suppression of excitatory glutamate outputs to the nucleus accumbens (Liu et al., 2007). Neuroanatomical evidence suggests that the effects of 5-HT_{2C} receptor activation in the VTA and cortex are mediated by the stimulation of gamma-aminobutyric acid (GABA) interneurons (Bubar and Cunningham, 2006, 2007; Liu et al., 2007); more research is needed to validate this proposal. Further investigation is warranted to fully elucidate the role of 5-HT_{2C} receptors in mediating anti-stimulant effects of 5-HT releasers. Additionally, the potential of 5-HT_{2C} agonists as medications for substance use disorders deserves to be examined (Higgins and Fletcher, 2003; Bubar and Cunningham, 2006).

Potential adverse effects of 5-HT releasers

The clinical use of 5-HT-releasing agents as medications has been associated with a number of adverse effects (Rothman et al., 1999; Rothman and Baumann, 2002; Zolkowska et al., 2006). Based primarily on experience with D,L-fenfluramine and its more potent isomer D-fenfluramine, three potentially serious side effects need to be considered when 5-HT releasers are developed as treatment agents: valvular heart disease (VHD), idiopathic pulmonary arterial hypertension (IPAH) and neurotoxicity. Fenfluramines were commonly prescribed anorectics until their removal from the market in 1997 due to the occurrence of VHD in some patients (Connolly et al., 1997; Connolly and McGoon, 1999). Fenfluramine-associated VHD is characterized by thickening of valve leaflets and increased regurgitation of blood, most often detected by echocardiography. While initial findings suggested that fenfluramines induce VHD in a high percentage of patients, more recent evidence shows a much smaller risk. For example, a meta-analysis of available clinical data demonstrates that the incidence of clinically significant valvular regurgitation was 12% in fenfluramine-treated patients vs. 6% in untreated controls (Sachdev et al., 2002).

Because fenfluramines are potent 5-HT releasers (Baumann et al., 2000; Rothman et al., 2003a) and 5-HT has established mitogenic effects (Nemecek et al., 1986; Seuwen et al., 1988), investigators initially speculated that serotonergic mechanisms might contribute to VHD (Connolly et al., 1997; Connolly and McGoon, 1999). To this end, we carried out an investigation to determine whether stereoisomers of fenfluramine, or the *N*-dealkylated metabolite norfenfluramine, might activate mitogenic 5-HT receptors (Rothman et al., 2000a). A number of other test drugs were included in these experiments as positive and negative controls. 'Positive controls' were ergot alkaloids known to increase the risk of VHD, such as methysergide, its active metabolite methylergonovine and ergotamine (Bana et al., 1974; Bredberg et al., 1986; Hendrikx et al., 1996). 'Negative controls' were drugs that interacted with monoamine transporters but did not cause VHD, and these drugs included phentermine, fluoxetine and its metabolite norfluoxetine. We also tested the antidepressant trazodone and its active metabolite m-chlorophenylpiperazine (mCPP) as negative controls (Ishida et al., 1995; Otani et al., 1997). mCPP has agonist activity at a wide range of 5-HT receptor subtypes (Hoyer et al., 1994, 2002) and is capable of releasing neuronal 5-HT via a transporter-mediated mechanism similar to that of fenfluramines (Baumann et al., 1993, 2001).

Our working hypothesis was that fenfluramines, norfenfluramines and positive control drugs would share the ability to activate a mitogenic 5-HT receptor subtype expressed in heart valves, while the negative control drugs would not. An initial

receptorome screen led to a detailed evaluation of the binding of these drugs to the 5-HT_2 family of receptors (Rothman et al., 2000a). Table 2 reports binding data, and Table 3 reports the functional effects of these compounds at cloned human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. Interestingly, fenfluramines have low affinity for all 5-HT_2 receptor subtypes. By contrast, we found that norfenfluramines displayed high affinity and efficacy at the 5-HT_{2B} receptor subtype (K_I = 10–50 nM), consistent with the findings of others (Porter et al., 1999; Fitzgerald et al., 2000).

