Pharmacology, Biochemistry and Behavior 156 (2017) 30–38

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Role of *d*-amphetamine and *d*-methamphetamine as active metabolites of benzphetamine: Evidence from drug discrimination and pharmacokinetic studies in male rhesus monkeys

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ARTICLE INFO

Keywords:
Cocaine
Discrimination
Benzphetamine
Rhesus monkey
Pharmacokinetic
Pharmacodynamic

ABSTRACT

Benzphetamine is a Schedule III anorectic agent that is a prodrug for *d*-amphetamine and *d*-methamphetamine and may have utility as an "agonist" medication for cocaine use disorder treatment. This study evaluated the pharmacokinetic-pharmacodynamic profile of benzphetamine using a drug discrimination procedure in rhesus monkeys. The potency and time course of cocaine-like discriminative stimulus effects were compared for benzphetamine (10–18 mg/kg, intramuscular (IM)) and *d*-amphetamine (0.032–0.32 mg/kg, IM) in monkeys (n = 3–4) trained to discriminate IM cocaine (0.32 mg/kg) from saline in a two-key food-reinforced discrimination procedure. Parallel pharmacokinetic studies in the same monkeys determined plasma benzphetamine, *d*-methamphetamine and/or *d*-amphetamine levels for correlation with behavioral effects. *d*-Amphetamine produced dose-dependent, time-dependent, and full cocaine-like effects, i.e. ≥ 90% cocaine-appropriate responding, in all monkeys without altering response rates. The time course of *d*-amphetamine's cocaine-like discriminative stimulus effects correlated with plasma *d*-amphetamine levels. Benzphetamine was 180-fold less potent than *d*-amphetamine and produced full cocaine-like effects in only 2 of 4 monkeys while significantly decreasing response rates. Benzphetamine administration increased plasma *d*-methamphetamine (peak at 100 min) and *d*-amphetamine (peak at 24 h) levels, but the time course of behavioral effects did not correlate with increased levels of benzphetamine, *d*-methamphetamine or *d*-amphetamine. These results suggest that benzphetamine yields *d*-amphetamine and *d*-methamphetamine as active metabolites in rhesus monkeys, but generation of these metabolites is not sufficient to account for benzphetamine behavioral effects. The incomplete cocaine substitution profile and protracted *d*-amphetamine plasma levels suggest that benzphetamine may still warrant further evaluation as a candidate pharmacotherapy for cocaine use disorder treatment.

1. Introduction

Cocaine use disorder remains a significant and global public health problem for which no Food and Drug Administration-approved pharmacotherapies exist (Acri and Skolnick, 2013). "Agonist"-based pharmacotherapies share pharmacodynamic mechanisms of action with the target abused drug, and this approach has shown potential for cocaine use disorder (for review, see (Herin et al., 2010, Negus and Henningfield, 2015, Stoops and Rush, 2013)). For example, chronic treatment with the monoamine transporter substrate *d*-amphetamine has demonstrated efficacy to decrease cocaine-maintained behaviors in both preclinical (Czoty et al., 2011; Negus, 2003; Thomsen et al., 2013) and human laboratory (Greenwald et al., 2010; Rush et al., 2010) studies, and in clinical trials (Grabowski et al., 2001; Mariani et al., 2012; Nuijten et al., 2016). However, the broad clinical deployment of *d*-amphetamine as a pharmacotherapy for cocaine use disorder treatment has been hindered by undesirable effects that include high abuse liability.

