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Reinforcing effect of pseudoephedrine isomers and the mechanism of action

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Abstract

It has been proposed that ephedrine and its isomers may have abuse potential. When made available to rhesus monkeys ($n=4$) for self-administration, (+)-pseudoephedrine functioned as a positive reinforcer in all monkeys, as did (−)-pseudoephedrine in two of three monkeys. Pseudoephedrine isomers were 10- to 33-fold less potent than cocaine. In in vitro binding in monkey brain tissue, both isomers had low affinity for dopamine and serotonin transporters by at least 200-fold relative to cocaine, but comparable affinity for norepinephrine transporters. (+)-Pseudoephedrine also blocked dopamine uptake in 293 hDAT cells with low potency relative to cocaine. When given in vivo (+)-pseudoephedrine significantly displaced radioligand binding to dopamine transporters with a potency comparable to that in self-administration. Therefore, pseudoephedrine isomers can function as reinforcers and the mechanism at dopamine transporters may underlie this effect. However, pseudoephedrine appears to be a weak reinforcer and may have relatively low abuse potential.

Keywords: Self-administration; (Rhesus monkey); Ephedrine; Pseudoephedrine; Dopamine transporter

1. Introduction

Ephedrine is an alkaloid extract from the herb *Ephedra sinica* (Foster and Tyler, 1999; Levy and Ahlquist, 1971). Ephedrine is structurally similar to methamphetamine, but has a hydroxyl group in the β-carbon position instead of the hydrogen in methamphetamine (Hoffman and Lefkowitz, 1996). Because of its two chiral centers, ephedrine has four isomers, (+)-ephedrine, (−)-ephedrine, (+)-pseudoephedrine and (−)-pseudoephedrine. Ephedrine isomers are sympathomimetic agents, causing increased heart rate and vasoconstriction, thus elevating the blood pressure (Hoffman and Lefkowitz, 1996). (−)-Ephedrine and (+)-pseudoephedrine are over-the-counter sympathomimetic drugs intended to be used as bronchodilators and nasal decongestants, respectively.

In addition to its peripheral action, ephedrine has been shown to stimulate the central nervous system (Schulte et al., 1940; Fairchild and Alles, 1967). Ephedrine has been readily accessible to consumers in dietary supplements containing the herb *Ephedra*. Ephedrine products have been used as appetite suppressants, metabolism-enhancers and energizers. Ephedrine has also been popular among college students and body-builders as a legal stimulant. With the passage of the Dietary Supplement Health and Education Act (DSHEA) in 1994, the manufacture and usage of ephedrine products dramatically increased. Correspondingly, there has been an increasing concern in its misuse and abuse (e.g. Ephedrine Alkaloid Consumer Protection Act, H.R. 3066), prompting researchers to investigate the abuse liability of ephedrine in its own right.

Several experiments have been conducted to examine behavioral effects of ephedrine isomers related to its potential for abuse. Ephedrine substituted for amphetamine (Holloway et al., 1985; Huang and Ho, 1974) and methamphetamine (Bondareva et al., 2002; Ando and Yanagita, 1992) as discriminative stimuli in rats. Similar results have been reported in pigeons (Ercil and France, 2003). Conversely, amphetamine and cocaine fully substituted for (−)-

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ephedrine in rats (Young and Glennon, 1998; Gauvin et al., 1993). With respect to reinforcing effects, ephedrine functioned as a positive reinforcer in dogs (Shannon and DeGregorio, 1982) and in monkeys (Anderson et al., 2001; Gold and Balster, 1996). In human subjects, Martin et al. (1971) reported euphoria and drug-liking with ephedrine, comparable to amphetamine, in subjects with drug-abuse histories. Chait (1994) also reported euphoria with ephedrine in individuals without a drug abuse history. However, in Chait (1994) study, ephedrine did not serve as a positive reinforcer, though it should be noted that the dose of ephedrine used in that study (25 mg) was substantially lower than the dose tested in the Martin et al. (1971) study (75 or 150 mg/70 kg). Chronic ephedrine use and dependence have been reported in some female weightlifters (Gruber and Pope, 1998). Taken together, these data suggest that ephedrine has some potential for abuse.

It is somewhat surprising that ephedrine can have reinforcing effects in animals. The mechanism of action has been known for some time to include increased noradrenergic neurotransmission (De Moraes and de Carvalho, 1968; Frost et al., 1978; Rothman et al., 2001), an action not usually associated with reinforcing effects (De Wit and Wise, 1977; Woolverton, 1987). In fact, dopaminergic actions have been suggested to mediate the stereotypy behavior induced by ephedrine (Angrist et al., 1977; Christie and Crow, 1971). Specifically, (−)-ephedrine increased the rate of dopamine release, inhibited dopamine uptake (Zarrindast, 1981; Rothman et al., 2001), and increased extracellular dopamine (Bowyer et al., 2000; Wellman et al., 1998). This dopaminergic action of ephedrine may underlie reinforcing effects of the drug. Recently, McMahon and Cunningham (2003) have suggested that the discriminative stimulus of (−)-ephedrine was mediated by α-adrenergic as well as dopaminergic receptor activation.

