Separate and combined impact of acute naltrexone and alprazolam on subjective and physiological effects of oral *d*-amphetamine in stimulant users

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Abstract

Rationale Opioid antagonists (e.g., naltrexone) and positive modulators of γ -aminobutyric-acid_A (GABA_A) receptors (e.g., alprazolam) modestly attenuate the abuse-related effects of stimulants like amphetamine. The use of higher doses to achieve greater efficacy is precluded by side effects. Combining naltrexone and alprazolam might safely maximize efficacy while avoiding the untoward effects of the constituent compounds.

Objectives The present pilot study tested the hypothesis that acute pretreatment with the combination of naltrexone and alprazolam would not produce clinically problematic physiological effects or negative subjective effects and would reduce the positive subjective effects of *d*-amphetamine to a greater extent than the constituent drugs alone.

Methods Eight nontreatment-seeking, stimulant-using individuals completed an outpatient experiment in which oral *d*-amphetamine (0, 15, and 30 mg) was administered following acute pretreatment with naltrexone (0 and 50 mg) and alprazolam (0 and 0.5 mg). Subjective effects, psychomotor task performance, and physiological measures were collected.

Results Oral *d*-amphetamine produced prototypical physiological and stimulant-like positive subjective effects (e.g., VAS ratings of Active/Alert/Energetic, Good Effect, and High). Pretreatment with naltrexone, alprazolam, and their combination did not produce clinically problematic acute physiological effects or negative subjective effects. Naltrexone and alprazolam each significantly attenuated some of the subjective effects of *d*-amphetamine. The combination attenuated a greater number of subjective effects than the constituent drugs alone.

Conclusions The present results support the continued evaluation of an opioid receptor antagonist combined with a GABA_A-positive modulator using more clinically relevant experimental conditions like examining the effect of chronic dosing with these drugs on methamphetamine self-administration.

Keywords *d*-Amphetamine · Naltrexone · Alprazolam · Subjective effects

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Introduction

The illicit use of amphetamine remains a significant public health concern. Amphetamines are the most widely used class of illicit drugs worldwide, with the exception of cannabis (United Nations Office of Drugs and Crime 2012). In the USA, amphetamine abuse and dependence accounted for 111,885 drug treatment admissions in 2011 (Substance Abuse and Mental Health Services Administration, Center for Behavioral Health Statistics and Quality 2012). Despite extensive efforts by the scientific and treatment communities, an effective medication has not yet been identified for amphetamine abuse (e.g., Elkashef et al. 2008; Karila et al. 2010; Rush et al. 2009).

One strategy for medications development is to block the effects of amphetamines. Central dopamine systems play a prominent role in mediating the abuse-related behavioral effects of stimulants (i.e., reinforcing and positive subjective effects) (for review, see Pierce and Kumaresan 2006). However, using dopamine antagonists at doses sufficient to block the behavioral effects of amphetamines is problematic because of side effects (Meltzer 2013). An alternative approach is to use compounds that indirectly attenuate the behavioral effects of amphetamines and have an improved safety and tolerability profile.

Naltrexone is an antagonist at mu opioid receptors, which are colocalized with dopamine D₂ receptors on mesolimbic dopamine neurons (Ambrose et al. 2004; Pollard et al. 1977). Consistent with this neuroanatomical overlap, rodent microdialysis studies have demonstrated that opioid antagonists (e.g., naloxone and naltrexone) reduce amphetamine-induced dopamine release in the striatum and nucleus accumbens (Hooks et al. 1992; Schad et al. 1995). The results of preclinical laboratory experiments have also demonstrated that opioid antagonists attenuate the behavioral effects of amphetamines (Dettmar et al. 1978; Haggkvist et al. 2009; Holtzman 1974; Jimenez-Gomez et al. 2010; Trujillo et al. 1991). For example, naltrexone pretreatment decreased intravenous *d*-amphetamine self-administration in rhesus monkeys by approximately 50 % (Jimenez-Gomez et al. 2010).

