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A preclinical evaluation of the discriminative and reinforcing properties of lisdexamfetamine in comparison to D-amfetamine, methylphenidate and modafinil

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ABSTRACT

Lisdexamfetamine dimesylate, which consists of L-lysine covalently bound to D-amfetamine, is the first prodrug for treating ADHD. Its metabolic conversion to yield D-amfetamine by rate-limited, enzymatic hydrolysis is unusual because it is performed by peptidases associated with red blood cells. Other stimulants shown to be effective in managing ADHD include D-amfetamine, methylphenidate and modafinil. All have the potential for misuse or recreational abuse. The discriminative and reinforcing effects of these compounds were determined in rats using a 2-choice, D-amfetamine (0.5 mg/kg, i.p.)-cued drug-discrimination test, and by substitution for intravenous cocaine in self-administration. Lisdexamfetamine (0.5–1.5 mg/kg [D-amfetamine base], p.o.) generalised to saline when tested 15 min post-dosing, but dose-dependently generalised to D-amfetamine at 60 min. At 120 min, its D-amfetamine-like effects were substantially diminished. At 15 min, methylphenidate (3.0–10 mg/kg, p.o.) and D-amfetamine (0.1–1.5 mg/kg, p.o.) dose-dependently generalised to the intraperitoneal D-amfetamine cue. Switching to the intraperitoneal route reduced the interval required for lisdexamfetamine to be recognised as D-amfetamine-like, but did not alter its potency. Switching to intraperitoneal injection increased the potency of methylphenidate and D-amfetamine by 3.4× and 2.2×, respectively. Modafinil (50–200 mg/kg, i.p.) generalised partially, but not fully, to D-amfetamine. Methylphenidate (0.1, 0.3, 1.0 mg/kg/injection, i.v.) maintained robust self-administration at the 2 highest doses. Neither lisdexamfetamine (0.05, 0.15 or 0.5 mg/kg/injection [D-amfetamine base], i.v.) nor modafinil (0.166, 0.498 or 1.66 mg/kg/injection, i.v.) served as reinforcers. The results reveal important differences between the profiles of these stimulants. Lisdexamfetamine did not serve as a positive reinforcer in cocaine-trained rats, and although it generalised fully to D-amfetamine, its discriminative effects were markedly influenced by its unusual pharmacokinetics.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a childhood-onset, psychiatric, cognitive and behavioural disorder that is widely treated with the catecholaminergic stimulants, D-amfetamine and methylphenidate. These drugs are effective in managing the symptoms of approximately three quarters of children and adults (Spencer et al., 1996; Elia et al., 1999; Heal and Pierce, 2006; Heal et al., 2009, 2012a; Buitelaar and Medori, 2010). Although these stimulants are undoubtedly effective, they have two major shortcomings. First, D-amfetamine and methylphenidate have relatively short half-lives that require the drugs to be administered several times a day, which makes them particularly unsuitable for

Abbreviations: ADHD, attention deficit hyperactivity disorder; AMF, amfetamine; C-II, Schedule 2 Controlled Drug; C-IV, Schedule 4 Controlled Drug; CD, controlled drug; C_{max}, maximum plasma drug concentration; DAT, dopamine re-uptake transporter; FR, fixed ratio; IACUC, Institutional Animal Care and Use Committee; i.p., intraperitoneal; IR, immediate release; i.v., intravenous; PET, positron emission tomography; PFC, prefrontal cortex; p.o., *per os* (oral); SAL, saline; SR, sustained release; t_{max}, time to reach maximum plasma drug concentration.

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use by individuals whose disorder is characterised by inattention, distractibility and impulsivity. Second, when these catecholaminergic drugs are taken at doses above those recommended in the prescribing instructions and often by non-clinical routes, e.g. nasal insufflation ("snorting") or intravenous injection, they have powerful psychostimulant and euphoriant properties which makes them liable to diversion and recreational abuse. Both shortcomings have to some extent been addressed by the development of long-acting formulations and by the use of novel delivery systems, e.g. osmotically controlled release or transdermal patches, that are also tamper deterrent (see reviews by Heal and Pierce, 2006; Heal et al., 2009, 2012a); nonetheless, all formulations of methylphenidate and D-amfetamine are classified as Schedule 2 Controlled Drugs (C-II) in the UK, USA and many other countries.