Less research has been done with pseudoephedrine and its potential for abuse. Amphetamine-like discriminative properties and reinforcing effects of (+)-pseudoephedrine have been reported in rats (Tongjaroenbuangam et al., 1998) and in monkeys (Anderson et al., 2001), respectively. There has not been any research on the reinforcing effect of pseudoephedrine in human subjects. The purpose of the present study was to further examine the reinforcing effects of (+)- and (−)-pseudoephedrine in monkeys and to examine potential mechanisms of action for this effect. In a previous study (Anderson et al., 2001), (+)-but not (−)-pseudoephedrine maintained self-administration by monkeys. However, the poor solubility of (−)-pseudoephedrine base made it difficult to test a wide range of doses. Therefore, in the present study, the reinforcing effect of (−)-pseudoephedrine was re-evaluated using the water-soluble hydrochloride salt. Since the reinforcing effect of many sympathomimetic amines has been associated with their affinity for the dopamine transporter, the interaction of (+)- and (−)-pseudoephedrine with the dopamine transporters was examined. Affinity of both drugs for the dopamine transporters was measured by in vitro binding in monkey brain tissue. The ability of (+)-pseudoephedrine to block dopamine uptake was also examined in vitro using HEK-293 cells transfected with the human dopamine transporters. In addition, dopamine transporter binding in vivo was assessed in rat brain for (+)-pseudoephedrine using an ex vivo binding assay.

2. Materials and methods

The animals used in this study were maintained in accordance with the United States Public Health Service *Guide for Care and Use of Laboratory Animals* and all procedures were approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

2.1. Self-administration

2.1.1. Subjects and apparatus

The subjects were four male rhesus monkeys (*Macaca mulatta*) weighing between 9.0 and 11.6 kg at the beginning of the study. All the monkeys had histories of drug self-administration. Monkey 341 had a history of self-administration of cocaine and other monoamine transporter inhibitors under the same conditions as this experiment (Wee and Woolverton, in press). Monkeys L638 and AP78 had recent histories of self-administration of a piperidine-based cocaine analog (+)-CPCA under a progressive-ratio schedule (Woolverton et al., 2002). Monkey AP01 was recently involved in the experiment of self-administration of combinations of scopolamine/cocaine under a fixed-ratio schedule (Ranaldi and Woolverton, 2002). There was no drug-free period between experiments. All monkeys were provided with sufficient food to maintain stable body weight (120–180 g/day, Teklad 25% Monkey Diet, Herlan/Teklad, Madison, WI) and had unlimited access to water. Fresh fruit and a vitamin supplement were provided daily and three times a week, respectively.

The monkeys were individually housed in the experimental cubicles (1.0 m^3, Plaslabs, Lansing, MI). Each monkey was fitted with a stainless-steel harness attached by a tether to the rear wall of the cubicle. The front door of the cubicle was made of transparent plastic and the remaining walls were opaque plastic. Two response levers (PRL-001, BRS/LVE, Beltsville, MD) were mounted on the inside of the door. Four jeweled stimulus lights, two red and two white, were mounted above each lever. Drug injections were delivered by a peristaltic infusion pump (Cole-Parmer, Chicago, IL). A Macintosh computer with custom interface and software controlled all events in an experimental session.

2.1.2. Procedure

Monkeys were implanted with a silastic catheter (0.26 cm o.d. × 0.076 cm i.d.; Cole-Parmer, Chicago, IL) into

the jugular (internal or external) or femoral vein under isoflurane anesthesia and strict aseptic conditions. Brachial veins were implanted with a microrenethane catheter (0.08″ o.d. × 0.04″ i.d.; Braintree Scientific, Braintree, MA) heated and drawn to approximately half size. The proximal end of the catheter was inserted into the vein and terminated in the vena cava near the right atrium. The distal end was threaded subcutaneously to exit the monkey between the scapulae, threaded through the spring arm, out the rear of the cubicle and connected to the peristaltic pump. In the event of catheter failure, surgery was repeated using another vein, after the veterinarian confirmed the health of the monkey.

Experimental sessions began at noon each day and were conducted seven days per week. Thirty minutes before each session started, catheters were filled with drugs for the sessions without infusing the drugs into monkeys. At the start of a session, the white lights were illuminated above both levers and responding on the right lever resulted in the delivery of a drug injection for 10 s. During the injection, the white lights were extinguished and the red lights were illuminated. Pressing the left lever was counted but had no other programmed consequence. The response requirement was 25 lever-presses per injection and each session lasted for 2 h. After the session, the catheters were flushed with 0.9% saline containing heparin (20 or 40 units/ml).