Table 2. K_I values of test drugs at 5-HT_2 receptors

Drug	Human		
	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
(±)-Fenfluramine	5216±423	4134±1281	3183±637
(+)-Fenfluramine	11,107±2303	5099±1173	6245±874
(−)-Fenfluramine	5463±600	5713±2285	3415±922
(±)-Norfenfluramine	2316±278	52.1±21	557±61
(+)-Norfenfluramine	1516±150	11.2±7.3	324±12
(−)-Norfenfluramine	3841±614	47.8±30.6	814±98
Ergotamine	9.0±1.0	3.0±0.4	12±1.5
Methysergide	15.0±4.0	9.1±4.9	1.8±0.2
Methylergonovine	12.6±1.0	0.49±0.16	12.4±1.0
Fluoxetine	299±53	5030±1960	50±10
Norfluoxetine	638±108	5063±1974	286±60
Trazodone	19.8±2.4	73.6±36	402±44
mCPP	391±47	3.2±1.0	59±11
5-HT	614±74	4.0±1.9	12.2±1.3
Phentermine	>10,000	>10,000	>10,000

Values are mean±SD for $n = 3$ experiments. Data taken with permission from Rothman et al. (2000a).

Table 3. Functional activity of test drugs at 5-HT_2 receptors

Drug	Human 5-HT_{2A}		Human 5-HT_{2B}		Human 5-HT_{2C}	
	K_{act} (nM±SD)	V_{max} (percent of 5-HT±SD)	K_{act} (nM±SD)	V_{max} (percent of 5-HT±SD)	K_{act} (nM±SD)	V_{max} (percent of 5-HT±SD)
(±)-Fenfluramine	4131±2448	15±4	ND	ND	ND	ND
(+)-Fenfluramine	>10,000	ND	379±120	38±14	362±109	80±10
(−)-Fenfluramine	5279±998	43±7.2	1248±430	47±5	360±155	84±15
(+)-Norfenfluramine	630±240	88±9	18.4±9	73±6	13±4	100±11
(−)-Norfenfluramine	1565±323	93±9	357±180	71±15	18±9	80±17
Ergotamine	16±4	75±3	9.8±3	56±3	5±3	75±15
Methysergide	3.5±1.7	24±3	150±43	18±4	2.9±1.5	33±3.5
Methylergonovine	1.3±0.4	70±7	0.8±0.5	40±3	2.5±1.2	103±7
Fluoxetine	ND	ND	ND	ND	Antagonist	K_I = 616±172
Norfluoxetine	ND	ND	ND	ND	Antagonist	K_I = 43±17
Trazodone	Antagonist		Antagonist		Antagonist	
mCPP	65±17	55±11	64±27	43±14	0.64±0.3	79±15
5-HT	66±26	100	2.4±1.5	100	0.6±0.18	100
Phentermine	ND		ND		1394±450	66±10

Values are mean±SD for $n = 3$ experiments. Data taken with permission from Rothman et al. (2000a).

Methysergide acts as a partial agonist at the 5-HT_{2B} receptor, while the metabolite methylergonovine has even greater affinity and efficacy. Ergotamine is a potent partial agonist at the 5-HT_{2B} receptor. Among the negative control drugs tested, only mCPP exhibits agonist activity at the 5-HT_{2B} site. It is noteworthy that trazodone binds to the 5-HT_{2B} receptor with moderate affinity but functions as an antagonist. Thus, when trazodone is metabolized to mCPP in vivo (Ishida et al., 1995; Otani et al., 1997), the 5-HT_{2B} actions of mCPP are probably blocked by antagonist actions of the parent compound.

Our results with the various positive control drugs strongly implicate the 5-HT_{2B} receptor as a major culprit in the development of drug-induced VHD, and accumulating data support this hypothesis (Porter et al., 1999; Fitzgerald et al., 2000; Setola and Roth, 2005; Roth, 2007). 5-HT_{2B} receptors are abundantly expressed on aortic and mitral valves (Fitzgerald et al., 2000), and these receptors are known to stimulate mitogenesis (Lopez-Ilasaca, 1998; Hafizi et al., 2000). Further evidence for the role of 5-HT_{2B} receptors in drug-induced VHD is based on the effects of ergot medications such as cabergoline and pergolide. Both of these medications increase the risk of VHD in human patients and are also potent 5-HT_{2B} receptor agonists (for review, see Roth, 2007). Setola et al. (2003) showed that the illicit amphetamine analogue 3,4-methylenedioxymethamphetamine (MDMA) and its *N*-demethylated metabolite, 3,4-methylenedioxyamphetamine (MDA), are 5-HT_{2B} receptor agonists. These drugs stimulate prolonged mitogenic responses in human valvular interstitial cells via activation of 5-HT_{2B} receptors (Setola et al., 2003). As predicted by this study, a recent clinical report found that heavy MDMA users display a significantly higher incidence of valvular regurgitation compared to control subjects (Droogmans et al., 2007). More clinical investigations are needed to clearly establish the link between illicit MDMA use and the risk of developing VHD.