Lisdexamfetamine dimesylate (Vyvanse®) is a relatively recent entry to the portfolio of ADHD medications. It is a D-amfetamine prodrug, which comprises the naturally occurring amino acid, L-lysine, covalently bound to D-amfetamine via an amide linking group. Lisdexamfetamine is the first prodrug to have been approved in the USA and Canada for the management of ADHD in children (age 6–12), adolescents (age 13–17) and adults. It is currently undergoing evaluation for the treatment of ADHD in a number of European countries. The metabolic route of conversion of lisdexamfetamine is unusual because after absorption into the bloodstream it is metabolised by red blood cells to yield D-amfetamine and the natural amino acid, L-lysine, by rate-limited, enzymatic hydrolysis (Pennick, 2010). The prodrug is pharmacologically inert *in vitro* and lacks affinity for a wide range of molecular targets that mediate the effects of drugs of abuse (data on file, Shire Pharmaceuticals). As a prodrug of D-amfetamine, lisdexamfetamine has been classified as C-II in both the USA and UK.

Modafinil is an unusual stimulant with enigmatic pharmacology (see reviews by Minzenberg and Carter, 2008; Heal et al., 2012a). Although its clinical development as a treatment for ADHD was terminated due to safety concerns, modafinil has been shown unequivocally to improve symptoms in children and adolescents with ADHD in several, randomised, double-blind, placebo-controlled, clinical trials (Biederman et al., 2006; Swanson et al., 2006; Greenhill et al., 2006). Modafinil has a C-IV classification in the USA, but it is not a CD in the UK.

Thus, all of these stimulants have to a greater or lesser extent the potential for misuse and/or recreational abuse. Drug-discrimination and self-administration studies are mandated by FDA and EMA for all novel CNS-active drugs for use in man (Center for Drug Evaluation and Research [CDER]/Food and Drug Administration [FDA], 2010; Committee for Medicinal Products for Human Use [CHMP]/European Medicines Agency [EMA], 2006), and for this reason, lisdexamfetamine and the other reference stimulants were tested in two established rodent models in laboratories where these protocols have been in use for more than 20 years and for which a wealth of data and experience with other reference abused and non-abused drugs exists. In this study, we have explored the discriminative effects of lisdexamfetamine in rats trained to discriminate between D-amfetamine and saline in a 2-choice lever-pressing model, and its ability to serve as a positive reinforcer in rats trained to intravenously self-administer low-dose cocaine. In these experiments, the profile of lisdexamfetamine has been compared with those of other stimulants that are effective ADHD medications, i.e. D-amfetamine, methylphenidate and modafinil.

2. Methods

2.1. Animals and environment

For the drug-discrimination study, 48 4-week old, female, PVG rats were obtained from Harlan UK. The animals were housed in groups of 4 in polypropylene cages with sawdust covered floors in a temperature and humidity controlled room. Animals were maintained on 12 h:12 h light–dark cycle with free access to food and tap water at all times when in their home cages. Rats were accustomed to these conditions for 1 week before the start of training.

For the self-administration study, 54 male, Sprague-Dawley rats (277–352 g at start of study) were purchased from Charles River UK, and 58 male, Sprague-Dawley rats (277 g–342 g at start of study) from Harlan, USA. Rats were housed individually in plastic cages containing rodent bedding and environmental enrichment on a 12 h:12 h light–dark cycle in a temperature and humidity controlled room. Animals were allowed to acclimatise to these conditions for at least 4 days before the study commenced, during which time they underwent daily weighing and handling. Rats were allowed free access to tap water and standard rodent diet during the acclimatisation period. After the acclimatisation period, food was restricted to 10 g/day over 5 days, after which daily food intake was restricted to ~90% of normal levels (calculation based on the mean daily food intake during the acclimatisation period). Rats were given sufficient food to maintain age-appropriate growth. Body weights were monitored and the amount of food given in home cages was altered when necessary. This regime was maintained throughout the remainder of the study, except during the recovery period after surgery.