Human laboratory experiments have demonstrated modest reductions in the subjective effects of *d*-amphetamine following acute pretreatment with naltrexone (Jayaram-Lindström et al. 2004, 2008b). Consistent with the laboratory studies, a double-blind, placebo-controlled clinical trial showed that patients maintained on 50 mg/day naltrexone provided more amphetamine-negative urine samples than their placebo-treated counterparts (Jayaram-Lindström et al. 2008a) and another trial found improved retention rates in a naltrexone implant-treated group (Tiihonen et al. 2012). Serious untoward effects, however, limit the use of higher doses. Liver enzymes are elevated following naltrexone doses ranging from 100 to 300 mg (Anton et al. 2006; Pfohl et al. 1986), and concerns have been raised regarding hepatotoxicity in those with compromised liver function (Garbutt 2010). Higher doses might also be unlikely to further impact the response to amphetamine. Oral doses of 50 mg naltrexone produce plasma levels sufficient to saturate mu opioid receptors for 72–108 h and provide near complete attenuation of the subjective effects of full mu agonists like hydromorphone, with no further attenuation observed following a fourfold increase in dose (Lee et al. 1988; Walsh et al. 1996).

Given the incomplete attenuation of subjective effects and amphetamine use at 50 mg/day naltrexone, novel strategies are needed to enhance its efficacy. Combining naltrexone with another drug that also modestly attenuates the effects of amphetamines might safely maximize efficacy in the management of amphetamine dependence. A series of elegant

preclinical studies demonstrated that combining low doses of two different drugs is an effective strategy to manage stimulant use disorders and avoid the untoward effects of the constituent compounds (Goeders et al. 1989, 1993; Goeders and Guerin 2008). Combining low doses of oxazepam and metyrapone that were ineffective when tested alone resulted in reduced cocaine self-administration in rats (Goeders and Guerin 2008). In agreement with the preclinical data, oxazepam-metyrapone combinations were well tolerated and significantly reduced cocaine use in a small clinical trial (Kablinger et al. 2012).

Evidence suggests that positive modulators of γ -aminobutyric-acid_A (GABA_A) receptors (e.g., benzodiazepines) might also be effective in the treatment of amphetamine dependence. Neuroanatomical studies indicate that GABA_A receptors are colocalized on dopamine cell bodies (Churchill et al. 1992), and studies in animals have demonstrated that GABA_A receptor modulators (e.g., lorazepam) attenuate methamphetamine-induced increases in dopamine levels in the striatum and nucleus accumbens (Dewey et al. 1997) and the behavioral response to stimulants (Barrett et al. 2005; Goeders et al. 1989, 1993; Weerts et al. 2005). For example, acute administration of alprazolam, a GABA_A receptor modulator, dose dependently reduced cocaine self-administration in baboons (Weerts et al. 2005).

Human behavioral pharmacology laboratory experiments have also demonstrated that acute and repeated alprazolam administration attenuates the behavioral effects of amphetamine (Lile et al. 2011a; Rush et al. 2004). In the more recent study, stimulant-dependent participants were maintained on extended-release alprazolam (i.e., alprazolam XR) or placebo (Lile et al. 2011a). Alprazolam XR produced a small, but orderly attenuation in some subjective effects of methamphetamine (i.e., Any Effect, High, Rush, and Stimulated) and was well tolerated. The use of larger doses that might more completely attenuate the abuse-related effects of amphetamine is problematic due to abuse potential (e.g., Chouinard 2004; Griffiths et al. 1985; Mumford et al. 1995a, b; Rush et al. 1993) and performance impairment (Lile et al. 2011a). The present experiment therefore tested the hypothesis that acute pretreatment with the combination of naltrexone and alprazolam would not produce clinically problematic physiological effects or negative subjective effects and that the combination would reduce the positive subjective effects of *d*-amphetamine to a greater extent than the constituent drugs alone.

Methods

Participants

The Institutional Review Board of the University of Kentucky Medical Center approved this study, and the participants gave

their sober, written informed consent prior to enrolling. During the consent process, participants were informed that they would receive various drugs including placebo, *d*-amphetamine, naltrexone, and alprazolam. Participants were instructed that these medications could be administered alone or in combination. Other than receiving this general information, participants were blind to the type and dose of drug administered. Participants were told that the purpose of the study was to determine how different drugs affect physiology, mood, and behavior. Other than this general explanation, they were given no instruction of what they were “supposed” to do or of what outcomes might be expected. Participants were compensated for their time and effort.