In baseline sessions, a baseline dose of cocaine (0.03 mg/kg/injection for AP01, AP78, M341; 0.01 mg/kg/injection for L638) or saline was made available. The baseline dose of cocaine was selected to maintain rates of responding that were clearly distinguishable from saline. When responding was stable (± 15% of the running mean number of injections for cocaine and less than 10 injections/session for saline), test sessions were inserted into baseline sessions. Test sessions were arranged among baseline sessions in a way that a test session came after two different baseline sessions, that is, C–S–T or S–C–T where C, S and T denote cocaine, saline and test sessions, respectively. A test session was identical to a baseline session, except that one of various doses of cocaine (0.003–0.3 mg/kg/injection), (+)-pseudoephedrine, or (−)-pseudoephedrine (0.03–3.0 mg/kg/injection) were made available. Cocaine and (+)-pseudoephedrine were tested in all the monkeys while (−)-pseudoephedrine was tested in three monkeys. The drugs were tested in a different sequence in each monkey and individual doses of each drug were tested in a random order. After a test session, monkeys were returned to baseline conditions until cocaine- and saline-maintained responding were again stable. Doses were tested twice, once the day after a cocaine baseline session and once the day after a saline baseline session. In monkey M341, the dose–response function of (+)-pseudoephedrine was determined twice because this monkey exhibited high variability at three out of four doses of (+)-pseudoephedrine in initial test sessions.

2.1.3. Data analysis

Data were analyzed as injections/session for individual monkeys. Means of two determinations were calculated for each dose of each drug and the ranges of the two determinations served as the measure of individual variability. Variability of baseline sessions was expressed as a 95% confidence interval (CI) of regression lines for baseline saline and cocaine. The regression lines for baseline saline and cocaine were fit to the number of injections in sessions that immediately preceded test sessions, over the entire period of the study. When mean injections/session at a dose of a drug was beyond 95% CI of baseline saline and the range did not overlap with 95% CI of saline, the dose of the drug was considered to function as a positive reinforcer.

2.2. In vitro binding

2.2.1. Subjects and apparatus

Brain tissues for rhesus monkeys [caudate/putamen for the dopamine transporters; cerebellum for the norepinephrine transporter; frontal cortex for the serotonin transporter] were used. Each monkey had a history of drug self-administration and had been drug free for at least two months before sacrifice. During that period they were housed in stainless steel primate cages with food and water available ad libitum. Monkeys were not sacrificed specifically for the purposes of this experiment but as part of a general program to bank monkey brain tissue.

2.2.2. Procedure

The procedure was as previously described (Woolverton et al., 2002). In brief, monkeys were euthanized by an overdose of i.v. pentobarbital. Brains were collected immediately after sacrifice and the caudate nucleus, putamen, frontal cortex, and cerebellum were dissected according to the atlas of Snider and Lee (1961). Immediately after dissection, the tissue was frozen over dry ice (solid CO_2), with no additional preparation, then placed into an −80 °C freezer until assay.

On the day of binding study, frozen tissue was thawed, homogenized in Tris buffer (50 mM, pH 7.4) and centrifuged at 20,000 × *g* for 20 min at 4 °C. The resulting pellet was collected. This washing procedure was repeated two or three times, depending on the assay. After centrifugation, the pellet was suspended in an incubation buffer at the appropriate tissue concentration.

Specific assay conditions for the different transporters were as previously published (Woolverton et al., 2002). The displacement of the radioligand binding {dopamine transporters: [^{3}H] 3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl esters (WIN35,428), 86 Ci/mmol, 2 nM; norepinephrine transporters: [^{3}H]nisoxetine, 80 Ci/mmol, 3 nM; serotonin transporters: [^{3}H]paroxetine, 21.5 Ci/mmol, 0.1 nM} was performed with various concentrations of cocaine, (+) -or (−)-pseudoephedrine. Concentrations of cocaine, (+) - and (−)-pseudoephedrine ranged between 1

nM and 10.24 mM. Non-specific binding was measured in the presence of 10 μM cocaine, 1 μM mazindol and 1 μM sertraline for the dopamine, norepinephrine and serotonin transporters, respectively. All assays were initiated with the addition of tissue. After the incubation, reactions were terminated by rapid vacuum filtration. The collected membranes on the filters were rinsed with an ice-cold buffer and deposited into Packard Top Count™ deep well plates. Microscint-20™ cocktail (Packard Instruments, Downers Grove, IL) was added to each well and sat overnight. Radioactivity was determined using a Packard Top Count™ scintillation counter.

2.2.3. Data analysis

Data were analyzed by competition using non-linear regression analysis (Prism 3.0, GraphPad, San Diego, CA). Data were fit to models assuming a one-site interaction and two sites of interaction. The best of fit was determined by comparing the sum of squares of residuals using the following equation: $F=[(SS_1 - SS_2)/(df_1 - df_2)]/(SS_2/df_2)$, where SS_1 and df_1 are the sum of squares and degrees of freedom from a one-site model and SS_2 and df_2 are that from a two-site model. A two-site model was considered a significantly better fit if the F value was greater than that reported in the F-statistic table at $P<0.05$ for the numerator of $df_1 - df_2$ and the denominator of df_2. Ki values were calculated from IC_{50} values using the Cheng–Prusoff correction factor (Cheng and Prusoff, 1973).

