Serotonin and dopamine system interactions in the reinforcing properties of psychostimulants: A research strategy

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THE MAIN GOAL of the experiments described in this proposal is to increase our understanding of the interaction between serotonin (5-HT) and dopamine (DA) systems in mediating the subjective, discriminative stimulus, and reinforcing effects of psychostimulant drugs in humans. This proposal will use three primary approaches to study DA/5-HT interactions: first, a drug with mixed DA/5-HT properties, 3,4-methylenedioxymethamphetamine (MDMA), will be compared to drugs with more selective DA (e.g., d-amphetamine) vs. 5-HT (e.g., d-fenfluramine) mechanisms of action; second, d-amphetamine and d-fenfluramine will be co-administered in such a way as to produce mixed DA/5-HT effects which will be compared to the effects of the compounds administered alone; and third, serotonin-mediated effects will be blocked either by fluoxetine or tryptophan depletion in order to isolate the effects of dopamine with the expectation that this blockade will have differential effects on amphetamine, fenfluramine and MDMA.

Although amphetamine and fenfluramine are similar structurally, they have significantly different subjective, discriminative stimulus and reinforcing effects. These differences in dependence-related effects are presumably related to the fact that amphetamine produces prominent effects on DA systems whereas fenfluramine's effects are mediated primarily by 5-HT systems. Interestingly, MDMA, which structurally is an amphetamine derivative, has prominent effects on both 5-HT and DA brain systems but unlike fenfluramine has abuse potential more like that of amphetamine. Thus, MDMA may be a useful compound for investigating the interactions among neurochemical systems as they relate to effects of drugs that are dependence-related.

Although most psychostimulants that are abused alter norepinephrine (NE), DA, and to a lesser degree 5-HT levels, there are compelling data that DA systems play the major role in the reinforcing properties and other dependence-related effects of psychostimulant drugs. On the other hand, DA and 5-HT neurochemical systems are known to interact; for instance, recent studies have demonstrated a potentiation of impulse-dependent DA release by 5-HT. MDMA, whose effects have been attributed to its actions on 5-HT systems as well as DA has been shown in animal studies to share discriminative stimulus and reinforcing effects with amphetamine. On the other hand, humans have also reported that it produces hallucinogenic-like effects. Animal brain dialysis studies have shown that pretreatment with selective 5-HT reuptake inhibitors (SSRIs such as fluoxetine, Prozac) will prevent fenfluramine from producing 5-HT release. Similarly, the 5-HT releasing property of MDMA is blocked by pretreatment with the selective SSRIs. The SSRIs provides us with a useful tool for investigating in humans how the dependence related actions of MDMA are modified by manipulations of its effects on 5-HT.

Specific Aim 1:

To investigate the subjective, reinforcing, motor activating, and aggression modulating effects of two doses of MDMA (between 1.0 mg/kg and 2.5 mg/kg—see below) compared with placebo, 10 mg/70 kg and 20 mg/70 kg of d-amphetamine, and 15 mg/70 kg and 30 mg/70 kg of d-fenfluramine in recreational drug users. Subjective effects of drugs of abuse have provided important information about the abuse and dependence-producing properties of drugs. To date, there are no well designed, double-blind studies of MDMA compared with prototypical psychostimulants or 5-HT releasing drugs. Based on animal research and reports of its abuse, we are hypothesizing that MDMA will resemble d-amphetamine more than d-fenfluramine in terms of its mood-altering properties although it may produce additional hallucinogenic effects. In addition, we hypothesize that MDMA, like amphetamine, will increase motor activity. On the other hand, we hypothesize d-amphetamine and MDMA will differ in a laboratory test of aggression, the point subtraction aggression paradigm, with d-amphetamine at the doses being used increasing aggression and MDMA decreasing it.

Specific Aim 2:

To characterize the discriminative stimulus properties of MDMA in recreational drug users. Based upon the results of animal research we are hypothesizing that MDMA will share discriminative properties with d-amphetamine but not d-fenfluramine. Specifically we are hypothesizing that subjects trained to discriminate between placebo, d-amphetamine and d-fenfluramine will respond to MDMA as if it is d-amphetamine. Recreational drug users will be trained to discriminate 10 mg/70 kg d-amphetamine from 30 mg/70 kg d-fenfluramine and placebo and tested with two doses of MDMA (to be determined in pilot study) to evaluate its discriminative properties, i.e., whether it is more amphetamine-like or fenfluramine-like.