Prior to enrollment, all potential participants underwent a comprehensive physical and mental health screening (e.g., Sevak et al. 2010). Participants had to meet the following inclusion criteria: (1) self-reported recent amphetamine or cocaine use, (2) confirmation of recent amphetamine or cocaine use by a stimulant-positive urine screen during the initial screening, (3) fulfillment of the diagnostic criteria for amphetamine or cocaine dependence or abuse on a computerized version of the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders-IV (SCID DSM-IV), and (4) urine sample negative for opioids and absence of withdrawal signs and symptoms during the screening process. All participants were in good health with no contraindications to the study drugs.

General procedures

Participants were enrolled as outpatients at the Laboratory of Human Behavioral Pharmacology (LHBP) at the University of Kentucky Chandler Medical Center where they completed two drug-free practice sessions followed by one experimental session for each of the pretreatment + *d*-amphetamine dose combinations (12 total). Participants were instructed to abstain from taking all psychoactive drugs (with the exception of tobacco) throughout the study, caffeinated products and solid food for 4 h before a scheduled session, and alcohol and cocaine for 12 h before a scheduled session. Urine screens were conducted at the outset of every session for recent drug use and pregnancy. In order to participate in an experimental session, participants had to provide a urine sample negative for recent barbiturate and opiate use, as well as pregnancy, on each day of their participation. Participants were allowed to participate in an experimental session if they tested positive for recent cannabis use or for amphetamine or benzodiazepine use if it was determined that these last two drugs were given in a recent session and was likely that the result was positive due to experimental administration. Due to its shorter duration of action, cocaine-positive urine samples were permitted. Participants also completed a field sobriety test to ensure that they were not currently intoxicated. No sessions were rescheduled

due to failure to comply with the drug restrictions or pass the field sobriety test. Participants provided an expired air sample, which was assayed for the presence of alcohol using an Alco-Sensor breathalyzer (Intoximeters, St. Louis, MO). Five sessions were rescheduled due to failure to comply with alcohol restrictions.

Experimental sessions

Experimental sessions were approximately 7.5 h long. Sessions were generally conducted daily, Monday through Friday, and arrival time was held constant for each participant. For example, on each session day, participants arrived at the LHBP at approximately 0800 hours. Participants completed the pre-session tasks; vital signs were recorded at 15 min intervals between 0800 and 0830 hours, and participants were provided with a light breakfast with a decaffeinated beverage. The pretreatment dose (placebo, 50 mg naltrexone, 0.5 mg alprazolam, or the combination of 50 mg naltrexone and 0.5 mg alprazolam) was administered at approximately 1000 hours. *d*-Amphetamine (0, 15, and 30 mg) was administered at approximately 1030 hours. Subjective drug effect questionnaires, performance tasks, and physiological measures (described below) were completed at hourly intervals from 1100 to 1500 hours. Participants also completed a cognitive task battery at 1100 and 1200 hours. Participants were allowed to eat lunch at 1300 hours, which was provided by the LHBP. If no drug effects (physiological or behavioral) were detected at 5 h post-administration, participants were released from the laboratory.

This experimental timeline was devised based on the pharmacokinetics of *d*-amphetamine, naltrexone, and alprazolam. More specifically, peak *d*-amphetamine plasma concentrations occur approximately 2–3 h after oral administration with a half-life of 12 h (Angrist et al. 1987; Ermer et al. 2011). Peak naltrexone plasma concentrations occur approximately 1 h after oral administration and the half-life for its active metabolites (e.g., 2-hydroxy-3-methoxy-6 β -naltrexol) is 12 h (Meyer et al. 1984). Peak alprazolam plasma concentrations occur approximately 2 h after oral administration (Abernethy et al. 1983; Greenblatt et al. 1983; Greenblatt and Wright 1993). The elimination half-life of alprazolam and its active metabolites, 4-hydroxyalprazolam and α -hydroxyalprazolam, is approximately 9–16 h (Greenblatt et al. 1983; Greenblatt and Wright 1993; Smith and Kroboth 1987). *d*-Amphetamine was therefore administered 30 min after administration of naltrexone and alprazolam to ensure that onset of the pretreatment drugs preceded *d*-amphetamine.

