Themed Issue: Drug Addiction - From Basic Research to Therapies
Guest Editors - Rao Rapaka and Wolfgang Sadée

Dual Dopamine/Serotonin Releasers as Potential Medications for Stimulante and Alcohol Addictions

Submitted: November 9, 2006; Accepted: November 16, 2006; Published: January 5, 2007

Richard B. Rothman,[1] Bruce E. Blough,[2] and Michael H. Baumann[1]

[1]Clinical Psychopharmacology Section, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, MD
[2]Chemistry and Life Sciences Group, Research Triangle Institute International, Research Triangle Park, NC

Abstract

We have advocated the idea of agonist therapy for treating cocaine addiction. This strategy involves administration of stimulant-like medications (eg, monoamine releasers) to alleviate withdrawal symptoms and prevent relapse. A major limitation of this approach is that many candidate medicines possess significant abuse potential because of activation of mesolimbic dopamine (DA) neurons in central nervous system reward circuits. Previous data suggest that serotonin (5-HT) neurons can provide an inhibitory influence over mesolimbic DA neurons. Thus, it might be predicted that the balance between DA and 5-HT transmission is important to consider when developing medications with reduced stimulant side effects. In this article, we discuss several issues related to the development of dual DA/5-HT releasers for the treatment of substance use disorders. First, we discuss evidence supporting the existence of a dual deficit in DA and 5-HT function during withdrawal from chronic cocaine or alcohol abuse. Then we summarize studies that have tested the hypothesis that 5-HT neurons can dampen the effects mediated by mesolimbic DA. For example, it has been shown that pharmacological manipulations that increase extracellular 5-HT attenuate stimulant effects produced by DA release, such as locomotor stimulation and self-administration behavior. Finally, we discuss our recently published data about PAL-287 (naphthylisopropylamine), 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 might be useful therapeutic adjuncts for the treatment of cocaine and alcohol addiction, obesity, and even attention deficit disorder and depression.

Keywords: Alcohol, amphetamine, cocaine, dopamine, serotonin, treatment, transporter

Corresponding Author: Richard B. Rothman, Clinical Psychopharmacology Section, IRP, NIDA, NIH, PO Box 5180, 5500 Nathan Shock Drive, Baltimore, MD 21224. Tel: (410) 550-1598; Fax: (410) 550-2997; E-mail: rrothman@mail.nih.gov

Introduction

A major goal of this article is to review recent work from our lab that pertains to dual dopamine (DA)/serotonin (5-HT) releasers as potential medications for alcohol and psychostimulant addictions.[1-9] The term "psychostimulant" refers to drugs that produce a spectrum of effects in humans, including cardiovascular stimulation, mood elevation, and a decreased need for sleep. At higher doses, or after longer periods of use, stimulants can cause a range of disordered thought processes, including severe psychotic episodes. In laboratory animals, psychostimulants increase locomotor activity and are readily self-administered because of their powerful reinforcing properties. Psychostimulants are often described generally as "amphetamine-like," since amphetamine is the prototypical stimulant agent. Table 1 lists examples of stimulants, and Figure 1 depicts the chemical structures of drugs discussed in this article. It is noteworthy that many of these drugs are useful medications with long histories of efficacy and safety, whereas others are highly addictive substances[10-12] that are associated with considerable morbidity and mortality.[12,13] In some cases, such as amphetamine, the same drug can be a therapeutic entity or an abused substance, depending upon the context in which the drug is administered.

Most psychostimulants interact with monoamine neurons in the central nervous system (CNS). Neurons that synthesize, store, and release the monoamine transmitters norepinephrine (NE), DA, and 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.[14,15] Substantial evidence has shown that there are distinct transporter proteins expressed by NE neurons (NET), DA neurons (DAT), and 5-HT neurons (SERT). These proteins belong to a superfamily of Na^+/Cl^--dependent transporters that share genetic, structural, and functional homologies.[16,17] Under normal circumstances, the transporter-mediated uptake of monoamine transmitters is the principal mechanism for inactivation of monoaminergic signaling in the brain. The biogenic amine neurotransmitters and their receptors play a critical role in either the

Table 1. Examples of Psychostimulants*

Drugs	Indication
Therapeutic	
Methylphenidate	Attention deficit disorder
Amphetamine	Attention deficit disorder/narcolepsy
Phentermine	Anorectic
Diethylpropion	Anorectic
Phendimetrazine	Anorectic
Benzphetamine	Anorectic
Abused	
Cocaine	
Methamphetamine	
3,4-Methylenedioxymethamphetamine	

pathogenesis or the treatment of a wide range of psychiatric disorders.[18]

In general, drugs that target transporter proteins can be divided into 2 classes based on their precise mechanism of action: reuptake inhibitors and substrate-type releasers. Reuptake inhibitors bind to transporter proteins but are not themselves transported. These drugs elevate extracellular transmitter concentrations by blocking transporter-mediated recapture of transmitter molecules from the synapse. Substrate-type releasers bind to transporter proteins and are then transported into the cytoplasm of nerve terminals. "Releasers," a term used interchangeably with the term "substrates," elevate extracellular transmitter concentrations by a 2-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 vesicular monoamine transporter$_2$ (VMAT$_2$).[19,20] The exact mechanism underlying the transporter-mediated exchange mechanism is complex and still under intensive investigation.[21-23] The interaction with VMAT$_2$ can increase the pool of neurotransmitter available for release by transporter-mediated exchange. Because substrate-type releasing agents must be transported into nerve terminals to promote transmitter release, reuptake inhibitors can block the effects of releasers.