2.3. Dopamine uptake

2.3.1. Subjects and apparatus

Human embryonic kidney (HEK) 293 cells expressing human dopamine transporters (293-hDAT cells) were used. 293-hDAT cells were kindly provided by A. Galli (University of Texas, San Antonio, TX). The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Frederick, Maryland) supplemented with 100 units/ml penicillin G sodium, 100 μg/ml streptomycin, 2 mM L-glutamine and 10% fetal bovine serum (Sigma, St. Louis, MO) at 37 °C and 5% CO_2.

2.3.2. Procedure

The assay was modified from Saunders et al. (2000). 293-hDAT cells were seeded in 75 cm^2 tissue culture flasks and grown to 80% confluence. The cells were harvested using non-enzymatic cell dissociation solution (Sigma), transferred into test tubes and centrifuged at 3000 × g for 3 min at 37 °C. After discarding the supernatant, the cells were re-suspended into DMEM and centrifuged again in the same condition. After the washing procedure, the cells were suspended in warm Krebs–Ringer–HEPES buffer (130 mM NaCl, 1.3 mM KCl, 1.2 mM $MgSO_4$, 2.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 10 mM HEPES, 10 mM glucose, 0.1 mM ascorbic acid, 0.1 mM pargyline, pH 7.4). For K_m and V_{max} measurement, the cells were incubated with increasing concentrations of dopamine together with [^{3}H]dopamine. The range of dopamine concentrations was 0.125–20 μM. Non-specific dopamine uptake was measured in the presence of 10 μM mazindol. For the uptake inhibition experiment, cells were incubated with various concentrations of cocaine or (+)-pseudoephedrine in the presence of 2 μM [^{3}H]dopamine (60 Ci/mmol diluted to 0.1 Ci/mmol). The range of the concentrations of cocaine and (+)-pseudoephedrine was 5 nM to 2.6 μM and 5 μM to 2.6 mM, respectively. All uptake experiments were performed at 37 °C for 5 min as soon as the cells were added into the assays. Reactions were terminated by rapid vacuum filtration using a 24-well Brandel cell harvester (Brandel, Gaithersburg, MD) through glass fiber filters (#30; Schleicher and Schuell, Riviera Beach, FL). The collected cells were rinsed with ice-cold buffer. Radioactivity was measured in a liquid scintillation counter using ScintiSafe™ (Fisher Scientific, Houston, TX). Protein levels in assays were determined by the bicinchoninic acid method (Smith et al., 1985; kits from Pierce, Rockford, IL).

2.3.3. Data analysis

V_{max} and K_m values were calculated using non-linear regression analysis (Prism 3.0, GraphPad). Saturation data were fit to a curve assuming a single-site interaction for dopamine. Competition data were fit to a model assuming a one-site interaction, set to 100 for the maximum value (Prism 3.0, GraphPad). Ki values were calculated from IC_{50} values using the Cheng–Prusoff correction factor (Cheng and Prusoff, 1973).

2.4. Ex vivo binding

The procedure used here was as previously described (Woolverton et al., 2002).

2.4.1. Subjects and apparatus

Male Sprague–Dawley rats, weighing between 250 and 300 g, were used. They were initially housed in groups of 3 in plastic cages under a 12:12 light/dark cycle (lights on at 06:00). Food and water were available ad libitum.

2.4.2. Procedure

Rats were surgically implanted with polyethylene catheters into femoral veins under pentobarbital (50 mg/kg, i.p.) anesthesia. The distal end of the catheter was threaded subcutaneously to exit the rat between the scapulae and was sealed. After surgery, rats were individually housed at least for 2 days then used experimentally. Catheterized rats were placed in a plastic restrainer. After examining the patency of the catheter, doses of (+)-pseudoephedrine were infused via the catheter with an injection volume of 1 ml/kg over ten seconds. Doses of (+)-pseudoephedrine ranged from 3 to 100 mg/kg. Doses higher than 100 mg/kg were not evaluated because of lethality in two rats at 300 mg/kg. At 30 min after the (+)-pseudoephedrine infusion, [^{3}H]WIN 35,428 (10 μCi/0.4 ml/rat) was infused. In a preliminary experiment, a 30-

min interval between the administration of (+)-pseudoephedrine and the radioligand for the dopamine transporters was found to be optimal for inhibition of binding. All rats were sacrificed 45 min after [^{3}H]WIN35,428 infusion, the time at which specific [^{3}H]WIN35,428 binding has been found to be asymptotic (see Woolverton et al., 2002). After decapitation, brains were immediately removed and the striatum and cerebellum were dissected. The striatum was chosen because of its high density of the dopamine transporters, and the cerebellum was used for the measurement of non-specific binding (Kaufman and Madras, 1992). The dissected striatum and cerebellum were solubilized in Solvable™ (10 μl/mg tissue; Perkin-Elmer, Boston MA) overnight. After the tissue was completely solubilized, glacial acetic acid (1 μl/mg tissue) was added and 200 μl aliquots were placed in 24-well scintillation plates. Microscint-20™ cocktail (Packard Instruments) was then added to each well and radioactivity was counted.