Experimental measures

The subjective drug effect questionnaires and a performance task were administered on an Apple Macintosh computer

(iMac, Cupertino, CA) in fixed order. The subjective drug effect questionnaires included the Drug Effect Questionnaire (Rush et al. 2003) and the Adjective Rating Scale (Oliveto et al. 1992). The Drug Effect Questionnaire used a 100-unit visual analog scale and the Adjective Rating Scale used a five-response Likert-type scale.

Digit symbol substitution test

The Digit Symbol Substitution Test (DSST) is widely used in human behavioral pharmacology research to assess changes in psychomotor performance following drug administration (McLeod et al. 1982). The dependent measures were the number of geometric patterns the participant attempted to enter (i.e., trials attempted) and the number of patterns the participant entered correctly (i.e., trials correct) in 90 s.

Physiological measures

Blood pressure and heart rate were recorded with an automated monitor (Dinamap Pro 1000 Vital Signs monitor; Critikon Company LLC, Tampa, FL). If heart rate had exceeded 130 bpm, systolic blood pressure exceeded 180 mm Hg, or diastolic blood pressure exceeded 120 mm Hg, a participant would have been excluded from participation, but this did not occur.

Cognitive tasks

Participants also completed a cognitive task battery that included the Visual Dot Probe (e.g., Ehrman et al. 2002), Cued-Go/No Go (Fillmore et al. 2003), Cocaine Stroop (Liu et al. 2011), and Balloon Analogue Risk Task (Lejuez et al. 2002). *d*-Amphetamine effects were limited, with no significant impact of pretreatment, and will not be discussed further.

Drug administration

All drugs were administered in a double-blind fashion and were prepared by over-encapsulating commercially available drug (50 mg naltrexone and 15 and 30 mg *d*-amphetamine, Barr Laboratories INC, Pomona, NY; 0.5 mg alprazolam, Greenstone, Ltd. Peapack, NJ). Cornstarch was used to fill the remainder of all the capsules. The naltrexone dose (50 mg) was selected based on the clinical recommendations for alcohol dependence, which has subsequently been used in human laboratory experiments and clinical trials for stimulant abuse (Lee et al. 1988; Jayaram-Lindström et al. 2004, 2008a, b; Volpicelli et al. 1992). The alprazolam dose (0.5 mg) was selected based on previous human laboratory research in which acute alprazolam (0.5 mg) and extended-release alprazolam (1 mg/day) attenuated the behavioral and subjective effects of *d*-amphetamine (Lile et al. 2011a; Rush et al.

2004). In order to match the pharmacokinetic profile of immediate-release naltrexone and avoid the possibility that drug effects would persist beyond the 7.5-h outpatient session, immediate-release alprazolam (0.5 mg) was selected. *d*-Amphetamine doses were selected based on a previous research that has tested its behavioral effects in individuals with a history of stimulant use (Oliveto et al. 1998; Stoops et al. 2004; 2007). Placebo was also tested and capsules contained only cornstarch.

The order of dose administration in each experiment was random, with the following exceptions. First, the lower dose of *d*-amphetamine alone was administered prior to the higher *d*-amphetamine dose. Second, the lower dose of *d*-amphetamine was administered in combination with naltrexone, alprazolam, or the combination before dose combinations with the higher *d*-amphetamine dose. References below to *d*-amphetamine alone pertain to those instances in which 15 or 30 mg was administered following pretreatment with 0 mg naltrexone and 0 mg alprazolam. References to naltrexone alone or alprazolam alone pertain to those instances in which 0 mg *d*-amphetamine was administered following pretreatment with an active dose (i.e., 50 or 0.5 mg, respectively) of one drug when combined with 0 mg of the other drug. Naltrexone and alprazolam alone refer to administration of 0 mg *d*-amphetamine following pretreatment with the combination of the active doses of both drugs (50 and 0.5 mg, respectively). Placebo refers to the condition in which 0 mg of *d*-amphetamine, naltrexone, and alprazolam were administered. Each combination of pretreatment dose + *d*-amphetamine dose was tested in a single session, with 12 total dose conditions administered.