Epidemiological evidence indicates that fenfluramines increase the risk of developing IPAH, a debilitating and incurable disease (Abenhaim et al., 1996; Fishman, 1999). IPAH is characterized by pulmonary arterial vasoconstriction and hyperplasia that leads to severe hypertension. The pathogenesis of IPAH is complex and difficult to study, especially given the rarity of the disorder. Nonetheless, evidence from our laboratory and others implicates the involvement of 5-HT and SERT proteins in the pathogenesis of IPAH (Rothman et al., 1999; MacLean et al., 2000; Eddahibi et al., 2006). We demonstrated that the SERT substrate activity was a common feature of drugs associated with IPAH, but not all substrates increased the risk of the disease (Rothman et al., 1999). It is well established that blood platelets express SERT proteins identical to those expressed on neurons, and platelet SERT accumulates nearly 99% of circulating 5-HT into platelet storage (Ni and Watts, 2006). One hypothesis — the so-called '5-HT hypothesis' — has been invoked as a potential mechanism underlying fenfluramine-induced IPAH (Fishman, 1999; MacLean et al., 2000). This hypothesis postulates that fenfluramines increase the risk of IPAH by stimulating SERT-mediated release of 5-HT from platelets, thereby elevating plasma levels of 5-HT. Persistent elevations in plasma 5-HT would then cause pulmonary dysfunction. It is noteworthy that an analogous 5-HT hypothesis has been invoked to explain VHD, but as discussed already, drug-induced activation of 5-HT_{2B} receptors seems to be the predominant mechanism involved in this disease.

A key prediction of the 5-HT hypothesis is that fenfluramine increases plasma 5-HT to concentrations sufficient to produce vasoconstriction and mitogenesis, which then leads to serious side effects. Despite the widespread acceptance of the 5-HT hypothesis as an explanation for fenfluramine-associated IPAH, the effects of fenfluramine and related agents on plasma 5-HT have received little attention. Studies conducted in the 1990s do not support the 5-HT hypothesis, since they show that acute fenfluramine does not increase plasma 5-HT in rats and chronic fenfluramine treatment lowers blood 5-HT in humans (Martin and Artigas, 1992; Rothman et al., 2000b). Given the uncertainties regarding validity of the 5-HT hypothesis, we assessed the acute effects of fenfluramine and other amphetamines on

plasma levels of 5-HT in rats (Zolkowska et al., 2006). Specifically, we developed a novel microdialysis method to measure plasma levels of 5-HT in whole blood samples obtained from conscious catheterized rats. Using this method, baseline plasma 5-HT levels in rats were found to be 0.22 nM, or about 1 nM when corrected for dialysis probe recovery, which is similar to plasma 5-HT concentrations measured in human subjects (Herve et al., 1995). Importantly, systemic administration of fenfluramine, MDMA and other amphetamines evokes transient dose-dependent increases in plasma 5-HT ranging from 4 to 20 nM. The ability of drugs to increase plasma 5-HT is directly correlated with their ability to increase SERT-mediated 5-HT release in neurons, suggesting the involvement of platelet SERT proteins. These data show that fenfluramine and other 5-HT releasers are able to acutely increase plasma 5-HT, but the absolute levels of 5-HT are well below the concentrations required to contract pulmonary arteries or stimulate mitogenesis (Cortijo et al., 1997; Eddahibi et al., 1999).

From a medication development standpoint, a more relevant issue is whether chronic administration of 5-HT releasers can persistently increase plasma 5-HT. To address this question, we used microdialysis methods to examine the effects of 2-week minipump infusions of fenfluramine (3 and 10 mg/kg/day) or the 5-HT uptake blocker fluoxetine (3 and 10 mg/kg/day) on plasma levels of 5-HT in rats (Zolkowska et al., 2008). In this study, chronic administration of fenfluramine, but not fluoxetine, caused two- to fourfold increases in baseline dialysate 5-HT levels in blood. Given baseline plasma 5-HT concentrations of about 1 nM, fenfluramine-induced increases in plasma 5-HT are less than 5 nM. The data in Fig. 5 show that chronic exposure to fenfluramine or fluoxetine markedly reduces the ability of acute fenfluramine to evoke increases in plasma 5-HT. Thus, chronic exposure to fenfluramine minimizes the surges in plasma 5-HT caused by acute administration of the drug. Chronic minipump infusions of fenfluramine and fluoxetine in rats give rise to steady-state blood levels of drugs and their bioactive metabolites that are similar to those measured in human patients taking prescribed doses of these medications (Rothman et al., 2000b; Lundmark et al., 2001). Thus, our rat model system is relevant to human patients taking fenfluramine or fluoxetine.