2.4.3. Data analysis

The binding ratio of [^{3}H]WIN 35,428 in striatum versus cerebellum (S/C) was calculated as a function of dose of (+)-pseudoephedrine. The statistical significance in the displacement was obtained by a one-way analysis of variance with Dunnett's multiple comparison as a post-hoc test. When the binding ratio reached 1, it was considered that the drug completely displaced the specific binding of [^{3}H]WIN 35,428 to the dopamine transporters in the striatum. Binding ratio data were also analyzed by competition using non-linear regression analysis assuming a one-site interaction and the minimum value was set to one (Prism 3.0, GraphPad). The dose that displaced one-half of [^{3}H]WIN 35,428 binding was expressed as the ED_{50}. The ED_{50} was used as an index of the potency of binding to the dopamine transporters in vivo.

2.5. Drugs

The National Institute on Drug Abuse (Rockville, MD) provided cocaine HCl. (+)-and (−)-Pseudoephedrine were purchased from Sigma. The HCl salt of (−)-pseudoephedrine was prepared by A. Coop (Baltimore, MD). For the self-administration study, drugs were dissolved in 0.9% saline. Doses were expressed as the salt forms of the drugs. Radioligands [^{3}H]WIN35,428, [^{3}H]paroxetine, [^{3}H]nisoxetine, [^{3}H]dopamine were purchased from Perkin-Elmer (Boston, MA). For in vitro binding and uptake studies, drugs were freshly prepared for each experiment.

3. Results

3.1. Self-administration

Responding in the baseline sessions was stable over the course of the experiment. The mean injections/session for cocaine was between 32 (AP78) and 46 (M341), and less than six for saline (data not shown). When injections/session were analyzed over sessions by linear regression, there was no trend for an increase or decrease of baseline level over the course of study, i.e., the 95% C.I. of the slope of the regression line included 0.

Cocaine and (+)-pseudoephedrine served as positive reinforcers at least at two doses in all monkeys, with biphasic dose–response functions (Fig. 1). (−)-Pseudoephedrine served as a positive reinforcer in two (AP01, L638) of three monkeys tested. Based upon the doses that maintained the maximum number of injections/session, cocaine was approximately 10–33 times as potent as (+)- and (−)-pseudoephedrine. Subject M341 exhibited high variability in the initial tests of (+)-pseudoephedrine. On re-testing, variability was again high and consistently above saline levels at 3.0 mg/kg/injection. The two highest doses of (+)-pseudoephedrine (1.0 and 3.0 mg/kg/injection) showed observable behavioral effects such as staring and a decrease in food intake in all the monkeys. In addition, responding was at or below saline levels in the cocaine baseline sessions that followed test sessions with these two doses in three of four monkeys (data not shown). Similarly, the doses of 1.0 and 3.0 mg/kg/injection of (−)-pseudoephedrine decreased food intake in AP78 and L638.

3.2. In vitro binding

Cocaine, (+)- and (−)-pseudoephedrine completely displaced the binding of all three radioligands to monoamine transporters in a dose-dependent manner (data not shown). The data for (+)-pseudoephedrine and cocaine were fit best to a model assuming a one-site of interaction for all the transporters. On the other hand, the data for (−)-pseudoephedrine were fit best by a one-site model for the dopamine and serotonin transporters and by a two-site model for the norepinephrine transporters. (+)-and (−)-Pseudoephedrine were, respectively, approximately 780- and 260-fold less potent than cocaine at the dopamine transporters, and 470- and 400-fold less potent at the serotonin transporters (Table 1). (+)-Pseudoephedrine was 7-fold less potent than cocaine at the norepinephrine transporters. (−)-Pseudoephedrine had about an equal fraction of high and low affinity binding sites at the norepinephrine transporters and the potency at the high affinity binding sites was 3-fold less than that of cocaine.

3.3. Dopamine uptake

K_m and V_{max} values for [^{3}H]dopamine uptake in 293-hDAT cells were 1.9 ± 0.4 μM, and 36.6 ± 10.4 ρmol/mg protein/min, respectively. Cocaine and (+)-pseudoephedrine inhibited dopamine uptake in a dose-dependent manner (Fig. 2). Cocaine was approximately 220-fold more potent than (+)-pseudoephedrine.