Data analysis

Peak effect data (i.e., the maximum score observed following *d*-amphetamine administration) from the subjective drug effect questionnaires, physiological assessments, and DSST presented here were collected during experimental sessions, following pretreatment with the abovementioned doses of naltrexone and alprazolam. Area under the curve was also calculated, and the outcomes were nearly identical to those from the peak effects analysis, so they are not included for brevity. Data were analyzed using a two-factor repeated measures analysis of variance (ANOVA) with pretreatment (i.e., placebo, naltrexone, alprazolam, and naltrexone + alprazolam) and *d*-amphetamine (i.e., 0, 15, and 30 mg) as the factors. If a main effect of *d*-amphetamine or a statistically significant interaction (i.e., interaction of pretreatment and *d*-amphetamine) was detected, the mean square error term was used to conduct Fisher's least significant difference post hoc tests to determine potential differences between active doses of *d*-amphetamine alone and placebo. If a dose of *d*-amphetamine differed significantly from the placebo, additional post hoc tests were conducted to compare the effects of these doses of

d-amphetamine alone and during the pretreatment conditions. For all statistical analyses, effects were considered significant for $p \leq 0.05$.

This study was initially designed to evaluate the ability of pretreatment with naltrexone and alprazolam to attenuate the reinforcing effects of *d*-amphetamine. *d*-Amphetamine failed to reliably maintain self-administration under the modified progressive ratio procedure used with the first four participants and the fixed ratio procedure used with the last four participants. To ensure that participation in the protocols using different self-administration procedures did not influence other outcomes, data were also analyzed using a mixed model ANOVA with group (i.e., modified progressive ratio procedure and fixed ratio procedure) as a between-group factor and pretreatment (i.e., placebo, naltrexone, alprazolam, naltrexone, and alprazolam) and *d*-amphetamine (i.e., 0, 15, and 30 mg) as the within-group factors (Statview, Cary, NC). Self-administration group did not produce a systematic effect and will not be discussed further.

Results

Demographics

Eight nontreatment seeking individuals were enrolled in the protocol. Participants ranged in age from 25 to 54 years (mean, 41 years) and in weight from 64 to 106 kg (mean, 81 kg). Seven participants were male and one was female. Seven participants reported their race as African-American and one reported their race as Caucasian. Four had used amphetamines 2 to 20 times in their lifetime (mean, 14). All participants reported current cocaine use (preferentially via the smoked route) using 4 to 26 days (mean, 12 days) in the month prior to screening and from 9 to 33 years (mean, 18) in total. All reported consuming alcohol (range 1–20 beverages per week; mean, 12) and using cannabis (range 1 to 30 days; mean, 17) in the month prior to screening. Seven participants reported lifetime benzodiazepine use and one participant reported using benzodiazepines three times in the month prior to screening. All participants reported smoking tobacco cigarettes (3–20 per day; mean, 9). Participants did not meet the criteria for dependence on any substances other than stimulants or nicotine.

Drug Effect Questionnaire

A significant main effect of *d*-amphetamine was observed for eight items on the Drug Effect Questionnaire: Active/Alert/Energetic, Any Effect, Good Effect, Irregular/Heart Racing, High, Nervous/Anxious, Rush, and Shaky/Jittery ($F_{6,42}$ values=4.0–8.0; $p \leq 0.05$). Figure 1 shows the separate and combined effects of *d*-amphetamine, naltrexone, and

alprazolam for ratings on five items: Active/Alert/Energetic, High, Good Effect, Any Effect, and Rush. Post hoc comparisons indicated that both active doses of *d*-amphetamine alone significantly increased ratings of Active/Alert/Energetic, Good Effect, and High relative to placebo. For the items Active/Alert/Energetic and Good Effect, 15 mg *d*-amphetamine did not significantly increase ratings on these items following pretreatment with alprazolam and the combination of naltrexone and alprazolam. In addition, alprazolam and the combination attenuated ratings of Active/Alert/Energetic produced by 15 mg *d*-amphetamine relative to 15 mg *d*-amphetamine alone. For the item High, following pretreatment with the combination of naltrexone and alprazolam, 15 mg *d*-amphetamine did not significantly increase ratings of High relative to placebo.