Dual-Deficit Model of Stimulant Addiction

The initial use of stimulants such as cocaine and methamphetamine produces a "high" or "rush" that is likely caused by elevations in extracellular DA levels in mesolimbic circuits, although some evidence indicates that elevations in extracellular NE may also contribute.[4,24] Similarly, alcohol-induced increases in mesolimbic DA are thought to underlie the positive reinforcing effects of this substance.[25] Episodic use of stimulants, especially when they are self-administered via smoking or the intravenous route, can lead to severe addiction in susceptible individuals. The persistent abuse of stimulants and alcohol causes long-term changes in neurochemistry and brain circuitry via processes of synaptic plasticity.[25-27] Both preclinical and human data suggest that withdrawal from drugs of abuse is associated with impairments in 5-HT neuronal function[28-30] in addition to the well-accepted deficits in DA function.[31] Compelling clinical

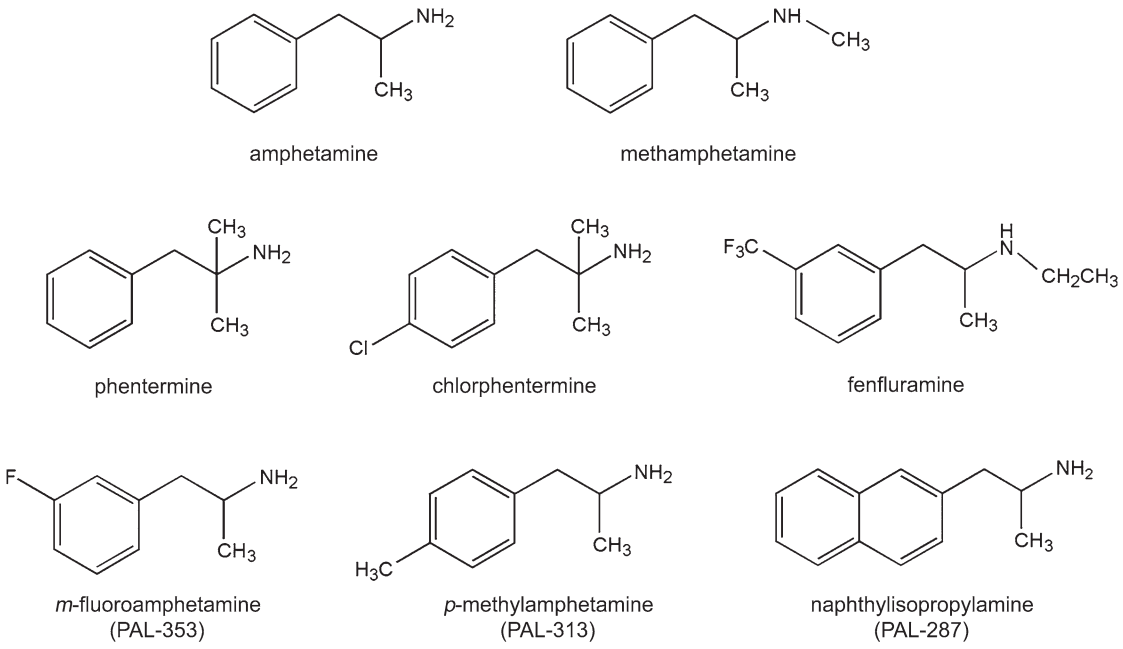

Figure 1. Chemical structures of selected psychostimulants.

evidence for 5-HT deficits in cocaine addiction is the occurrence of psychiatric symptoms resembling major depression following abstinence from binge cocaine use,[31,32] coupled with increased prevalence of suicidal ideation and suicide attempts among cocaine addicts.[33] A well-established role of 5-HT dysfunction in mediating depression and suicide[34] suggests that decreased synaptic 5-HT might play a role in cocaine and alcohol withdrawal states.[35] Indeed, the constellation of symptoms often reported by patients withdrawing from stimulant or alcohol use, such as depressed mood, suicidal ideations, obsessive thoughts of using drugs, intense drug craving, anhedonia, increased impulsivity, and susceptibility to drug-related cues presumably reflects long-term changes in brain function and structure. In particular, evidence points to deficits in DA and 5-HT neuronal function during withdrawal from alcohol and stimulant abuse.[30]

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.[1,30,36,37] According to the dual-deficit model, depicted diagrammatically in Figure 2, 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.[30,38-40] If abstinent stimulant addicts exhibit DA and 5-HT deficits, a logical prediction of the dual-deficit model would be that pharmacotherapies capable of correcting abnormalities in DA and 5-HT function should 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.[36,41-44] Indeed, administration of DA and 5-HT releasers alone, or in combination, decreases drug-seeking behavior in preclinical models of addiction. Acute and chronic administration of d-amphetamine, a DA-releasing agent, decreases cocaine self-administration behavior in rhesus monkeys.[45,46] As illustrated in Figure 3, the DA-releasing agent phentermine suppresses responding for cocaine without affecting food-reinforced behavior, and this effect is maintained by daily administration of

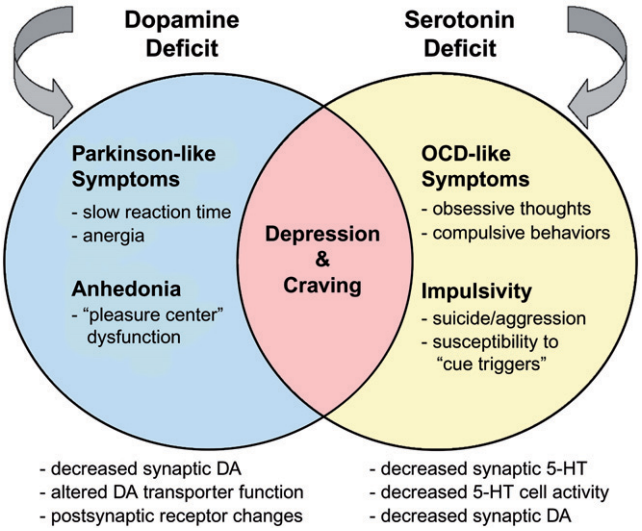

Figure 2. The dual-deficit model of psychostimulant addiction. According to the model, withdrawal from chronic stimulant use leads to decreased synaptic availability of DA and 5-HT that, in turn, contributes to withdrawal symptoms, drug craving, and relapse. DA dysfunction underlies anhedonia and psychomotor 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. Taken from Rothman and Baumann.[5] OCD indicates obsessive-compulsive disorder; DA, dopamine; 5-HT, serotonin.