Specific Aim 3:

To systematically measure the interaction of d-amphetamine and d-fenfluramine in healthy volunteers. The hypothesis is that increasing 5-HT release by the administration of d-fenfluramine will decrease the subjective stimulant effects of d-amphetamine and d-amphetamine will attenuate the typical aversive subjective effects of d-fenfluramine. A secondary hypothesis is that some of these drug combinations will result in subjective effects that resemble MDMA (mixed stimulant and hallucinogenic effects). Subjects will receive a fixed dose of d-amphetamine (10 mg/70 kg) in combination with a range of d-fenfluramine doses (15 mg/70 kg, 30 mg/70 kg, and placebo). A second group of subjects will receive a fixed dose of d-fenfluramine (15 mg/70 kg) in combination with a range of d-amphetamine doses (10 mg/70 kg, 20 mg/70 kg, and placebo).

The main goal of the experiments described in this proposal is to increase our understanding of the interaction between serotonin (5-HT) and dopamine systems in mediating the subjective, discriminative stimulus, and reinforcing effects of psychostimulant drugs in humans.

Specific Aim 4:

To evaluate the effect of pretreatment with fluoxetine on the behavioral and subjective effects of d-amphetamine, d-fenfluramine, and MDMA in recreational drug users. The hypothesis is that fluoxetine, by blocking the 5-HT transporter and thereby preventing the uptake of d-fenfluramine into neurons where it can release 5-HT, will block to a major degree its characteristic subjective effect profile. In contrast, pretreatment with fluoxetine will have only minor effects on the stimulant-like subjective and reinforcing effects of d-amphetamine. Although it is known that MDMA's effects on serotonergic neurons can be blocked by fluoxetine, we are hypothesizing that MDMA's stimulant-like and dependence-producing effects are mediated by DA, and that fluoxetine pretreatment would not significantly attenuate them. However, any of MDMA's effects which are mediated by 5-HT (e.g., changes in the LSD scale score of the ARCI, changes in the Hallucinogen Rating Scale), will be attenuated. Subjects will be randomized to receive pre-treatment with fluoxetine or placebo. Subjects in both groups will then be tested with single doses of placebo, d-fenfluramine (30 mg/70 kg), d-amphetamine (10 mg/70 kg), and MDMA (dose to be determined) under double-blind, balanced-order conditions. The subjective and reinforcing effects of the MDMA, d-amphetamine, d-fenfluramine and placebo will be compared across groups.

Specific Aim 5:

The tryptophan depletion paradigm, which transiently reduces brain 5-HT levels, will be used to investigate the effect of acute reduction in presynaptic 5-HT levels on the subjective and reinforcing properties of d-fenfluramine, d-amphetamine and MDMA. The hypothesis is that tryptophan depletion, but not sham depletion (tryptophan is added back to the amino-acid slurry), will attenuate the characteristic subjective and reinforcing effects of d-fenfluramine and produce only modest attenuation of the stimulant-like effects of d-amphetamine. As in the experiment with fluoxetine,

the effects of depletion on MDMA's effects will be a function of which neurochemical system is mediating its dependence-related effects. We are hypothesizing that depletion of 5-HT will attenuate the hallucinogenic effects of MDMA but have little effect on the stimulant and reinforcing effects of MDMA. Volunteers will be randomly assigned to one of four groups. Each subject will undergo both tryptophan and sham depletion paradigms under double-blind conditions. At the nadir of plasma tryptophan levels, each of the groups will receive either d-amphetamine (10 mg/70 kg), d-fenfluramine (30 mg/70 kg), or MDMA (dose to be determined), or placebo.

Specific Aim 6:

In conjunction with the studies outlined above blood samples will be collected for the measurement of homovanillic acid (HVA), the principal metabolite of dopamine, as well as cortisol and prolactin. These measures will provide objective data about the neurochemical and neurohormonal effects of the various drugs alone and in combination. This will allow the correlation of drug induced mood and behavioral changes with the neurochemical and neurohormonal changes mediated by DA and 5-HT. This series of studies will provide novel and scientifically valuable information about the role of DA/5-HT interactions in the subjective, discriminative stimulus, and reinforcing effects of psychostimulant drugs in humans. In addition, these studies will provide objective data on the neurochemical, neurohormonal, subjective and reinforcing effects of MDMA under standardized laboratory conditions.