It has been shown that 5-HT provokes contraction of human pulmonary arteries at concentrations ranging from 100 nM to 10 μM (Cortijo et al., 1997). The threshold concentration of 5-HT required to stimulate mitogenic responses in cultured human pulmonary artery smooth muscle

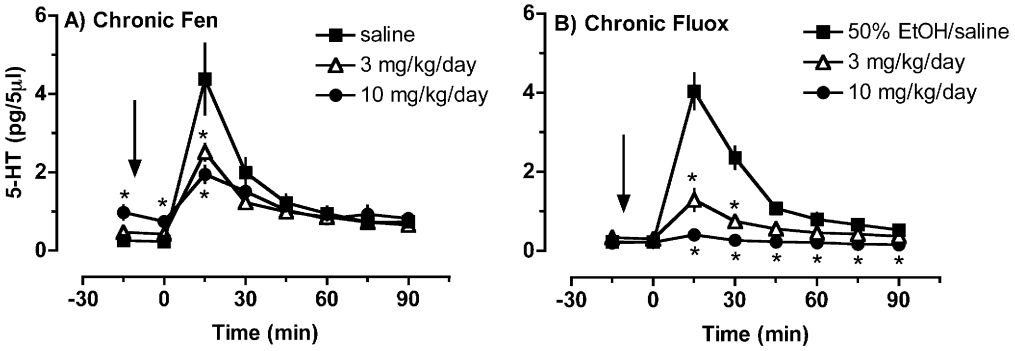

Fig. 5. Effects of acute fenfluramine administration on dialysate 5-HT levels measured in blood from conscious rats previously treated with chronic administration of fenfluramine (Panel A) or fluoxetine (Panel B). For chronic treatments, drugs were dissolved in sterile saline (fenfluramine) or 50% ethanol/saline (fluoxetine) and administered s.c. via osmotic minipumps for 2 weeks. On the day of test, fenfluramine was dissolved in saline and administered i.v. at 0 min. Serial blood samples were withdrawn at 15-min intervals and immediately dialyzed. 5-HT levels are mean ± SEM for $N = 9$ rats/group. * = $P < 0.05$, compared to saline controls at corresponding time points (Duncan's post hoc test). Data taken with permission from Zolkowska et al. (2008).

cells is about 10 nM (Eddahibi et al., 2001; Marcos et al., 2003), while much higher 5-HT concentrations are needed to stimulate mitogenic responses in rat pulmonary artery smooth muscle cells (Pitt et al., 1994; Eddahibi et al., 1999). Because we found that chronic fenfluramine elevates baseline plasma 5-HT to less than 5 nM in rats, it appears that fenfluramine-induced increases in plasma 5-HT are below the concentrations known to cause pulmonary side effects. Fenfluramine-induced elevations of plasma 5-HT are also much lower than those required to produce VHD in rats exposed to exogenous 5-HT (580–974 nM) (Gustafsson et al., 2005). Viewed collectively, these findings demonstrate that the 5-HT hypothesis cannot explain the mechanism of fenfluramine-associated IPAH or VHD. Of course, it is possible that two- to fourfold increases in plasma 5-HT could be enough to stimulate mitogenic responses in susceptible individuals and increase the risk of developing IPAH. However, this scenario seems unlikely, since treatment with lithium or MAO inhibitors produces two- to fourfold increases in plasma 5-HT without increasing the risk of IPAH (Artigas et al., 1989; Celada et al., 1992).

At present, the mechanisms underlying fenfluramine-associated IPAH remain enigmatic. One significant problem is that most animal models of IPAH require the induction of hypoxia, which is not a major factor in humans. Furthermore, the aetiology of fenfluramine-associated IPAH may differ substantially from that of non-drug-related IPAH. Despite these caveats, recent findings have provided novel hypotheses to explain how fenfluramine might cause IPAH. For example, Launay and colleagues (Launay et al., 2002) have provided evidence that activation of 5-HT_{2B} receptors in the lung is critical to the development of IPAH in a hypoxic mouse model. Since the stereoisomers of norfenfluramine are potent and selective 5-HT_{2B} agonists (Rothman et al., 2000a), a role for 5-HT_{2B} receptors seems feasible. On the other hand, a number of medications that produce VHD and activate 5-HT_{2B} receptors do not increase the risk of IPAH; these medications include methysergide, ergotamine, pergolide and cabergoline.