In both studies, animals were tested in the light part of the light–dark cycle.

2.2. Drug-discrimination training and testing

D-Amfetamine-cued drug-discrimination testing in rats was based on the method previously described by Heal et al. (1992). Briefly, female PVG rats were trained to distinguish between D-amfetamine (0.5 mg/kg, i.p.) and saline (1 ml/kg, i.p.) in a 2-choice lever-pressing task in response to a sweetened milk reward made available on a FR-5 reward schedule (i.e. 5 lever-presses for 1 reward). Rats were randomly allocated one lever for D-amfetamine (0.5 mg/kg, i.p.) and the other for saline. Once a rat had achieved approximately 60% correct lever-presses on most trials, it began the test regime.

On the test regime, rats were injected with drug cue or saline and then placed in the test chamber. The treatments during testing were alternated to prevent rats learning a particular sequence. On a test day, rats were not rewarded during the first 2.5 min of the session for presses on either lever and then rewarded on either lever for the remaining 7.5 min of the session.

The criterion for acceptable performance during testing was ≥75% correct lever-presses in response to the drug cue or saline in the initial 2.5 min of the 10 min test preceding a drug test and a mean of ≥75% correct lever-presses in 4 consecutive drug cue and saline cue tests. When rats had achieved 4 correct saline and amfetamine test sessions they progressed to the test drugs, routes and time periods evaluated in this study. Test compounds were assessed in the same manner i.e. the result for each rat was the percentage of responses on the amfetamine lever in the unrewarded 2.5 min of the test session.

Rats had to correctly complete one saline and D-amfetamine test and reinforcement session in a random order between each compound test. These sessions were repeated if a rat showed unacceptable performance in response to saline or D-amfetamine.

Training of the rats with saline (i.p.) and D-amfetamine (0.5 mg/kg, i.p.) was performed 3–4 days each week, but test compounds were tested only once per week. Prior to each rat being placed in a chamber, the levers and walls were swabbed with 10% ethanol solution to prevent olfactory stimuli from the previous rat influencing the subsequent rat's lever choice (Extance and Goudie, 1981).

In test sessions where the operant responding after administration of a test compound was markedly suppressed, i.e. ≥50% decrease in operant responding compared to the mean number of responses in the previous 4 sessions made by the same rat when tested with the training cue, i.e. D-amfetamine 0.5 mg/kg, i.p., the test was repeated 1 day later. If the result of ≥50% decrease in operant responding was confirmed the repeat test, suppressed operant responding was taken as the experimental outcome. On the other hand, if on repeat testing the rat showed an acceptable level of operant responding, the percentage generalisation to D-amfetamine was recorded and included in the analysis. When the dose of a test compound selected for testing produced ≥50% decrease in the operant responding for ≥50% of a group of rats, it was classified as "behavioural disruption" and testing at higher doses was not performed. In these experiments, behaviourally disruptive doses of lisdexamfetamine and the reference comparators, methylphenidate and D-amfetamine, were not encountered. In the case of modafinil, only the highest 200 mg/kg, i.p. dose of caused behavioural disruption.

2.3. Self-administration training and testing

Training sessions were conducted on a FR-1 schedule of food reinforcement (45 mg dustless pellets; F0021-B, Bilaney Consultants Ltd or PJAI-0045, Noyes Precision Pellets, Research Diets Inc., New Brunswick, New Jersey, USA.). Operant training sessions lasted for a maximum of 1.0 h, or finished once a rat had received 50 food pellet rewards. Once rats had learnt to lever-press to receive 50 pellets in a 1.0 h session, the response requirement was increased to FR-2 and the left lever was designated as the active lever. Thereafter only responses on the left lever resulted in the delivery of a reward.