Prodrugs of agonist medications represent one potential mechanism to slow the onset of drug effects, prolong the duration of action, and potentially reduce abuse liability while retaining therapeutic effectiveness of the active metabolite (Balster and Schuster, 1973; Huttunen et al., 2011; Schindler et al., 2009). For example, the clinically available *d*-amphetamine prodrug lisdexamfetamine displays a slower onset and longer duration of action compared to *d*-amphetamine in humans (Jasinski and Krishnan, 2009a, 2009b), rhesus monkeys (Banks et al., 2015), and rats (Rowley et al., 2012). In addition, lisdexamfetamine failed to maintain drug self-administration in rats (Heal et al., 2013),

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http://dx.doi.org/10.1016/j.pbb.2017.03.008
Received 13 February 2017; Received in revised form 28 March 2017; Accepted 30 March 2017
Available online 31 March 2017
0091-3057/

but retained some treatment efficacy to decrease cocaine vs. food choice in monkeys (Banks et al., 2015; Johnson et al., 2016). Overall, this body of literature supports the conceptual framework of *d*-amphetamine prodrug formulations as candidate medications for cocaine use disorder treatment.

Benzphetamine is a Schedule III anorectic agent that yields both *d*-amphetamine and *d*-methamphetamine as active metabolites in humans (Cody and Valtier, 1998). However, the relationship of benzphetamine behavioral effects to its pharmacokinetics is unknown. To address this issue, the present study used Pharmacokinetic-Pharmacodynamic (PK/PD) analysis (Negus and Banks, 2016) to compare benzphetamine cocaine-like discriminative stimulus effects to plasma benzphetamine, *d*-amphetamine, and *d*-methamphetamine levels in the rhesus monkeys. PK/PD analysis has been particularly useful for elucidating the role of active metabolites in the behavioral effects of prodrug formulations, such as lisdexamfetamine (Banks et al., 2015; Rowley et al., 2012). We hypothesized that benzphetamine would produce full substitution for cocaine with a time course that paralleled generation of *d*-amphetamine and/or *d*-methamphetamine as active metabolites. Drug discrimination and pharmacokinetic studies were also conducted with *d*-amphetamine for comparison to results with benzphetamine.

2. Methods

2.1. Subjects

Studies were conducted in 4 individually housed adult male rhesus monkeys (*Macaca mulatta*) that had an extensive experimental history behaving under the cocaine drug discrimination procedure (Banks, 2014; Banks et al., 2013; Banks et al., 2015). Monkeys could earn 1-g banana-flavored pellets (Grain-based Precision Primate Tablets; Test Diets, Richmond, IL) during daily experimental sessions (see below). In addition, monkeys received daily rations of food biscuits (Lab Diet High Protein Monkey Biscuits; PMI Feeds, St Louis, MO) and fresh fruit or vegetables delivered in the afternoons after behavioral sessions to minimize the effects of biscuit availability and consumption on food-maintained operant responding. Water was continuously available. A 12-h light/dark cycle was in effect (lights on from 0600 to 1800 h). Environmental enrichment, which consisted of movies displayed on a monitor in the housing room and foraging boards loaded with nuts, seeds or diced vegetables, was also provided after behavioral sessions. Facilities were licensed by the United States Department of Agriculture and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The Institutional Animal Care and Use Committee approved all experimental and enrichment protocols. Animal research and husbandry were conducted according to the 8th edition of the Guide for the Care and Use of Laboratory Animals.

2.2. Pharmacodynamic studies

Pharmacodynamic studies were conducted using a two-key food-reinforced cocaine discrimination procedure that has been described previously in detail (Banks et al., 2013). Experimental sessions were conducted in each monkey's home chamber. A custom operant response panel with three horizontally arranged square response keys was attached to the front of the home chamber, and only the left and right keys were used in the present studies. A pellet dispenser (Med Associates, ENV-203-1000, St. Albans, VT) was bolted to each panel. Equipment operation and data collection were accomplished with a Windows-based computer and MED-PC IV software (Med Associates).