Background and Significance

Psychostimulant abuse is a major public health problem in the United States. The popularity of stimulant drugs goes in cycles and there is recent evidence of increased amphetamine and methamphetamine use in southern California (Baberg et al., 1996). 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy) is a substituted amphetamine with both stimulant and hallucinogenic properties. In 1988, 39% of the students at a major United States university reported trying MDMA at least once (Peroutka, et al., 1987). A more recent NIDA report suggests that 2% of college students had taken MDMA within the past year (NIDA Caps 1993). Cuomo et al. (1994) reported an increase in the percentage of college students reporting having used MDMA at least once from 16% in 1986 to 24% in 1990 at a Southern University. MDMA is widely used at all-night parties ("raves") both in the United States and the United Kingdom (Randall 1992a,b; Solowij et al., 1992). It has been reported that 13-18% second year university students in the UK had taken LSD, amphetamine, or MDMA (Webb et al., 1996).

There is compelling evidence for the importance of DA systems in mediating the reinforcing properties of d-amphetamine and cocaine (Giros et al., 1996; Di Chiara and Imperato, 1988; Ritz and Kuhar, 1989). However, in humans, blocking the DA system with pimozide does not reliably block the subjective effects of d-amphetamine, suggesting that the stimulant effects of d-amphetamine are not simply due to activation of DA systems (Brauer and de Wit, 1996). Likewise, Volkow et al. (1996) found that blockade of DA transporter by methylphenidate did not block the "high" from a second dose of methylphenidate and suggested that other neurotransmitter systems may be involved in the "high." Furthermore, manipulation of 5-HT systems have been found to modulate the stimulant and reinforcing properties of d-amphetamine (Porrino et al., 1989; Leccese and Lyness, 1984).

Conflicting literature

There is a large, often conflicting literature describing functional 5-HT and DA system interactions both in vitro and in vivo (Korsgaard et al., 1985; Carroll et al., 1990; Peltier and Schenk, 1993). Some studies report an inhibition of DA systems by 5-HT (see review by Kapur and Remington, 1996; Prisco et al., 1994) while others report a 5-HT facilitation of impulse-dependent DA release (Benloucif et al., 1993; Iyer & Bradberry, 1996).

In behavioral paradigms, it appears that increased 5-HT activity does decrease the reinforcing properties of d-amphetamine. For instance, acute administration of the 5-HT precursor L-tryptophan (Leccese and Lyness, 1984), the 5-HT releasing agent d-fenfluramine (Olds and Yuwiler, 1992; Fletcher, 1995), the direct 5-HT agonist quipazine (Leccese and Lyness, 1984), and the 5-HT reuptake inhibitor fluoxetine (Porrino et al., 1989) decreased the rate of amphetamine self-administration, while chemical 5-HT depletions enhance the rate of responding for amphetamine (Leccese and Lyness, 1984). Furthermore, the 5-HT antagonist metergoline (Lyness and Moore, 1983) enhanced d-amphetamine self-administration, while cyproheptidine and methysergide reduced self-administration frequency (Leccese and Lyness, 1984). This inhibitory interaction between 5-HT and DA systems was consistent

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with a recent drug interaction study in humans in which a mutual antagonism of the mood altering properties of D,L-fenfluramine and phentermine were observed (Brauer et al., 1996). Interestingly, in a drug discrimination paradigm in rats, this combination was discriminated as more cocaine-like than either drug alone. This points to the importance of studying the interaction of d-amphetamine and fenfluramine in humans.

On the other hand, two studies in cocaine abusing human subjects report the opposite interaction. Satel et al., (1995) and Aronson et al. (1995) reported that tryptophan depletion, a procedure which acutely decreases 5-HT levels in the brain (Delgado et al., 1989; 1994) resulted in a decreased subjective response to intranasal administration of cocaine and to decreased cue-induced cocaine craving. Taken together, these different results may be a function of drug (cocaine versus d-amphetamine), different experimental paradigms, different brain regions being examined, different procedures, as well as different species. Nevertheless, this sort of conflicting data speaks to the need to conduct additional research in humans.