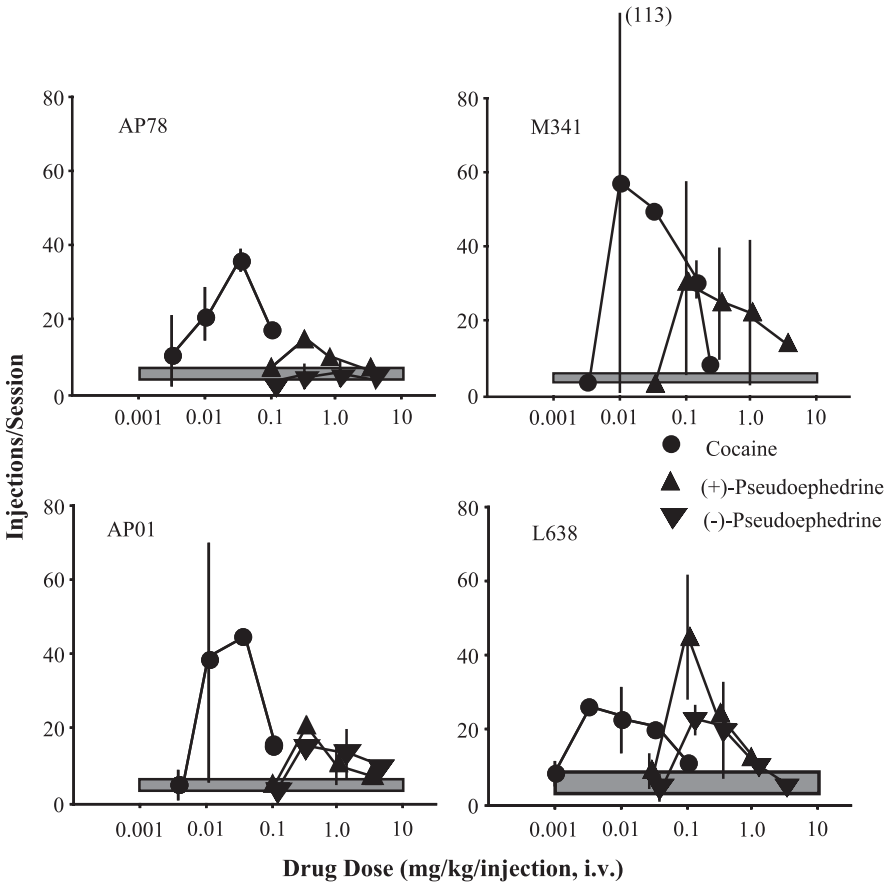

Fig. 1. Self-administration of cocaine, (+)-pseudoephedrine and (−)-pseudoephedrine by rhesus monkeys. Each point is the mean of two determinations of the effects of each dose of the drugs. Vertical bars indicate the range of the two determinations. The symbols without bars have the ranges inside of the points. Shaded horizontal bars represent 95% confidence interval of saline baseline sessions.

3.4. Ex vivo binding

Intravenous infusion of doses of (+)-pseudoephedrine between 3.0 and 100 mg/kg did not exert any observable behavioral effect on the rats (data not shown). However, when 300 mg/kg of (+)-pseudoephedrine was tested in two rats, both died immediately after the infusion. (+)-Pseudoephedrine significantly displaced [^{3}H]WIN 35,428 binding in a dose-related manner (Fig. 3). The ED_{50} value of (+)-pseudoephedrine was 56.7 mg/kg (281.2 μmol/kg). Under similar conditions, the ED_{50} value of cocaine was 8.8 μmol/kg (Woolverton et al., 2002), that is, cocaine was about 32-fold more potent than (+)-pseudoephedrine.

Table 1
Inhibition of radioligand binding to monoamine transporters in rhesus monkey brain

Drug	DAT	NET	SERT
	Ki (μM)		
(−)-Cocaine HCl	0.178 (0.160–0.198)	3.805 (2.646–5.473)	0.204 (0.155–0.271)
(+)-Pseudoephedrine HCl	139.0 (116.3–166.2)	26.07 (17.15–39.63)	179.3 (105.2–305.3)
(−)-Pseudoephedrine HCl	46.72 (41.33–52.82)	high 12.15 (3.47–42.53) low 117.7 (20.88–663.8)	233.5 (155.8–350.0)

Data are the mean and 95% confidence interval in parentheses based on duplicate determinations in three or four individual brains.
The caudate/putamen was used for the dopamine transporter (DAT) binding; the cerebellum, for the norepinephrine transporter (NET) binding; the frontal cortex, for the serotonin transporter (SERT) binding.