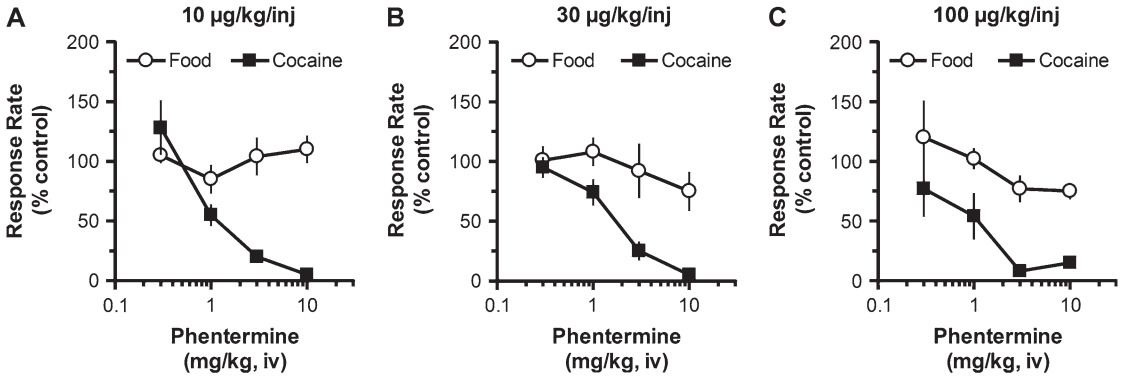

Figure 3. Phentermine, administered intravenously, decreases cocaine self-administration. Taken from Wojnicki et al.[9] Acute effects of phentermine on rates of responding maintained under an FR 30 food (○), FR 30 cocaine (■) schedule with different unit doses (10-100 µg/kg/injection, left-right) of cocaine. Abscissa: dose of phentermine (mg/kg). Ordinate: effect, expressed as the mean (± SEM) percentage of individual control rates of responding (n = 3-4); control variability (filled symbols), expressed as the average of individual coefficients of variation. Inj indicates injection; IV, intravenous; FR, fixed ratio.

phentermine.[9] These results provide a rationale for using DA-releasing agents as medications for treating cocaine addiction.[47,48]

Stimulation of 5-HT transmission can reduce drug-seeking behavior as well. Administration of the 5-HT precursor L-tryptophan, which increases brain 5-HT synthesis, to rats decreases self-administration of cocaine[49] and amphetamine.[50] The 5-HT releaser fenfluramine decreases responding for cocaine in rhesus monkeys. Interestingly, combined administration of phentermine and fenfluramine produces a 75% decrease in cocaine self-administration in monkeys.[51] The mixture of d-fenfluramine and phentermine reduces cocaine self-administration by 80% in rats, yet this mixture is not self-administered.[52] The 5-HT-releasing agents suppress cue-elicited cocaine-seeking behavior in rats[53] and decrease cocaine craving in cocaine-dependent patients.[54] These and other findings[36] indicate that combined treatment with DA and 5-HT releasers may have greater therapeutic value than treatment with either drug alone, in terms of decreasing stimulant self-administration and reducing cue-induced relapse. A growing body of evidence shows that DA-/5-HT-releasing agents may provide similar therapeutic benefits for alcohol dependence.[41,42,55]

Dual DA/5-HT Releasers as Agonist Treatments

The use of stimulant-like medications to treat stimulant addictions is known as agonist therapy. This strategy involves administering medications that are less potent and less addictive than cocaine or methamphetamine but decrease stimulant abuse because they share neurochemical properties with the illicit drugs.[56] Viewed from this perspective, agonist therapy is like neurochemical "normalization" therapy: by substituting for the abused drug, the treatment drug "normalizes" dysregulated neurochemistry.[5] Neurochemical normalization therapy has generated effective treatments for nicotine dependence[57] and opioid dependence[58,59] and has recently been explored for the treatment of cocaine dependence.[60-64] A major limitation of this approach as applied to stimulant addiction is that candidate medications often exhibit intrinsic abuse liability.[47] One possible advantage of treating stimulant and alcohol dependence with dual DA-/5-HT-releasing agents is that concurrent elevation of extracellular 5-HT can greatly reduce the typical psychomotor actions of DA releasers, including locomotor activity, reward, and self-administration behavior.

Several lines of evidence support the notion that elevations in synaptic 5-HT counteract the stimulant and reinforcing effects mediated by elevations in synaptic DA.[65,66] As noted above, L-tryptophan decreases cocaine[49] and amphetamine[50] self-administration in rats. Likewise, pretreatment with 5-HT reuptake inhibitors reduces intravenous cocaine self-administration in rats[67] and squirrel monkeys.[68] Cocaine analogs that have potent 5-HT transporter affinity[69] support less self-administration behavior than analogs with weak 5-HT transporter affinity. Moreover, as described in a recent review, agents that broadly activate brain 5-HT systems can reduce self-administration of stimulants and xother drugs of abuse.[70] The "antistimulant" effect of increasing extracellular 5-HT is readily observed after combined administration of 5-HT releasers and DA releasers, or after administration of single agents that release both neurotransmitters. As summarized in Table 2, drugs that release [^{3}H]DA more potently than [^{3}H]5-HT in vitro (amphetamine, *m*-fluoroamphetamine, phentermine) increase endogenous extracellular DA much more than extracellular 5-HT in vivo. Such indirect DA agonists are strong

Table 2. 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	1756	8.0	45 (0.3 mg/kg ip)	224 (0.3 mg/kg ip)	Yes	Strong
Phentermine	3511	262	32 (1.0 mg/kg ip)	156 (1.0 mg/kg ip)	Yes	Strong
PAL-353	1937	24.2	170 (1.0 mg/kg IV)	432 (1.0 mg/kg IV)	Yes	Strong
Fenfluramine	79.3	>10 000	215 (1.0 mg/kg ip)	20 (1.0 mg/kg ip)	No	None
Chlorphentermine	30.9	2650	228 (1.0 mg/kg ip)	86 (1.0 mg/kg ip)	No	None
Phentermine + fenfluramine	NA	NA	222 (1.0 + 1.0 mg/kg, ip)	144 (1.0 + 1.0 mg/kg ip)	No	Weak
PAL-313	53.4	44.1	544 (1.0 mg/kg IV)	130 (1.0 mg/kg IV)	Weak	Weak
PAL-287	3.4	12.6	464 (1.0 mg/kg IV)	133 (1.0 mg/kg IV)	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.[1-9] Microdialysis data for PAL-315 and PAL-353 are unpublished. 5-HT indicates serotonin; DA, dopamine; ip, intraperitoneally; PAL-353, *m*-fluoroamphetamine; IV, intravenously; PAL-313, *p*-methylamphetamine; PAL-287, naphthylisopropylamine.

locomotor stimulants and support self-administration behavior. Drugs that release [^{3}H]5-HT more potently than [^{3}H]DA (fenfluramine, chlorphentermine) increase endogenous extracellular 5-HT more than extracellular DA. Such indirect 5-HT agonists do not stimulate motor activity and do not support self-administration behavior.