Comparative studies

Experimental methods have been developed in humans which permit assessment of subjective (Johanson and de Wit, 1989), discriminative stimulus (Preston and Bigelow, 1991), drug interaction, and reinforcing effects of drugs (Griffiths et al., 1996). There are also validated paradigms to assess their effects on behaviors such as activity and aggression (Cherek et al., 1986; Miczek and Haney, 1994) as well as neurohormonal and neurochemical effects. In this proposal the effects of d-amphetamine, d-fenfluramine, and MDMA alone and in combination will be compared using behavioral, subjective, neurohormonal and neurochemical measures.

d-Amphetamine is a prototypical psychostimulant drug which is used clinically in the treatment of narcolepsy and attention-deficit hyperactivity disorder. In humans it has positive subjective effects, increasing vigor, elation, arousal, and positive mood (Johanson and Uhlenhuth, 1980), is clearly discriminated from caffeine (Chait and Johanson, 1988) and fenfluramine (Chait et al., 1986), but not phenmetrazine (Chait et al., 1986), is reinforcing (Johanson and Uhlenhuth, 1980), and has a biphasic response on aggression (Cherek et al., 1986) with low doses increasing aggressive responding and higher doses decreasing the aggressive responses.

d-Fenfluramine is a potent 5-HT releaser and reuptake blocker which has recently been approved by the FDA as a treatment for obesity. Much less research work has been done in humans with this more selective enantiomer than with its racemic (d,l-) form. Although the studies in this proposal will use d-fenfluramine, it is assumed that these effects will be similar to those produced by d,l-fenfluramine. d,l-Fenfluramine has been shown to have mildly aversive subjective effects (increased anxiety and confusion and decreased elation and positive mood scales on the POMS; Brauer et al., 1996), is discriminated from d-amphetamine (Chait et al., 1986), is not reinforcing in humans (Chait et al., 1987) or rhesus monkeys (Woods and Tessel, 1974). Although no clinical data from humans has indicated any long-term problems with fenfluramine, animals studies in rats, guinea pigs, and rhesus monkeys have indicated that there are long-term changes in 5-HT neurons that may even be irreversible in particular brain regions (Schuster et al 1986; Harvey and McMaster 1975; Neckers et al., 1976; Barnes et al., 1989). In monkeys the dose that produced these effects was 10 mg/kg/injection given twice daily for fourteen consecutive days. This dose is five times that necessary to produce a significant decrease in the monkeys eating behavior. Similar results have been obtained in rats at doses ranging 6.25 and 50 mg/kg/injection twice daily for four consecutive days. More recently, Ricaurte and his colleagues have shown similar long term effects on serotonin neurons following the repeated administration of d-fenfluramine which has recently been approved by the FDA for marketing as an anorectic medication. While these data must be taken seriously, it must be appreciated that world wide over 30 million people have been treated for obesity with the D,L-fenfluramine and 10 million with the d isomer. There is no credible evidence that at therapeutic doses fenfluramine produces any neurotoxic effects in humans. Because of its lack of abuse potential the Food and Drug Administration and the Drug Enforcement Administration have recently recommended that fenfluramine and its isomers be removed from the Controlled Substances Act.

The pharmacology of MDMA is well known from animal studies and there have been numerous studies comparing it to prototypic dopaminergic (e.g., amphetamine) and seroton-

ergic (e.g., fenfluramine) drugs (for reviews, see Green et al., 1995; White et al., 1996). There is evidence that MDMA is self-administered in monkeys (Beardsley et al., 1986) suggesting human abuse liability. However, there are no well-controlled studies comparing the subjective (reinforcing) or objective (changes in temperature, heart rate or blood pressure) effects of MDMA in human subjects to other drugs, such as d-amphetamine and/or d-fenfluramine (McCann and Ricaurte, 1993; Grob et al., 1992; Liester et al., 1992). The only data that are available are retrospective subjective reports of symptoms experienced following MDMA ingestion (Greer and Talbert 1986; Downing 1986; Peroutka et al., 1988). In these reports, users describe a wide range of subjective effects ranging from "altered time perception" or a sense of "closeness" with other people, increased alertness, luminescence of objects, and decreased "hostility." It is important to note that the MDMA was usually taken in a social context in which people were told what effects they would experience. These expectations may have been of great importance in modulating the subjective experiences produced by MDMA. Common side effects or adverse effects reported by MDMA users include: insomnia, nausea, tight jaw muscles, dry mouth, diaphoresis, trouble concentrating, palpitations, tremor, and increased body temperature. Much less commonly, there are single case reports of liver failure, accidents, and cerebral hemorrhage (see Green et al., 1995 for review). Grob et al., (1996) have recently reported that low doses of MDMA (1 mg/kg) cause robust increases in prolactin and adrenocorticotropin hormone levels compared with placebo.