For the items Any Effect and Rush, 30 mg *d*-amphetamine alone significantly increased ratings on these items relative to placebo. Following pretreatment with naltrexone, 30 mg *d*-amphetamine did not significantly increase ratings of Any Effect relative to placebo. Following pretreatment with alprazolam and the combination of naltrexone and alprazolam, 30 mg *d*-amphetamine did not significantly increase ratings of Rush relative to placebo. See Fig. 1 of the Supplementary Materials for the separate and combined effects of *d*-amphetamine, naltrexone, and alprazolam for ratings on the remaining three items: Irregular/Heart Racing, Nervous/Anxious, and Shaky/Jittery. There were no effects of *d*-amphetamine on the following items: Bad Effect, Euphoric, Like Drug, Nauseated/Queasy/Sick to Stomach, Performance Impaired, Performance Improved, Restless, Sluggish/Fatigued/Lazy, Talkative/Friendly, Willing to Pay For, and Willing to Take Again.

Adjective rating scale

A significant main effect of *d*-amphetamine ($F_{6,42}=13.0$; $p \leq 0.01$) was observed for scores on the Stimulant subscale of the Adjective Rating Scale (Fig. 1). Post hoc comparisons revealed that both active doses of *d*-amphetamine alone increased ratings on this scale relative to placebo. Following pretreatment with alprazolam and the combination of naltrexone and alprazolam, 15 mg *d*-amphetamine did not significantly increase ratings relative to placebo. In addition, pretreatment with alprazolam significantly decreased participant ratings on this stimulant subscale produced by 15 mg *d*-amphetamine relative to 15 mg *d*-amphetamine alone. There were no effects of *d*-amphetamine on the Sedative subscale (data not shown).

Physiological measures

A significant main effect of *d*-amphetamine was observed for systolic ($F_{6,42}=33.4$; $p \leq 0.01$) and diastolic blood pressure ($F_{6,42}=18.1$; $p \leq 0.01$) (Fig. 2). Post hoc comparisons indicated

Fig. 1 Mean peak subject ratings for oral *d*-amphetamine on the items Active/Alert/Energetic, High, Good Effect, Any Effect, and Rush from the Drug Effect Questionnaire and the Stimulant subscale of the Adjective Rating Scale following pretreatment with placebo, naltrexone and placebo, alprazolam (PL), naltrexone (NTX) and placebo, alprazolam (ALP) and placebo, and naltrexone and alprazolam (NTX + ALP). Symbols represent the mean of eight subjects. Filled symbols indicate a significant difference from the placebo-placebo condition (i.e., the data point above the leftmost PL on the x-axis). An asterisk indicates a significant difference from the corresponding *d*-amphetamine dose under the placebo pretreatment condition. Error bars indicate 1 standard error of the mean (SEM)

that 30 mg *d*-amphetamine significantly increased systolic and diastolic blood pressure readings relative to placebo. Following pretreatment with alprazolam and the combination of naltrexone and alprazolam, 30 mg *d*-amphetamine did not significantly increase systolic blood pressure relative to placebo. Alprazolam alone significantly decreased systolic blood pressure relative to placebo. Diastolic blood pressure was significantly elevated by 30 mg *d*-amphetamine following pretreatment with naltrexone relative to placebo and alprazolam pretreatment. There were no effects of *d*-amphetamine on heart rate (data not shown).

DSST

A significant interaction of pretreatment and *d*-amphetamine was observed for the number of trials attempted ($F_{6,42}=2.5$; $p\leq 0.05$) on the DSST (Fig. 2 Supplementary Materials). Pretreatment with naltrexone alone significantly increased the number of trials attempted relative to placebo and alprazolam alone. A significant main effect of *d*-amphetamine was observed for the number of correct trials ($F_{6,42}=5.0$; $p\leq 0.05$) on

the DSST; however, post hoc comparisons did not detect any significant differences between conditions.

Discussion

Previous research has demonstrated that both positive modulation of the GABA_A receptor subtype and opioid receptor antagonism modestly attenuate the neurochemical and behavioral response to stimulants (e.g. Comer et al. 2013; Dewey et al. 1997; Jayaram-Lindström et al. 2008b; Jimenez-Gomez et al. 2010; Lile et al. 2011a). However, as described above, untoward side effects limit the use of higher doses of these drugs that might be necessary to enhance their clinical utility. The goal of this pilot study was therefore to test the hypothesis that pretreatment with the combination of naltrexone and alprazolam would produce a greater reduction in the abuse-related effects of *d*-amphetamine relative to the constituent compounds and without side effects.