Monkeys were trained to discriminate 0.32 mg/kg cocaine intramuscularly (IM) from saline. Training was conducted 5 days per week during daily sessions composed of multiple components. Each component consisted of a 5-min response period, during which the right and left response keys were transilluminated red and green, respectively, and monkeys could earn up to 10 food pellets by responding under a fixed-ratio (FR) 30 schedule of food presentation. Training sessions were composed of three components presented at 2-h intervals, and either saline or cocaine (0.32 mg/kg) was administered IM approximately 15 min prior to the start of each component. On training days, monkeys received a sequence of saline (S) and cocaine (C) injections in the order SSS, SSC, SCS, CSS, SCC, CSC, CCS, or CCC. These sequences were presented to engender daily experience with randomized sequences of saline- and cocaine-appropriate components. The 2 h inter-component interval exceeded the time course of discriminative stimulus effects produced by the cocaine-training dose (Lamas et al., 1995), and thereby minimized effects of cocaine administered in earlier trials on performance during later trials on the same day. Following saline administration, only responding on the green key (the saline-appropriate key) produced food, whereas following 0.32 mg/kg cocaine administration, only responding on the red key (the cocaine-appropriate key) produced food. Responses on the inappropriate key reset the FR requirement on the appropriate key. The criterion for accurate discrimination was ≥ 85% injection-appropriate responding before delivery of the first reinforcer, ≥ 90% injection-appropriate responding for the entire component, and rates of responding ≥ 0.1 responses/s (sufficient to earn at least one pellet) for all components during 7 of 8 consecutive sessions.

Test sessions were identical to training sessions except that (a) responding on either key produced food, (b) monkeys received only one injection of vehicle or a test drug dose at the start of the session, and (c) 5-min response components began 10, 30, 56, 100, 180, 300, and 560 min after the injection to assess the time course of drug effects. If > 50% cocaine-appropriate responding was still observed in any monkey after 560 min, then additional response components began after 24 h in all monkeys. The drugs and dose ranges tested were: benzphetamine (10–18 mg/kg) and *d*-amphetamine (0.032–0.32 mg/kg). Test sessions were generally conducted on Tuesdays and Fridays with training sessions conducted on Mondays, Wednesdays, and Thursdays. Test sessions were conducted only if performance during the previous two training sessions met the criteria for accurate discrimination (described above). Benzphetamine doses were tested twice, whereas *d*-amphetamine doses were tested once in each monkey. All studies with a drug were completed in a given monkey before testing the next drug in that monkey, and vehicle (saline) test sessions were conducted before or after evaluation of each test drug. The order of both drug doses and drugs was counterbalanced across monkeys.

2.3. Pharmacokinetic studies

After completion of behavioral studies, the same monkeys were used to evaluate pharmacokinetics of behaviorally active benzphetamine and *d*-amphetamine doses. Monkeys were transferred to primate restraint chairs and fitted with temporary intravenous catheters (24 gauge Exel safelet catheter, Fisher scientific, Pittsburg, PA) inserted into a saphenous vein. Blood samples (1–2 mL) were collected in Vacutainer© tubes containing 3 mg of sodium fluoride and 6 mg sodium ethylenediaminetetraacetic acid before and 10, 30, 56, 100, 180, 300 min, 24 h, and 48 h after 18 mg/kg (IM) benzphetamine or 0.32 mg/kg *d*-amphetamine administration. Drugs were tested in a counterbalanced order across monkeys with at least two weeks between experiments. Samples were immediately centrifuged at 1000*g* for 10 min. The plasma supernatant was transferred into a labeled storage tube and frozen at − 80 °C until analyzed. The identification and quantification of benzphetamine, *d*-methamphetamine, and *d*-amphetamine were accomplished using an API-5000 with a turbo V source for Turbolon Spray (Applied Biosystems, Foster City, CA) run in multiple reaction monitoring mode (MRM) and attached to a Waters Acquity UPLC system (Milford, MA) controlled by Analyst 1.4.2 software. The column used was a Phenomenex (Torrance, CA) Synergi Hydro-RP (50 × 2 mm, 4 μm) with mobile phases consisting of A: 10 mM ammonium bicarbonate, pH 7.0, and B: Acetonitrile:Methanol (50:50) and a flow rate of 500 μL/