4. Discussion

In the present study, cocaine functioned as a positive reinforcer, with a typical biphasic dose–response function in all monkeys. Consistent with previous findings (Anderson et al., 2001), (+)-pseudoephedrine maintained self-administration above saline levels, also with a biphasic dose–response function. Interestingly, (−)-pseudoephedrine served as a positive reinforcer in two of three monkeys at 1.0 and 3.0 mg/kg as well as at the doses (0.1 and 0.3 mg/kg) which did not maintain self-administration in the previous study (Anderson et al., 2001). Cocaine was approximately 10- to 33-fold more potent than pseudoephedrine isomers, a potency relationship similar to that reported previously for ephedrine (Shannon and DeGregorio,

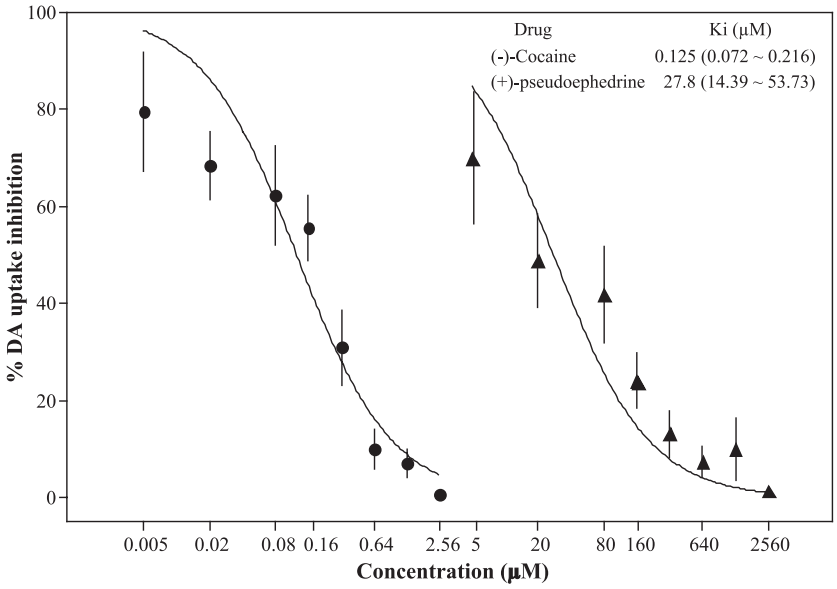

Fig. 2. Inhibition of dopamine uptake in 293-hDAT cells. Data were analyzed using a non-linear regression assuming a one-site interaction. Each point is the mean ± S.E.M. from five experiments. Ki values are expressed as the mean and 95% confidence interval in parentheses.

1982). In drug-discrimination studies, ephedrine and its isomers have been found to have low potency cocaine-and d-amphetamine-like discriminative effects (Anderson et al., 2001; Ercil and France, 2003; Bondareva et al., 2002). Together these findings suggest that ephedrine isomers can have abuse potential of the psychomotor stimulant type.

Since ephedrine and its isomers are well known to be noradrenergic agonists, and reinforcing effects are generally associated more with dopaminergic than noradrenergic effects (e.g., Ritz et al., 1987), various aspects of the central nervous system pharmacology of the pseudoephedrines were examined. Both (+)- and (−)-pseudoephedrine displaced radioligand binding to all three monoamine transporters in monkey brain in vitro. As predicted, both isomers had relatively high affinities for the norepinephrine transporters and less affinity for the dopamine and serotonin transporters. (+)-Pseudoephedrine also blocked dopamine uptake in the 293-hDAT cells. These findings demonstrate affinity and activity at the dopamine transporters for (+)-pseudoephedrine. Based purely on these results, the in vitro potency of pseudoephedrine at the dopamine transporters was in μM range, arguably too low to be relevant in vivo. However, in the ex vivo binding assay, [^{3}H]WIN35,428 binding decreased by approximately 70% after sublethal doses of (+)-pseudoephedrine, with an ED_{50} of 56.71 mg/kg (281.2 μmol/kg). This result is consistent with significant dopamine transporter binding by (+)-pseudophedrine in vivo, although the possibility of some effect of (+)-pseudoephedrine on dopamine release may not be ruled out (see Rothman et al., 2001). Interestingly, it has been reported that 40 mg/kg of i.p. ephedrine produced 7.2 ± 1.2 μM ephedrine in rat caudate/putamen as measured by microdialysis (Bowyer et al., 2000), a concentration that approximates the (+)-pesudoephedrine Ki for dopamine uptake. Kumarnsit et al. (1999) have reported that 40–80 mg/kg of i.p. pseudoephedrine significantly induced Fos-like immunoreactivity in rat striatum and nucleus accumbens, which was blocked by (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol (SCH23390), dopamine D1 receptor antagonist, suggesting the activation of dopamine D1 receptors by pseudoephedrine. A previous study showed that the ED_{50} dose of cocaine in this same procedure was 8.8 μmol/kg (Woolverton et al., 2002), indicating that (+)-pseudoephedrine was approximately 32-fold less potent than cocaine at the dopamine transporters in vivo. This potency difference is compatible with the 10- to 33-fold potency difference seen in the self-administration study. Taken together, these data support the conclusion that binding at the dopamine