The participants enrolled in this study reported past month stimulant use and met the criteria for cocaine abuse

Fig. 2 Mean peak systolic and diastolic blood pressure following administration of *d*-amphetamine and the four pretreatment conditions. An asterisk indicates a significant difference from the corresponding *d*-amphetamine dose under the placebo pretreatment condition. A hash indicates a significant difference from the corresponding *d*-amphetamine dose under the alprazolam pretreatment condition. Other details are as in Fig. 1

or dependence. Oral *d*-amphetamine produced prototypical physiological and stimulant-like positive subjective effects (e.g., VAS ratings of Active/Alert/Energetic, Good Effect, and High; Stimulant scale of Adjective Rating Scale) and negative subjective effects (e.g., VAS ratings of Irregular/Heart Racing, Nervous/Anxious, and Shaky/Jittery) in these participants. These oral *d*-amphetamine effects were similar to what was observed in previous studies with stimulant users (Fillmore et al. 2003; Stoops et al. 2004) and healthy participants (e.g., Jayaram-Lindström et al. 2004; Makris et al. 2007). In contrast, in a recent human laboratory experiment in cocaine users that sought to replicate the ability of naltrexone to attenuate the effects of *d*-amphetamine (i.e., healthy participants, Jayaram-Lindström et al. 2004; amphetamine-dependent individuals, Jayaram-Lindström et al. 2008b), *d*-amphetamine (10 and 20 mg) did not produce sufficient positive subjective effects to determine the impact of concurrent naltrexone administration (Comer et al. 2013). Given that both Comer et al. (2013) and the present study enrolled participants of similar age and stimulant use history, the discrepancy in results might be attributed to methodological differences. Comer et al. (2013) administered *d*-amphetamine (0, 10, and 20 mg) in ascending order within a single session and collected VAS ratings every 15 min following each *d*-amphetamine dose. In contrast, the present study administered *d*-amphetamine (0, 15, and 30 mg) in separate sessions and

measured VAS ratings hourly. Additionally, the Comer study included experimental sessions in which cocaine was administered, potentially producing a “contrast effect” between the subjective effects of oral *d*-amphetamine and smoked cocaine (Comer et al. 2013).

Naltrexone pretreatment alone did not produce any physiological or subjective effects, which is generally consistent with previous laboratory studies, although others have sometimes reported mild fatigue or gastrointestinal disturbance (Hollister et al. 1981; Jayaram-Lindström et al. 2004). Following *d*-amphetamine administration, naltrexone modestly attenuated both positive and negative subjective effects of *d*-amphetamine. These results are consistent with other human laboratory studies that demonstrated a decrease in subjective effects during naltrexone treatment relative to placebo (Jayaram-Lindström et al. 2004, 2008b). To the extent that both the positive and negative subjective effects of *d*-amphetamine are the result of elevated synaptic dopamine, the blockade of this response is consistent with the ability of naltrexone to reduce *d*-amphetamine-mediated increases in dopamine (Hooks et al. 1992; Schad et al. 1995).

Alprazolam pretreatment alone did not produce performance impairment or subjective effects. Alprazolam alone did decrease systolic blood pressure relative to placebo; however, these values were statistically but not clinically significant. When administered prior to *d*-amphetamine, alprazolam pretreatment modestly attenuated some subjective effects of *d*-amphetamine. These reductions in subjective effects were similar to what was observed in previous studies using acute pretreatment doses of 0.5 mg alprazolam and maintenance doses of 1.0 mg/day alprazolam XR (Lile et al. 2011a; Rush et al. 2004). Studies using GABA_A positive modulators have also found reductions in drug-appropriate responding in animals and humans discriminating stimulants (Gatch et al. 2005; Negus et al. 2000; Rush et al. 2004). Together, these results are consistent with the ability of benzodiazepines to attenuate the interoceptive effects of stimulants. Importantly, the lack of alprazolam effects on the DSST suggests that the attenuation of the subjective effects of *d*-amphetamine were not the result of general behavioral impairment.