Combining a DA releaser with a 5-HT releaser (phentermine plus fenfluramine), or a single molecule that has similar potencies for releasing DA and 5-HT, results in elevation of both extracellular 5-HT and DA. When the elevations in 5-HT are somewhat greater than the elevations in DA, there is minimal locomotor activation, coupled with minimal or no self-administration. The "antireward" effect of increasing extracellular 5-HT is also seen in the conditioned place preference (CPP) assay, shown in Figure 4, where a low dose of fenfluramine greatly reduces the positive CPP induced by phentermine. These considerations led us to predict that a single molecule that releases both 5-HT and DA would be able to decrease stimulant self-administration yet have minimal abuse liability. Such an agent would thereby provide the advantages of agonist therapy without the untoward side effects of a potential drug of abuse.

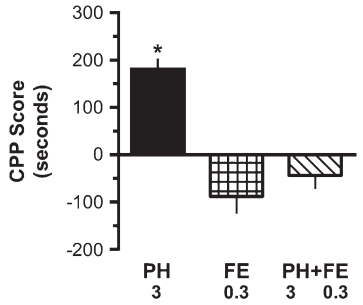

Figure 4. FE (0.3 mg/kg) reduces PH (3 mg/kg) CPP. Ordinate: mean difference (sec) between time spent in the drug- and vehicle-paired sides of the test chamber. Abscissa: drug dose (mg/kg). Each column represents the mean conditioning score of 9 to 10 rats. Data taken from Rea et al.[3] *Significant place conditioning (Wilcoxon test, $P < .05$). FE indicates fenfluramine; PH, phentermine; CPP, conditioned place preference.

Potential Adverse Effects of 5-HT Releasers

Unfortunately, 5-HT-releasing agents are associated with several adverse effects of their own.[71] Based primarily on experience with fenfluramine, 3 potentially serious adverse effects need to be addressed when 5-HT releasers are developed as treatment agents: cardiac valvulopathy, serotonergic neurotoxicity, and primary pulmonary hypertension (PPH). The association of fenfluramine with an increased prevalence of cardiac valve disease led to its withdrawal from the marketplace in September 1997.[72] Recent investigations have demonstrated that norfenfluramine, the *N*-deethylated metabolite of fenfluramine, activates 5-HT$_{2B}$ receptors on heart valves to stimulate mitogenesis, and this action may represent the principal mechanism underlying cardiac valve disease.[73-75] The term "5-HT neurotoxicity," when used in the present context, refers to the fact that high-dose administration of selective 5-HT releasers (eg, fenfluramine) often causes persistent depletion of brain tissue 5-HT and SERT binding. A key observation is that not all 5-HT releasers deplete 5-HT.[2,76] As shown in Figure 5, repeated administration of the 5-HT-releasing agent m-chlorophenylpiperazine (mCPP) fails to deplete brain 5-HT, despite producing elevations of extracellular 5-HT comparable to those produced by fenfluramine.[2] These data indicate that SERT-mediated release of 5-HT is necessary but not sufficient to produce long-term depletion of brain 5-HT.

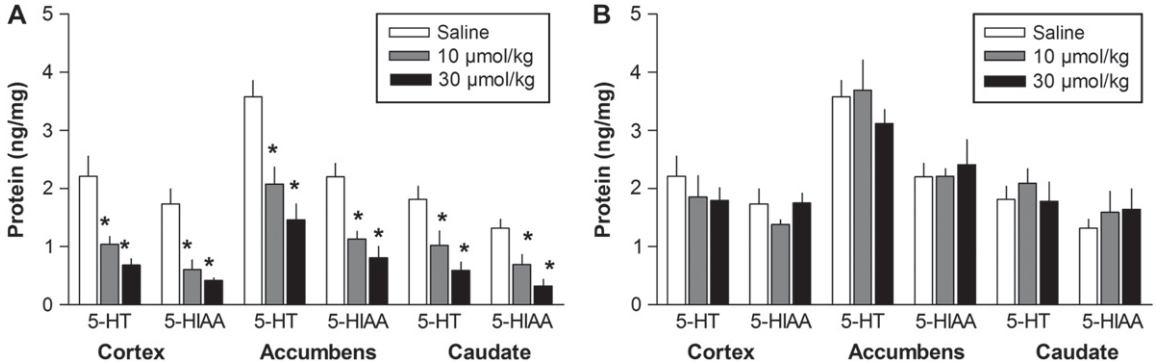

Figure 5. Effects of dFEN and mCPP on brain tissue 5-HT and 5-HIAA. (A) Effects of high-dose dFEN or saline on postmortem tissue levels of 5-HT and 5-HIAA in cingulate cortex, nucleus accumbens, and caudate nucleus. dFEN was administered ip at 10 or 30 µmol/kg, every 2 hours, for 4 doses. Rats were killed 2 weeks after the dosing regimen. (B) Effects of high-dose mCPP or saline on postmortem tissue levels of 5-HT and 5-HIAA in cingulate cortex, nucleus accumbens, and caudate nucleus. mCPP was administered ip at 10 or 30 µmol/kg, every 2 hours, for 4 doses. Rats were killed 2 weeks after the dosing regimen. These doses of dFEN and mCPP produced comparable increases in extracellular 5-HT. Data are mean ± SEM expressed as ng/mg protein for 4 to 6 rats/group. *$P < .05$ compared with saline-treated group. Data taken from Baumann et al.[2] dFEN indicates d-fenfluramine; mCPP, m-chlorophenylpiperazine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; ip, intraperitoneally.