MDMA has more serotonergic activity than d-amphetamine and like fenfluramine, high doses have been associated with selective damage to serotonergic systems with a sparing of DA systems (Ricaurte et al., 1985; Ricaurte et al., 1988) in rats and non-human primates. Interestingly, in mice, MDMA caused DA not 5-HT neurotoxicity (Logan et al., 1988). Moreover, unlike other neurotoxins such as 1-methyl-1,2,3,6-tetrahydropyridine [MPTP] (Langston et al., 1983, 1984) which has been associated with severe depletion of dopamine and clinical symptoms of Parkinson's disease, there is no clear evidence that MDMA use results in any neurotoxicity in humans. Ricaurte and associates (1990) have reported decreased cerebral spinal fluid 5-HIAA (the primary serotonin metabolite) levels in subjects who had previously taken MDMA (as well as other drugs)—although there were no physical or psychological symptoms associated with these changes nor was MDMA use verified or the potential influence of others factors controlled. Several human deaths have been reported following MDMA ingestion. The majority of the deaths have been attributed to underlying medical conditions, such as arrhythmias, accidents, and most recently to dehydration and hyperthermia at "rave" parties (Screaton et al., 1992; Henry et al., 1992).

Summary

MDMA is a widely used illicit drug in this country. The mechanism by which the drug exerts its unique effects in humans is not well understood and cannot be reliably extrapolated from the animal literature or retrospective reports in humans (Johanson and de Wit, 1989; Schuster, 1989). The studies in this application are designed to provide important information about the effects of MDMA in humans.

In the proposed studies, several subjective (Johanson and Uhlenhuth, 1980) and behavioral measures will be used to compare across drugs. In addition to standard subjective effects, the discriminative stimulus effects of amphetamine, fenfluramine, and MDMA will be compared. Drug discrimination procedures provide a powerful technique to classify and differentiate closely related compounds and provide important information concerning their mechanisms of action (Appel et al., 1991; Preston and Bigelow, 1991). MDMA is discriminated as amphetamine in d-amphetamine trained rats (Glennon et al., 1988), pigeons (Evans and Johanson 1986), and rhesus monkeys, (Kamien et al., 1986) but also as fenfluramine in fenfluramine-trained rats (Schechter 1986). In rats trained to discriminate MDMA, d-amphetamine did not substitute for MDMA, while fenfluramine partially substituted (Baker et al., 1995). Evans, et al. (1990), showed in a three-way discrimination (amphetamine, fenfluramine, placebo) in pigeons that MDMA had both amphetamine-like and fenfluramine-like effects with differences across individuals and across doses.

This study is being conducted in "recreational drug users." Individuals who have tried drugs of abuse but have not developed drug abuse problems are ideal from an ethical viewpoint since their failure to become drug abusers, even though they have passed through the age of risk for severe drug dependence (Anthony et al., 1994; Breslau et al., 1993) and

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have sampled some drugs of abuse, means that exposure to amphetamine in a laboratory study would be an insignificant risk factor. Amphetamine and fenfluramine are therapeutic drugs and are frequently given to naive patients as part of outpatient therapy with little evidence of iatrogenic dependence (Schuster, 1989). Finally, d-amphetamine in the 10 mg/70 kg to 35 mg/70 kg dose proposed in the present have been given to normal volunteers in previous studies with no reports of serious adverse reactions (Wolkin et al., 1987; Angrist et al., 1987; Heishman and Henningfield, 1991; Johanson and de Wit, 1989; Foltin and Fischman, 1991).

In addition to comparison studies using subjective, discriminative stimulus, and reinforcing effects, drug interaction studies may help elucidate the mechanism of action mediating various subjective, behavioral and physiological effects of drugs. In the present studies, 5-HT systems are being manipulated by acute treatments with fluoxetine (5-HT transporter inhibitor) and with tryptophan depletion. These studies, will provide important information regarding which effects of MDMA and d-amphetamine are mediated by serotonin. It might be expected for example that the activity increasing effects of MDMA would be unaffected by manipulations which decrease serotonin activity whereas any hallucinogenic effects of MDMA would be attenuated.•

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