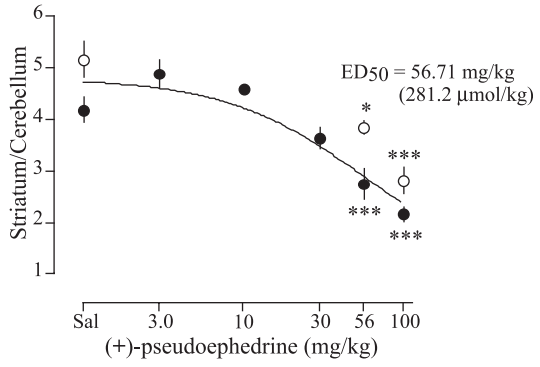

Fig. 3. Inhibition of [^{3}H]WIN 35,428 binding by (+)-pseudoephedrine in rat striatum. (+)-Pseudoephedrine was administered 30 min before the administration of [^{3}H]WIN 35,428. Rats were sacrificed 45 min after the administration of the radioligand. Each point represents the mean ± S.E.M., $n = 3$. *$P < 0.05$, ***$P < 0.001$ statistically significant when compared with saline data. Open circles: 3-min pretreatment time before the administration of [^{3}H]WIN 35,428.

transporters and blockade of dopamine uptake contribute to the reinforcing effect of pseudoephedrines.

The substantial difference in the potency of (+)-pseudoephedrine relative to cocaine at the dopamine transporters between in vitro experiments (780-fold, in vitro binding; 220-fold, dopamine uptake) and in vivo experiments (32-fold, ex vivo binding; 10- to 33-fold, self-administration) was surprising. Because of decreased penetration of the blood brain barrier for (+)-pseudoephedrine relative to cocaine, one might expect the potency difference to be larger in vivo than in vitro. Among the possible sources of this discrepancy are pretreatment time in vivo and/or incubation time in vitro. In the ex vivo binding assay, (+)-pseudoephedrine was given 30 minutes before the radioligand whereas cocaine was given three minutes before. However, in in vitro binding and dopamine uptake experiments, the same incubation time was allowed for both drugs without the adjustment for each drug (2 h, in vitro binding; 5 min, dopamine uptake). If the association rate at the dopamine transporters were slow for (+)-pseudoephedrine, then cocaine but not (+)-pseudoephedrine might exhibit maximal binding with a 2-h or 5-min incubation time. However, in preliminary experiments ($n = 1$) with three-and four-hour incubation times in vitro (data not shown), affinity estimates at the dopamine transporters were unchanged for either (+)-pseudoephedrine or cocaine. Additionally, a preliminary ex vivo binding study ($n = 3$) revealed that the binding of (+)-pseudoephedrine at the dopamine transporters decreased with a three-minute pretreatment time (Fig. 3, open circles), increasing the potency ratio between (+)-pseudoephedrine and cocaine approximately two-fold, still compatible with the self-administration potency relationship. It is also conceivable that a metabolite of (+)-pseudoephedrine accounts for the dopamine transporter binding in vivo. However, it has been reported in humans that 90–98% of pseudoephedrine was excreted unchanged in the urine, with a 3–6 h elimination half-life (Lai et al., 1979; Moffat et al., 1986; Wellington and Jarvis, 2001), arguing against this mechanism. In the end, further research will be required to clarify reason(s) for this apparent discrepancy.

Although ephedrine isomers, including pseudoephedrines, appear to bind the dopamine transporters, block dopamine uptake, and function as postive reinforcers, it can be concluded that they are relatively weak reinforcers. Anderson et al. (2001) showed that rhesus monkeys stopped self-administering ephedrine more rapidly than cocaine as a response requirement/injection increased. In the present study, responding was sometimes variable in repeated test sessions and in dose–effect redeterminations, suggestive of relatively weak reinforcing effects. This result has not been found with cocaine in the present procedure. Any of several mechanisms could contribute to this diminished efficacy as a reinforcer. Our observation that pseudoephedrine needed a long pretreatment time to optimally displace the radioligand at the dopamine transporters for the ex vivo assay, suggests that the onset of action of pseudoephedrine is slower than for cocaine. Slower onset of action has been associated with reduced reinforcing effects for dopamine transporter ligands (Woolverton et al., 2002; Lile et al., 2003). The noradrenergic actions of the ephedrines may diminish their reinforcing effects. Nisoxetine, a drug with more selectivity and higher affinity for the norepinephrine transporters than the ephedrines, has been reported not to serve as a positive reinforcer in monkeys (Woolverton, 1987). Logan et al. (1997) have demonstrated that the abused doses of cocaine in humans occupied approximately 50% of the dopamine transporters. Here, ex vivo binding data demonstrated that (+)-pseudoephedrine can achieve greater than 50% of dopamine transporter occupancy in vivo without any observable adverse effects in rats. However, full dopamine transporter occupancy, as has been reported for cocaine (Wilcox et al., 2000; Woolverton et al., 2002), was not seen with sub-lethal doses. Thus, reduced maximal dopamine transporter occupancy may limit the efficacy of the ephedrines as reinforcers. Taken together, it was suggested that pseudoephedrine is a weaker reinforcer than cocaine and it may have potential for abuse. In any case, relatively weak reinforcing effects of ephedrines predict relatively low potential for abuse, a prediction that appears to be born out in the human population.

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