Increasing evidence indicates that SERT sites are involved in the mechanism by which fenfluramine increases the risk of developing PPH (for review, see Rothman and Baumann[71] and references therein). For example, medications that increase the risk for PPH have in common the ability to release 5-HT by a SERT-mediated process. On the other hand, not all 5-HT releasers are associated with PPH. The antidepressant trazodone is not associated with PPH, yet its major metabolite, mCPP, is a potent SERT substrate, as noted above.[2] Experimental data from a mouse model of hypoxic pulmonary hypertension suggest that 5-HT_{2B} receptors may also contribute to the pathogenesis of PPH.[77] The relevance of these findings to drug-induced PPH is not clear, since aminorex, a 5-HT releaser that caused an epidemic of PPH in the 1960s,[78] has minimal activity at 5-HT_{2B} receptors. Viewed collectively, the available data suggest that it should be possible to develop dual DA/5-HT releasers devoid of fenfluramine-like adverse effects. In particular, we have suggested that a lead drug molecule should be chemically distinct from the phenylethylamine structure shared by amphetamine-like agents and should lack significant agonist activity at 5-HT_{2B} receptors.[79]

PAL-287, a Non-Amphetamine DA/5-HT Releaser

Based in part on the above rationale, we sought to identify and characterize a non-amphetamine transporter substrate that would be a potent releaser of DA and 5-HT without affecting the release of NE. After an extensive evaluation of over 350 compounds, we found it virtually impossible to dissociate NE-and DA-releasing properties, perhaps because of phylogenetic similarities between NET and DAT. The first lead compound from our search was PAL-287 (naphthylisopropylamine; see structure in Figure 1), a novel non-amphetamine monoamine releaser.[7] The in vitro potency of PAL-287 at releasing tritiated transmitter from DAT, NET, and SERT is 12.6 ± 0.4 nM, 11.1 ± 0.9 nM, and 3.4 ± 0.2 nM, respectively (Table 2). Figure 6 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 than on 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.[80] The 5-HT_{2C} agonist activity may also contribute to the minimal reinforcing properties of PAL-287 despite the potent DA-releasing actions of the drug (see Czoty et al[81] for a review). The relatively weak potency at 5-HT_{2A} and 5-HT_{2B} receptors, as compared with its activity at SERT, suggests that PAL-287 may not activate 5-HT_{2A} and 5-HT_{2B} receptors in vivo.

As reported in Figure 6, PAL-287 produces minimal ambulation and stereotypy (approximately one third of that produced by (+)-amphetamine) when administered to rats. Repeated high-dose administration of PAL-287 to rats (18 mg/kg intraperitoneally [ip] q 2 hours × 3 doses) fails to affect brain tissue 5-HT levels when assessed 2 weeks after injections, unlike (+)-methamphetamine (6.0 mg/kg ip q 2 hours × 3 doses) and (±)-3,4-methylenedioxymethamphetamine (MDMA) (7.5 mg/kg ip q 2 hours × 3 doses), which cause significant 5-HT depletions. The data in Figure 7 show that PAL-287 does not support self-administration behavior, and chronic administration of the drug decreases cocaine self-administration in rhesus monkeys. A dose of 1.0 mg/kg/hr PAL-287 significantly reduces both cocaine- and

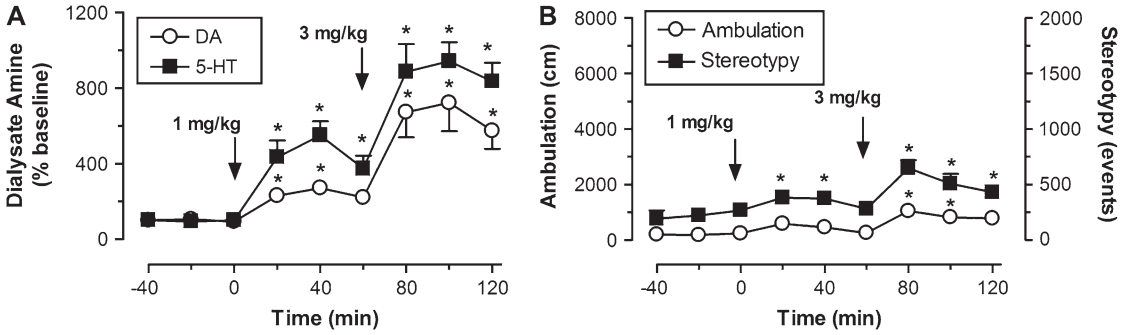

Figure 6. Effects of PAL-287 on neurochemical and locomotor measures in rats. Taken from Rothman et al.[7] (A) Dose-response effects of PAL-287 on extracellular DA and 5-HT in rat prefrontal cortex, as determined by in vivo microdialysis. Rats received IV injection of 1 mg/kg PAL-287 at time 0, followed by 3 mg/kg 60 minutes later. Data are mean ± SEM for 7 rats/group, expressed as percentage of baseline. Baseline levels of DA and 5-HT were 0.43 ± 0.07 and 0.27 ± 0.06 pg/5 µL. (B) Dose-response effects of PAL-287 on ambulation and stereotypy in rats undergoing microdialysis sampling. Rats received IV injections of 1 mg/kg PAL-287 at time 0, followed by 3 mg/kg 60 minutes later. Data are mean ± SEM for 7 rats/group, expressed as distance traveled in cm (ambulation) and number of repetitive movements (stereotypy). **P* < .05 compared with preinjection control, Duncan's post hoc test. DA indicates dopamine; 5-HT, serotonin; PAL-287, naphthylisopropylamine; IV, intravenous.