Additional evidence from our laboratory disputes a role for 5-HT_{2B} sites in fenfluramine-associated IPAH (Rothman and Baumann, 2006). Aminorex is a SERT substrate that caused an epidemic of IPAH in the 1960s (Gurtner, 1985; Fishman, 1999), and case reports implicate the related designer drug 4-methylaminorex as a cause of the disease (Gaine et al., 2000). If 5-HT_{2B} receptors are involved in the pathogenesis of drug-associated IPAH, then one would suspect aminorex to target 5-HT_{2B} sites. While aminorex does interact with cloned human 5-HT_{2B} receptors, the half-maximum effective concentration (EC_{50}) of the drug for 5-HT_{2B} receptor activation (870 nM) is 30-fold higher than its EC_{50} for NE release (26.4 nM). Moreover, the activity of aminorex at 5-HT_{2B} sites is nearly 50-fold less than that of D-norfenfluramine. It seems plausible that metabolites of aminorex may act more potently at 5-HT_{2B} receptors, and this possibility deserves to be examined. However, the available data argue against an important role for 5-HT_{2B} receptors in the pathogenesis of anorectic-associated IPAH.

Recent studies by Eddahibi et al. (2006) focus on 5-HT produced locally in the lung as a critical player in pathogenesis of IPAH. These investigators reported that endothelial cells in the pulmonary microvasculature synthesize 5-HT, which is then released as a growth factor. It has been proposed that dysregulation of 5-HT production in endothelial cells, along with over-expression of SERT by the pulmonary artery smooth muscle cells, contributes to hyperplasia observed in IPAH. Immunohistochemical studies show that the pulmonary microvascular endothelium does not express SERT (Eddahibi et al., 2006), indicating that fenfluramine cannot release 5-HT from these cells. Based on the findings of Eddahibi et al., administration of the 5-HT precursor L-5-hydroxytryptophan (5-HTP) would be predicted to increase 5-HT synthesis in pulmonary endothelial cells, since this compound bypasses the rate-limiting enzyme tryptophan hydroxylase. 5-HTP is a commonly used dietary supplement with a well-established history of safety (Das et al., 2004), and its use is not known to increase the risk of IPAH. Although speculative, this observation suggests that an increase in 5-HT synthesis in the pulmonary microvascular

endothelial cells may be necessary, but is not sufficient, to increase the risk of IPAH.

Experiments in laboratory animals show that high-dose administration of fenfluramine or D-fenfluramine can cause long-term depletion of 5-HT and loss of SERT binding sites in the brain (Zaczek et al., 1990; McCann et al., 1997). The persistent nature of fenfluramine-induced 5-HT deficits in the CNS has been interpreted as evidence for neurotoxicity, although this hypothesis and its clinical relevance are still a matter of debate (Rose et al., 1996; Rothman et al., 2003b). The mechanisms underlying fenfluramine-induced 5-HT depletions are not well understood, but acute 5-HT release has been implicated because 5-HT uptake blockers and synthesis inhibitors can prevent long-term 5-HT depletions (Steranka and Sanders-Bush, 1979; Halladay et al., 2001). An important observation is that not all SERT substrates deplete 5-HT (Nichols et al., 1990; Cozzi et al., 1998; Baumann et al., 2001). As noted previously, mCPP interacts with SERT to release 5-HT from neurons, and mCPP is equipotent with D-fenfluramine in this regard (Baumann et al., 1995b, 2001; Eriksson et al., 1999). The data in Fig. 6 demonstrate that repeated high-dose administration of mCPP fails to affect postmortem tissue levels of 5-HT in rat brain whereas fenfluramine causes profound loss of 5-HT. These data indicate that SERT-mediated 5-HT release is separable from long-term 5-HT depletion.

Elucidating the mechanisms responsible for the adverse effects of 5-HT releasers will have important implications for the future development of SERT substrates as pharmacotherapies. The findings reviewed here provide clues for designing dual DA/5-HT releasers devoid of fenfluramine-like adverse effects. In particular, any lead drug molecule must lack 5-HT_{2B} agonist activity to prevent the risk of VHD. Additionally, candidate drugs should be chemically distinct from the phenylethylamine structure shared by amphetamine-like agents, as non-amphetamine 5-HT releasers have a reduced capacity for causing neurotoxic effects and possibly IPAH.