There appears to be only a single previously published report in which naltrexone and alprazolam were given in combination to human subjects under controlled laboratory conditions (Janssen and Arntz 1999). In that study, experimenter administered pain and anxiety were assessed in participants with a spider-specific phobia. Safety-related outcomes such as heart rate were not significantly affected by naltrexone, alprazolam, or the combination. There do not appear to be any previous studies, however, in which naltrexone, alprazolam, and *d*-amphetamine were administered together. Thus, one purpose of this pilot study was to test whether the combination of all three compounds produces problematic physiological or negative subjective effects.

In agreement with the Janssen and Arntz (1999) study, the present results demonstrated that administration of the combination of naltrexone and alprazolam (i.e., sessions in which placebo *d*-amphetamine was administered) did not alter blood pressure or heart rate across the session. Following *d*-amphetamine administration, the combination did not produce problematic physiological effects. The combination alone did not produce subjective effects or performance impairment. When the combination was administered prior to *d*-amphetamine, naltrexone and alprazolam attenuated a greater number of subjective effects (eight) than naltrexone (three) or alprazolam (five) alone. Importantly, the combination significantly attenuated ratings of High, which was not observed following individual pretreatment with naltrexone or alprazolam. These results suggest that the combination of naltrexone and alprazolam might more effectively reduce the abuse-related effects of amphetamine compared to the constituent compounds.

Several limitations of the present experiment warrant discussion in an effort to guide the design of future studies. First, the present study tested *d*-amphetamine whereas methamphetamine is also a commonly abused amphetamine. Although *d*-amphetamine and methamphetamine produce a similar constellation of effects (Sevak et al. 2009), there are neuropharmacological differences between these congeners (e.g., norepinephrine and serotonin release; Rothman et al. 2001) that might translate to a differential impact of naltrexone and alprazolam treatment on methamphetamine-related effects. Second, *d*-amphetamine was administered orally in the present study. Drug effects that onset rapidly, such as those following intranasal or smoked administration of methamphetamine, might be more difficult to attenuate. Third, a higher dose of *d*-amphetamine might have been more difficult to attenuate. Indeed, pretreatment with the constituent compounds and the combination attenuated a greater number of subjective effects produced by 15 mg *d*-amphetamine relative to 30 mg *d*-amphetamine. The present doses are consistent with previous studies administering *d*-amphetamine acutely (e.g., 2.5–30 mg in stimulant users; Oliveto et al. 1998; Rush et al. 2004; Stoops et al. 2004), but amphetamine users might take higher doses. Fourth, acute pretreatment was used instead of maintenance, and the impact of a potential therapeutic might differ based on whether administration was acute or repeated (see Lile et al. 2011b for discussion). Important to note, however, is that naltrexone and alprazolam have produced generally consistent effects under both dosing regimens (e.g., Jayaram-Lindström et al. 2008a, b; Lile et al. 2011a; Rush et al. 2004). In addition, acute dosing provides valuable information about initial side effects (e.g., sedation with benzodiazepines). Fifth, only a single dose of naltrexone and alprazolam was tested. A more robust attenuation of the effects of *d*-amphetamine might have been observed with a different dose combination. Sixth, a relatively small number of participants were enrolled, which might have limited the ability to detect effects that might be observed in

larger samples. Seventh, four participants were amphetamine-naïve prior to enrollment in the study, which might have affected the response to *d*-amphetamine. However, participants with a history of drug use in the class under study are suitable because they are able to use their past drug experiences to evaluate drug effects (Griffiths et al. 2003), and as noted, cocaine and amphetamine produce similar behavioral effects (Johanson et al. 2006; Lamb and Henningfield 1994; Oliveto et al. 1998; Sevak et al. 2009). Further, an amphetamine-dependent sample would likely be comprised of users of smoked methamphetamine who, like cocaine users, do not necessarily have experience with or prefer oral routes of administration. A final consideration is that because the study was conducted on an outpatient basis, the use of drugs such as cocaine and cannabis that were permitted outside of experimental sessions might have affected study outcomes.

In summary, pretreatment with the combination of naltrexone and alprazolam did not produce clinically problematic physiological effects or negative subjective effects and attenuated more subjective effects of *d*-amphetamine than the constituent compounds. These findings support continued research to determine the ability of an opioid receptor antagonist combined with a GABA_A-positive modulator to attenuate the reinforcing and other abuse-related effects of amphetamine, which would more directly model a clinical treatment scenario.

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