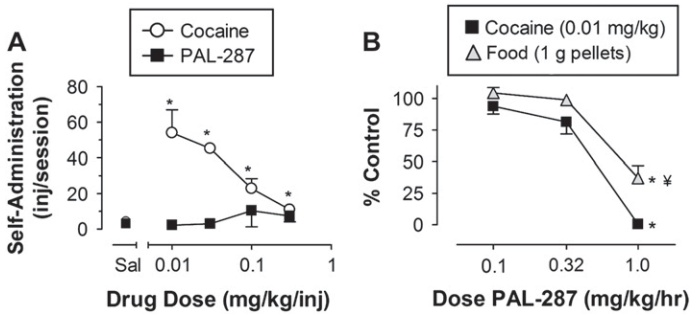

Figure 7. Effects of PAL-287 in the monkey self-administration assay (taken from Rothman et al[7]). (A) Self-administration of cocaine and PAL-287 by rhesus monkeys. Drugs were available under an FR 25 schedule of reinforcement for 2 hours/day. Each point is the mean of 2 sessions of access to each dose of the drugs. Data are mean ± SEM for n = 4 monkeys. Symbols without bars have variability smaller than the points. **P* < .05 compared with saline-injected control, Duncan's post hoc test. (B) Effects of chronic 7-day treatment with PAL-287 on cocaine- and food-maintained responding. Abscissa: Dose PAL-287 in mg/kg/hr (log scale). Ordinate: Percent control levels of cocaine- and food-maintained responding. Control values were defined as levels of cocaine- or food-maintained responding observed during 7 days of saline treatment. Each point shows mean data ± SEM for 3 monkeys collected during the last 3 days of each 7-day treatment. Two-way ANOVA indicated a significant effect of PAL-287 dose [$F(2,4) = 167$, $P = .0001$] but not a significant effect of reinforcer type [$F(2,2) = 7.26$, $P = 0.114$) or a significant interaction [$F(2,4) = 3.86$, $P = .116$]. Post hoc analysis was conducted with the Newman-Keuls test. *Indicates significant effect of PAL-287 dose in comparison to control for a given reinforcer, $P < .05$. ¥ Indicates that food-maintained responding was significantly greater than cocaine-maintained responding at that dose of PAL-287, $P < .05$. PAL-287 indicates naphthylisopropylamine; inj, injection; FR, fixed ratio.

food-maintained responding; however, the suppression of cocaine self-administration is greater than the reduction in food-maintained responding.

Our results with PAL-287 confirm the hypothesis that a non-amphetamine substrate at DAT and SERT will release DA and 5-HT from neurons in vivo, be minimally reinforcing, and suppress ongoing cocaine self-administration. PAL-287 displays several desirable qualities for a candidate treatment medication, including minimal locomotor activation, lack of long-term 5-HT neurotoxicity, and low abuse potential. Future studies will be necessary to determine the potential of PAL-287 for increasing the risk for PPH, perhaps by determining its potency at human voltage-gated K^+ channels.[82] 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/hr 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.[4]

Conclusions

Our findings with PAL-287 in monkeys are similar to the suppression of cocaine self-administration produced by (+)-amphetamine, although amphetamine displays greater selectivity than PAL-287 in reducing cocaine self-administration as opposed to food-maintained responding.[83] Grabowski et al[61,84] showed that a slow-release formulation of (+)-amphetamine is effective in keeping cocaine addicts in treatment and reducing illicit cocaine use. We predict that agents with mixed DA-/5-HT-releasing activity, such as PAL-287, will possess the therapeutic effects of amphetamine-type monoamine releasers, while minimizing the adverse effects associated with the phenylethylamine structure. Based on observations that dual DA/5-HT releasers also suppress alcohol ingestion,[41,42,55] it is also likely that PAL-287 agents should also be tested as potential treatment agents for alcohol addiction, especially since dual DA/5-HT releasers suppress alcohol withdrawal seizures.[41] 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 biogenic amine release by acting as substrates at multiple biogenic amine transporters. It seems possible that drugs with a similar mode of action will provide neurochemical normalization therapy for the treatment of cocaine and alcohol addiction, and might be useful for treating depression, obsessive-compulsive disorder, attention deficit disorder, and obesity.

Acknowledgments

This research was supported in part by the Intramural Research Program of the National Institutes of Health, National Institute on Drug Abuse; and grant number NIDA R01 DA12970 to Bruce Blough.

References

1. 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;36:102-113.

2. 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.

3. Rea WP, Rothman RB, Shippenberg TS. Evaluation of the conditioned reinforcing effects of phentermine and fenfluramine in the rat: concordance with clinical studies. *Synapse*. 1998;30:107-111.

4. Rothman RB, Baumann MH, Dersch CM, et al. Amphetamine-type central nervous system stimulants release norepinephrine more

potently than they release dopamine and serotonin. *Synapse*. 2001;39:32-41.

5. Rothman RB, Baumann MH. Monoamine transporters and psychostimulant drugs. *Eur J Pharmacol*. 2003; 479:23-40.

6. Rothman RB, Baumann MH. Serotonin releasing agents, Neurochemical, therapeutic and adverse effects. *Pharmacol Biochem Behav*. 2002;71:825-836.

7. Rothman RB, Blough BE, Woolverton WL, et al. Development of a rationally designed, low abuse potential, biogenic amine releaser that suppresses cocaine self-administration. *J Pharmacol Exp Ther*. 2005;313:1361-1369.

8. Wee S, Anderson KG, Baumann MH, Rothman RB, Blough BE, Woolverton WL. Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. *J Pharmacol Exp Ther*. 2005;313:848-854.

9. Wojnicki FHE, Rothman RB, Rice KC, Glowa JR. Effects of phentermine on responding maintained under multiple fixed-ratio schedules of food and cocaine presentation in the rhesus monkey. *J Pharmacol Exp Ther*. 1999;288:550-560.

10. Castro FG, Barrington EH, Walton MA, Rawson RA. Cocaine and methamphetamine: differential addiction rates. *Psychol Addict Behav*. 2000;14:390-396.

11. Musto DF. Cocaine's history, especially the American experience. *Ciba Found Symp*. 1992;166:7-14.discussion 14-19.

12. Das G. Cocaine abuse in North America: a milestone in history. *J Clin Pharmacol*. 1993;33:296-310.

13. Centers for Disease Control and Prevention (CDC). Increasing morbidity and mortality associated with abuse of methamphetamine—United States, 1991-1994. *MMWR Morb Mortal Wkly Rep*. 1995;44:882-886.

14. Amara SG, Kuhar MJ. Neurotransmitter transporters: recent progress. *Annu Rev Neurosci*. 1993;16:73-93.

15. Masson J, Sagne C, Hamon M, el Mestikawy S. Neurotransmitter transporters in the central nervous system. *Pharmacol Rev*. 1999;51:439-464.

16. Blakely RD, De Felice LJ, Hartzell HC. Molecular physiology of norepinephrine and serotonin transporters. *J Exp Biol*. 1994;196:263-281.

17. Uhl GR, Johnson PS. Neurotransmitter transporters: three important gene families for neuronal function. *J Exp Biol*. 1994;196:229-236.