PAL-287, a non-amphetamine DA/5-HT releaser

Partially based on the above rationale, we sought to identify and characterize a non-amphetamine transporter substrate that would release DA and 5-HT, without affecting release of NE. After an extensive evaluation of over 350 compounds, we found it impossible to dissociate NE- and DA-releasing properties, perhaps due to the

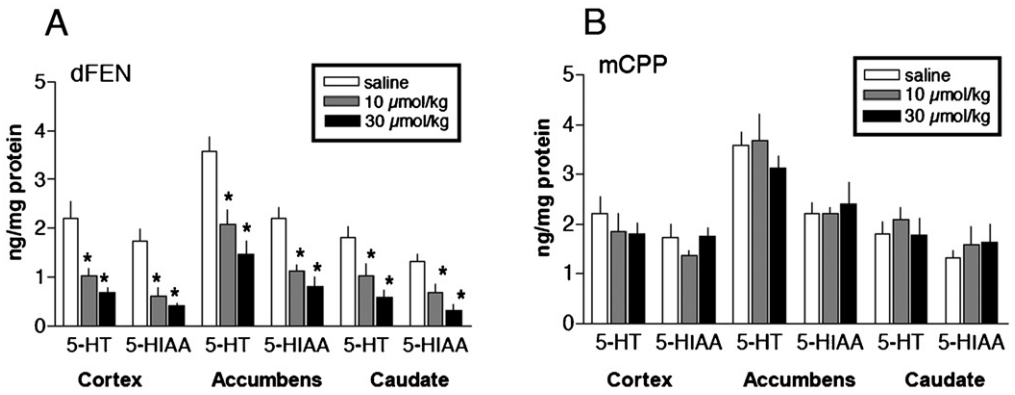

Fig. 6. Effects of high-dose administration of D-fenfluramine (D-FEN) or mCPP on postmortem tissue levels of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in rat brain. D-FEN or mCPP was administered i.p. at doses of 10 or 30 µmol/kg, every 2 h, for four doses. Rats were killed 2 weeks after the dosing regimen. Postmortem tissue levels of 5-HT and 5-HIAA in the prefrontal cortex, nucleus accumbens and caudate nucleus were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). These doses of D-FEN and mCPP produce equivalent increases in extracellular 5-HT. Data are mean ± SEM, expressed as ng/mg protein for $N = 4$–6 rats/group. $^{*}P < 0.05$, compared to saline-treated group (Duncan's post hoc test). Data taken with permission from Baumann et al. (2001).

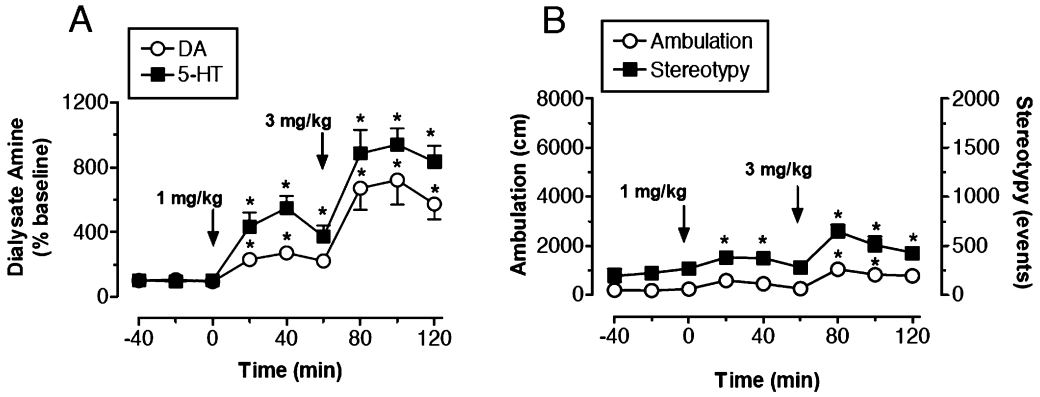

Fig. 7. Effects of PAL-287 on neurochemical and locomotor parameters in rats undergoing in vivo microdialysis in the prefrontal cortex. Rats received i.v. injections of 1 mg/kg PAL-287 at time zero, followed by 3 mg/kg 60 min later. Panel A: Concentrations of DA and 5-HT in dialysate samples are mean ± SEM for $N = 7$ rats/group, expressed as percentage of the baseline. Baseline levels of DA and 5-HT were 0.43 ± 0.07 and 0.27 ± 0.06 pg/5 μl, respectively. Panel B: Locomotor parameters are mean ± SEM for $N = 7$ rats/group, expressed as distance traveled in cm (ambulation) and number of repetitive movements (stereotypy). $^{*}P < 0.05$, compared to pre-injection control (Duncan's post hoc test). Data taken with permission from Rothman et al. (2005).