18. Amara SG, Sonders MS. Neurotransmitter transporters as molecular targets for addictive drugs. *Drug Alcohol Depend*. 1998;51:87-96.

19. Rudnick G, Clark J. From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. *Biochim Biophys Acta*. 1993;1144:249-263.

20. Rudnick G. Mechanisms of biogenic amine transporters. In: Reith MEA, ed. *Neurotransmitter Transporters: Structure, Function and Regulation.* Totowa, NJ: Humana Press; 1997:73-100.

21. Blakely RD, Defelice LJ, Galli A. Biogenic amine neurotransmitter transporters: just when you thought you knew them. *Physiology (Bethesda)*. 2005;20:225-231.

22. Sitte HH, Freissmuth M. Oligomer formation by Na+-Cl--coupled neurotransmitter transporters. *Eur J Pharmacol*. 2003;479:229-236.

23. Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol*. 2005;75:406-433.

24. Alexander M, Rothman RB, Baumann MH, Endres CJ, Brasic JR, Wong DF. Noradrenergic and dopaminergic effects of (+)-amphetamine-like stimulants in the baboon Papio anubis. *Synapse*. 2005;56:94-99.

25. Koob GF. Alcoholism: allostasis and beyond. *Alcohol Clin Exp Res*. 2003;27:232-243.

26. Volkow ND, Li TK. Drug addiction: the neurobiology of behaviour gone awry. *Nat Rev Neurosci*. 2004;5:963-970.

27. Hyman SE. Addiction: a disease of learning and memory. *Am J Psychiatry*. 2005;162:1414-1422.

28. Weiss F, Parsons LH, Schulteis G, et al. Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. *J Neurosci*. 1996;16:3474-3485.

29. Parsons LH, Koob GF, Weiss F. Serotonin dysfunction in the nucleus accumbens of rats during withdrawal after unlimited access to intravenous cocaine. *J Pharmacol Exp Ther*. 1995;274: 1182-1191.

30. Baumann MH, Rothman RB. Alterations in serotonergic responsiveness during cocaine withdrawal in rats: similarities to major depression in humans. *Biol Psychiatry*. 1998;44: 578-591.

31. Dackis CA, Gold MS. New concepts in cocaine addiction: the dopamine depletion hypothesis. *Neurosci Biobehav Rev*. 1985;9:469-477.

32. Gawin FH, Kleber HD. Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. *Arch Gen Psychiatry*. 1986;43:107-113.

33. Garlow SJ, Purselle D, D'Orio B. Cocaine use disorders and suicidal ideation. *Drug Alcohol Depend*. 2003;70: 101-104.

34. Mann JJ. Neurobiology of suicidal behaviour. *Nat Rev Neurosci*. 2003;4:819-828.

35. Lesch KP. Alcohol dependence and gene x environment interaction in emotion regulation: is serotonin the link? *Eur J Pharmacol*. 2005;526:113-124.

36. Rothman RB, Elmer GI, Shippenberg TS, Rea W, Baumann MH. Phentermine and fenfluramine: preclinical studies in animal models of cocaine addiction. *Ann N Y Acad Sci*. 1998;844:59-74.

37. Baumann MH, Rothman RB. Serotonergic dysfunction during cocaine withdrawal: implications for cocaine-induced depression. In: Karch SB, ed. *Drug Abuse Handbook.* Boca Raton, FL: CRC Press; 1998:463-484.

38. Lin D, Koob GF, Markou A. Differential effects of withdrawal from chronic amphetamine or fluoxetine administration on brain stimulation reward in the rat—interactions between the two drugs. *Psychopharmacology (Berl)*. 1999;145:283-294.

39. Markou A, Koob GF. Postcocaine anhedonia. An animal model of cocaine withdrawal. *Neuropsychopharmacology*. 1991;4:17-26.

40. Levy AD, Baumann MH, Van de Kar LD. Monoaminergic regulation of neuroendocrine function and its modification by cocaine. *Front Neuroendocrinol*. 1994;15:85-156.

41. Yu YL, Fisher H, Sekowski A, Wagner GC. Amphetamine and fenfluramine suppress ethanol intake in ethanol-dependent rats. *Alcohol*. 1997;14:45-48.

42. 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.

43. Hitzig P. Combined dopamine and serotonin agonists: a synergistic approach to alcoholism and other addictive behaviors. *Md Med J*. 1993;42:153-157.

44. Rothman RB, Gendron TM, Hitzig P. Combined use of fenfluramine and phentermine in the treatment of cocaine addiction: a pilot case series. *J Subst Abuse Treat*. 1994;11:273-275.

45. Glowa JR, Wojnicki FHE, Matecka D, Rice KC, Rothman RB. Effects of dopamine reuptake inhibitors on food- and cocaine-maintained responding, II: comparisons with other drugs and repeated administrations. *Exp Clin Psychopharmacol*. 1995;3:232-239.

46. Negus SS, Mello NK. Effects of chronic d-amphetamine treatment on cocaine- and food-maintained responding under a progressive-ratio schedule in rhesus monkeys. *Psychopharmacology (Berl)*. 2003;167:324-332.

47. Grabowski J, Shearer J, Merrill J, Negus SS. Agonist-like, replacement pharmacotherapy for stimulant abuse and dependence. *Addict Behav*. 2004;29:1439-1464.

48. Rothman RB, Blough BE, Baumann MH. Appetite suppressants as agonist substitution therapies for stimulant dependence. *Ann N Y Acad Sci*. 2002;965:109-126.

49. McGregor A, Lacosta S, Roberts DC. L-tryptophan decreases the breaking point under a progressive ratio schedule of intravenous cocaine reinforcement in the rat. *Pharmacol Biochem Behav*. 1993;44:651-655.

50. Smith FL, Yu DS, Smith DG, Leccese AP, Lyness WH. Dietary tryptophan supplements attenuate amphetamine self-administration in the rat. *Pharmacol Biochem Behav*. 1986;25:849-855.

51. Glowa JR, Rice KC, Matecka D, Rothman RB. Phentermine/fenfluramine decreases cocaine self-administration in rhesus monkeys. *Neuroreport*. 1997;8:1347-1351.

52. Glatz AC, Ehrlich M, Bae RS, et al. Inhibition of cocaine self-administration by fluoxetine or D-fenfluramine combined with phentermine. *Pharmacol Biochem Behav*. 2002;71:197-204.

53. Burmeister JJ, Lungren EM, Neisewander JL. Effects of fluoxetine and d-fenfluramine on cocaine-seeking behavior in rats. *Psychopharmacology (Berl)*. 2003;168:146-154.

54. Buydens-Branchey L, Branchey M, Hudson J, Rothman M, Fergeson P, McKernin C. Effect of fenfluramine challenge on cocaine craving in addicted male users. *Am J Addict*. 1998;7:142-155.

55. Halladay AK, Wagner GC, Sekowski A, Rothman RB, Baumann MH, Fisher H. Alterations in alcohol consumption, withdrawal seizures, and monoamine transmission in rats treated with phentermine and 5-hydroxy-L-tryptophan. *Synapse*. 2006;59:277-289.

56. Gorelick DA. The rate hypothesis and agonist substitution approaches to cocaine abuse treatment. *Adv Pharmacol*. 1998;42:995-997.

57. Henningfield JE. Nicotine medications for smoking cessation. *N Engl J Med*. 1995;333:1196-1203.

58. Kreek MJ. Opiates, opioids and addiction. *Mol Psychiatry*. 1996;1:232-254.

59. Ling W, Rawson RA, Compton MA. Substitution pharmacotherapies for opioid addiction: from methadone to LAAM and buprenorphine. *J Psychoactive Drugs*. 1994;26:119-128.

60. Grabowski J, Roache JD, Schmitz JM, Rhoades H, Creson D, Korszun A. Replacement medication for cocaine dependence: methylphenidate. *J Clin Psychopharmacol*. 1997;17:485-488.

61. Grabowski J, Rhoades H, Schmitz J, et al. Dextroamphetamine for cocaine-dependence treatment: a double-blind randomized clinical trial. *J Clin Psychopharmacol*. 2001;21:522-526.

62. Kampman KM, Rukstalis M, Pettinati H, et al. The combination of phentermine and fenfluramine reduced cocaine withdrawal symptoms in an open trial. *J Subst Abuse Treat*. 2000;19:77-79.

63. Walsh SL, Haberny KA, Bigelow GE. Modulation of intravenous cocaine effects by chronic oral cocaine in humans. *Psychopharmacology (Berl)*. 2000;150:361-373.

64. Alim TN, Jr, Rosse RB, Jr, Vocci FJ, Jr, Lindquist T, Deutsch SI. Diethylpropion pharmacotherapeutic adjuvant therapy for inpatient treatment of cocaine dependence: a test of the cocaine-agonist hypothesis. *Clin Neuropharmacol*. 1995;18:183-195.

65. Daw ND, Kakade S, Dayan P. Opponent interactions between serotonin and dopamine. *Neural Netw*. 2002;15:603-616.

66. Burmeister JJ, Lungren EM, Kirschner KF, Neisewander JL. Differential roles of 5-HT receptor subtypes in cue and cocaine reinstatement of cocaine-seeking behavior in rats. *Neuropsychopharmacology*. 2004;29:660-668.

67. Carroll ME, Lac ST, Asencio M, Kragh R. Fluoxetine reduces intravenous cocaine self-administration in rats. *Pharmacol Biochem Behav*. 1990;35:237-244.

68. Howell LL, Byrd LD. Serotonergic modulation of the behavioral effects of cocaine in the squirrel monkey. *J Pharmacol Exp Ther*. 1995;275:1551-1559.

69. Roberts DC, Phelan R, Hodges LM, et al. Self-administration of cocaine analogs by rats. *Psychopharmacology (Berl)*. 1999;144:389-397.

70. Higgins GA, Fletcher PJ. Serotonin and drug reward: focus on 5-HT2C receptors. *Eur J Pharmacol*. 2003;480:151-162.

71. Rothman RB, Baumann M. Therapeutic and adverse actions of serotonin transporter substrates. *Pharmacol Ther*. 2002;95:73-88.

72. Connolly HM, McGoon MD. Obesity drugs and the heart. *Curr Probl Cardiol*. 1999;24:745-792.

73. Fitzgerald LW, Burn TC, Brown BS, et al. Possible role of valvular serotonin 5-HT_{2B} receptors in the cardiopathy associated with fenfluramine. *Mol Pharmacol*. 2000;57:75-81.

74. Rothman RB, Baumann MH, Savage JE, et al. Evidence for possible involvement of 5-HT_{2B} receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation*. 2000;102:2836-2841.

75. Setola V, Hufeisen SJ, Grande-Allen KJ, et al. 3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. *Mol Pharmacol*. 2003;63:1223-1229.

76. 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;33:703-710.

77. Launay JM, Herve P, Peoc'h K, et al. Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. *Nat Med*. 2002;8:1129-1135.

78. Gurtner HP. Aminorex and pulmonary hypertension. *Cor Vasa*. 1985;27:160-171.

79. Rothman RB, Baumann MH. Neurochemical mechanisms of phentermine and fenfluramine: therapeutic and adverse effects. *Drug Dev Res*. 2000;51:52-65.

80. Vickers SP, Clifton PG, Dourish CT, Tecott LH. Reduced satiating effect of d-fenfluramine in serotonin 5-HT(2C) receptor mutant mice. *Psychopharmacology (Berl)*. 1999;143:309-314.

81. Czoty PW, Ginsburg BC, Howell LL. Serotonergic attenuation of the reinforcing and neurochemical effects of cocaine in squirrel monkeys. *J Pharmacol Exp Ther*. 2002;300:831-837.

82. Michelakis ED, Weir EK. Anorectic drugs and pulmonary hypertension from the bedside to the bench. *Am J Med Sci*. 2001;321:292-299.

83. Negus SS, Mello NK. Effects of chronic d-amphetamine treatment on cocaine- and food-maintained responding under a second-order schedule in rhesus monkeys. *Drug Alcohol Depend*. 2003;70:39-52.

84. Grabowski J, Rhoades H, Stotts A, et al. Agonist-like or antagonist-like treatment for cocaine dependence with methadone for heroin dependence: two double-blind randomized clinical trials. *Neuropsychopharmacology*. 2004;29:969-981.