phylogenetic similarities between NET and DAT. The first lead compound from our search was PAL-287 (1-napthyl-2-aminopropane, see structure in Fig. 1), a novel non-amphetamine monoamine releaser (Rothman et al., 2005). The in vitro potency of PAL-287 at releasing radiolabeled transmitters from DAT, NET and SERT is 12.6 ± 0.4 nM, 11.1 ± 0.9 nM and 3.4 ± 0.2 nM, respectively (see Table 2). Figure 7 shows that administration of PAL-287 to rats increases extracellular 5-HT and DA in a dose-dependent manner, with larger effects on 5-HT compared to DA. Functional studies with cloned human 5-HT_2 receptors reveal that PAL-287 is a full agonist at 5-HT_{2B} receptors ($EC_{50} = 40$ nM) and 5-HT_{2A} receptors ($EC_{50} = 466$ nM). The drug is a potent partial agonist at 5-HT_{2C} receptor sites ($EC_{50} = 2.3$ nM, $E_{MAX} = 20\%$), an effect that suggests possible anorectic actions of PAL-287 (Vickers et al., 1999; Nilsson, 2006). 5-HT_{2C} agonist activity may also contribute to the minimal reinforcing properties of PAL-287 despite potent DA-releasing actions of the drug (see Czoty et al., 2002; Higgins and Fletcher, 2003). The weaker potency of PAL-287 at 5-HT_{2A} and 5-HT_{2B} receptors, as compared to its activity at SERT, suggests that the drug may not activate 5-HT_{2A} and 5-HT_{2B} receptors in vivo.

PAL-287 produces minimal locomotor activation despite substantial elevations in extracellular DA (Fig. 7). In particular, the amount of ambulation produced by 3 mg/kg PAL-287 is one-third the amount produced by 1 mg/kg D-amphetamine, even though both drug treatments cause equivalent DA release. These data suggest that 5-HT-releasing properties of PAL-287 limit the stimulant effects of concurrent DA release. Repeated high-dose administration of PAL-287 to rats (18 mg/kg i.p., every 2 h, for three doses) fails to affect brain tissue 5-HT levels when assessed 2 weeks after injections, unlike D-methamphetamine (6.0 mg/kg i.p., every 2 h, for three doses) and MDMA (7.5 mg/kg i.p., every 2 h, for three doses), which cause significant 5-HT depletions. The data in Fig. 8 show that PAL-287 does not support self-administration behaviour, and chronic administration of the drug decreases cocaine self-administration in rhesus monkeys. A dose of 1.0 mg/kg/h PAL-287 significantly reduces both cocaine- and food-maintained responding, but the suppression of cocaine self-administration is somewhat greater than the reduction in food-maintained responding.

Our results with PAL-287 confirm the hypothesis that a non-amphetamine substrate at DAT

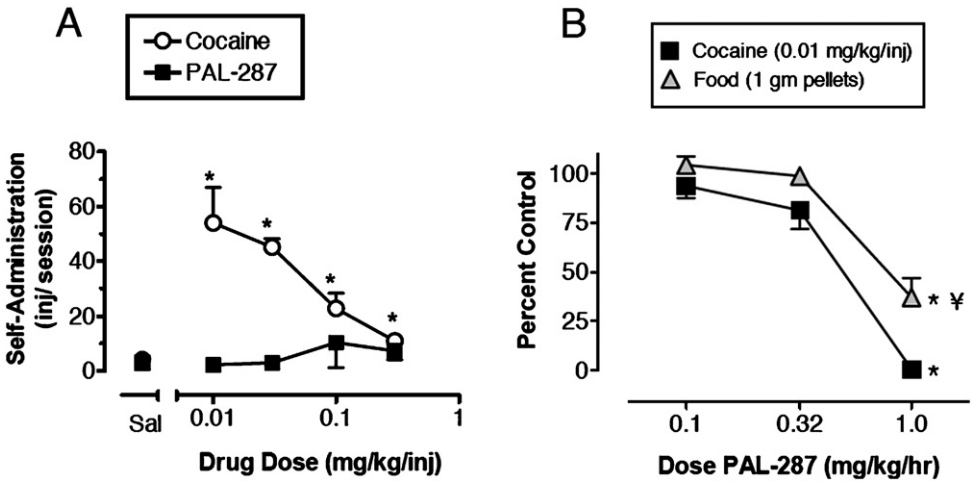

Fig. 8. Effects of PAL-287 in the monkey self-administration assay. Panel A: Self-administration of cocaine and PAL-287 by rhesus monkeys. Drugs were available under a fixed ratio (FR) 25 schedule of reinforcement for 2 h/day. Each point is the mean of two sessions of access to each dose of the drugs. Data are mean ± SEM for $N = 4$ monkeys. Symbols without bars have variability smaller than the points. $^{*}P < 0.05$, compared to saline-injected control (Newman–Keuls post hoc test). Panel B: Effects of chronic 7-day treatment with PAL-287 on cocaine- and food-maintained responding. Control levels of responding were defined as levels of cocaine- or food-maintained responding observed during 7 days of saline treatment. Each point shows mean ± SEM for three monkeys, with data collected during the last 3 days of each 7-day treatment. $^{*} = P < 0.05$, compared to control for a given reinforcer (Newman–Keuls post hoc test). ¥ = $P < 0.05$, compared to cocaine-maintained responding at that dose of PAL-287 (Newman–Keuls post hoc test). Data taken with permission from Rothman et al. (2005).

and SERT will release DA and 5-HT from neurons in vivo, be minimally reinforcing and also suppress ongoing cocaine self-administration. PAL-287 displays a number of desirable qualities for a candidate treatment medication, including minimal locomotor activation, lack of long-term 5-HT neurotoxicity and low abuse potential. Further studies will be necessary to determine the potential of PAL-287 for increasing the risk of VHD and IPAH, especially given the 5-HT_{2B} agonist effects of the drug. The present data with PAL-287 support the use of monoamine releasers as agonist medications for the treatment of stimulant addictions. A dose of 1.0 mg/kg/h PAL-287 virtually eliminated cocaine self-administration in rhesus monkeys by the end of the 7-day treatment, although this effect was not entirely selective for cocaine vs. food. We also note that the role of NE in the actions of PAL-287 is an important issue awaiting additional study (Rothman et al., 2001).

Conclusions

Our findings with PAL-287 in monkeys are similar to the suppression of cocaine self-administration produced by D-amphetamine, although D-amphetamine displays greater selectivity in reducing cocaine self-administration as opposed to food-maintained responding (Negus and Mello, 2003a). Grabowski et al. (2001, 2004b) showed that a slow-release formulation of D-amphetamine is effective in maintaining cocaine addicts in treatment and reducing illicit cocaine use. We predict that agents such as PAL-287, which have mixed DA/5-HT-releasing activity, will possess the therapeutic effects of amphetamine-type monoamine releasers, while minimizing the adverse effects associated with the phenethylamine structure. Based on observations that dual DA/5-HT releasers suppress alcohol ingestion (Yu et al., 1997; Halladay et al., 1999, 2006), it seems that PAL-287 or similar agents should be tested as potential treatments for

alcohol addiction. Additionally, combined treatment with DA and 5-HT releasers blocks alcohol withdrawal seizures (Yu et al., 1997). Although further work remains to refine PAL-287, in particular to reduce its potency at 5-HT_{2B} receptors, we believe that PAL-287 represents the prototype for a new generation of drugs that enhance monoamine release by acting as substrates at multiple transporters.

While compounds such as PAL-287 move slowly from the preclinical arena towards clinical development, it is possible to test the concept of administering dual DA/5-HT releasers in humans by implementing clinically available compounds. For example, the utility of DA/5-HT releasers as treatments for addictive disorders can be tested by administration of the DA releaser D-amphetamine along with the 5-HT precursor 5-HTP. It is noteworthy that 5-HTP must be co-administered with the peripheral decarboxylase inhibitor carbidopa to selectively increase extracellular 5-HT in the CNS (see Halladay et al., 2006). Moreover, the utility of DA/5-HT releasers could also be tested using phentermine and 5-HTP/carbidopa, a drug combination with predicted efficacy as an appetite suppressant (Rothman and Baumann, 2008). In summary, we suggest that drugs with a mode of action similar to that of PAL-287 will provide neurochemical normalization therapy for stimulant addictions and might also be useful for treating depression, obsessive compulsive disorder, attention deficit hyperactivity disorder and obesity.

Abbreviations

CNS	central nervous system
CPP	conditioned place preference
DA	dopamine
DAT	dopamine transporter
EC_{50}	half-maximal effective concentration
E_{MAX}	maximal efficacy
GABA	gamma-aminobutyric acid
5-HT	5-hydroxytryptamine or serotonin
5-HTP	*L*-5-hydroxytryptophan
IPAH	idiopathic pulmonary arterial hypertension
mCPP	*m*-chlorophenylpiperazine
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
NE	norepinephrine
NET	norepinephrine transporter
PAL-287	1-naphthyl-2-aminopropane
PAL-313	*p*-methylamphetamine
PAL-353	*m*-fluoroamphetamine
Ro 60-0175	(S)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine
SB 242084	6-chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline
SERT	serotonin transporter
VHD	valvular heart disease
VMAT2	vesicular monoamine transporter type 2
VTA	ventral tegmental area

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