Intravenous self-administration of 4-methylaminorex in primates

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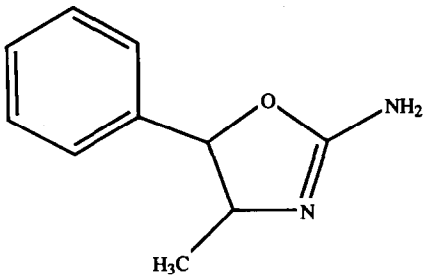
The reinforcing effects of (±)-*cis*-2-Amino-4-methyl-5-phenyl-2-oxazoline (4-methylaminorex) were determined in two models of intravenous drug self-administration in primates. In baboons, lever pressing was maintained under a fixed-ratio (FR) 80- or 160-schedule of intravenous cocaine delivery (0.32 mg/kg per injection). Each drug injection was followed by a 3-h time-out allowing a maximum of 8 injections per day. Vehicle or 4-methylaminorex doses were substituted for cocaine for a period of 15 or more days. One of the two 4-methylaminorex doses evaluated (0.32 mg/kg per injection) maintained self-administration behavior above vehicle control levels in all four animals. This dose of 4-methylaminorex maintained cyclic patterns of self-injection behavior across days and produced signs of psychomotor stimulant toxicity. In rhesus monkeys, 4-methylaminorex (0.0003 – 0.1 mg/kg per injection) was made available to animals trained to self-administer cocaine (0.01 or 0.033 mg/kg per injection) under an FR 10 schedule of reinforcement during daily 1-h sessions. Each of the three monkeys self-administered at least two doses of 4-methylaminorex at rates exceeding those maintained by vehicle injections. Taken together with reports of recreational abuse of 4-methylaminorex, the present results indicate that this drug has a potential for abuse similar to that of other psychomotor stimulants.

Key words: 4-methylaminorex; self-administration; baboon; rhesus monkey; stimulant abuse

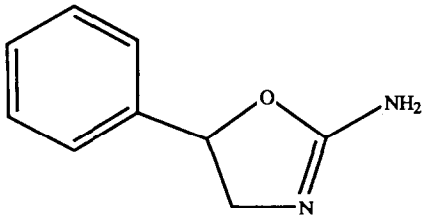
Introduction

(±)-*cis*-2-Amino-4-methyl-5-pheynl-2-oxazoline (4-methylaminorex) is an analog of aminorex, an appetite suppressant which was marketed in Europe in the 1960s under the proprietary name Menocil but removed from the market when its use was found to be correlated with an increased incidence of pulmonary hypertension (Byrne-Quinn and Grover, 1972). Aminorex and 4-methylaminorex share a common phenetylamine backbone with amphetamine (Fig. 1). 4-Methylaminorex has been described as a potent amphetamine-like sympathomimetic agent (Yelnosky and Katz, 1963). Its reported psychomotor stimulant properties include appetite suppression, seizure production, and amphetamine-like discriminative stimulus effects (Yelnosky and Katz, 1963; Roszkowski and Kelley, 1963; White et al., 1990; Glennon and Misenheimer, 1990).

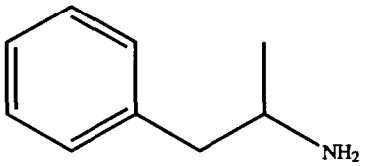
Recent reports of recreational abuse of 4-methylaminorex (DEA, 1988), including one documented fatality (Davis and Brewster, 1988), and its emergency scheduling under the U.S. Controlled Substances Act, led to the undertaking of the present abuse potential evaluation. Intravenous self-administration of drugs in laboratory animals is considered to be a useful means to predict reinforcing effects of the same drugs in human populations (Johanson and Balster, 1978). Two models of drug self-adminstration were used to characterize the reinforcing effects of 4-methylaminorex in this study, one involving spaced injections in baboons, and the other involving limited periods of continuous access in rhesus monkeys. A number of abused sympathomimetic stimulant



4-Methylaminorex



Aminorex



Amphetamine

Fig. 1. Chemical structures of 4-methylaminorex, aminorex, and amphetamine.

compounds have been previously shown to support responding under each procedure (Griffiths et al., 1976; Griffiths et al., 1979b; Beardsley et al., 1986; Lamb and Griffiths, 1987).

Methods

Baboon experiment

Subjects and apparatus. Four male baboons (*Papio cynocephalus*) weighing 23–26 kg were used as subjects. Baboons were surgically prepared with chronically indwelling silastic catheters implanted in either femoral or jugular veins under pentobarbital or halothane anesthesia using methods described by Lukas et al. (1982). All baboons had served in studies of intravenous self-administration with a variety of drugs. Animals had continuous access to water via a drinking tube and to food pellets (as described below). Animals received two pieces of fresh produce and a multivitamin daily.

Baboons were housed in standard stainless steel primate cages with a bench running the length of one wall of the cage. Each cage, which served as the experimental chamber, was surrounded by a sound attenuating double walled plywood external enclosure which was continuously illuminated with a 20 W frosted bulb. The catheter was protected by a harness/vest system that allowed the baboons virtually unrestricted movement within the cage (Lukas et al., 1982).

The drug delivery system was similar to that described by Findley et al. (1972). The catheter was attached to a valve system that allowed slow continuous administration (approx. 55–60 ml/24 h) of heparinized saline (5 units/ml) via a peristaltic pump to maintain catheter patency. Drug was injected, in 5-ml volume, into the valve system by means of a second pump and then flushed into the animal with 5 ml of saline from a third pump. This system necessitated a delay of approximately 20 s between the onset of drug delivery and actual injection into the vein. Drugs were delivered within a 2-min period.

An aluminum panel (0.7 × 1.0 m) containing levers and associated stimulus lights (approx. 1 cm in diameter) was mounted on the rear wall of the cage. A Lindsley lever (No. G6310, Gerbrands Corp., Arlington MA) (lower left of panel), a leaf lever (lower right of panel), and a food hopper with stimulus light (lower left of panel) were mounted on the panel. A 5 × 5 cm translucent Plexiglas panel that could be transilluminated with green light was mounted on the aluminum panel in the upper left corner. A speaker for delivery of white noise and tones was mounted behind the panel. A feeder (model PDC 050, BRS/LVE, Beltsville MD or model G5210, Gerbrands Corp.) for delivering food pellets into the food pellet tray was mounted on the top of the wooden enclosure. Experimental control and data collection were accomplished by PDP8 computers (Digital Equipment Corp.,

Maynard MA) programmed in SUPERSKED (State Systems, Kalamazoo, MI). Temporal patterning of responses was also recorded using cumulative recorders (Gerbrands Corp.).

Procedure. Animals were trained to self-inject cocaine (0.32 mg/kg per injection) under a fixed ratio 80 (subject GA) or 160 (subjects AC, DI and JB) (FR 80 or 160) response schedule on the Lindsley lever. Drug injections were available every 3 h and were signaled by a 5-s tone and by the illumination of the jewel light over the Lindsley lever. When the jewel light was illuminated, each response on the Lindsley lever produced a 0.1-s feedback tone. Upon completion of the response requirement, the jewel light was extinguished and the drug injection was begun, followed by a flush injection. Following the completion of the response requirement, the 5 × 5 cm translucent panel was illuminated for a 1-h period and the 3-h time-out period was begun. There was no time limit for the completion of the response requirement.

When criterion cocaine self-injection performance (6 or more injections per day for 3 consecutive days) was obtained, a dose of 4-methylaminorex or vehicle was substituted for cocaine for 15 or more days. Cocaine (0.32 mg/injection) self-injection performance was re-established, and when criterion performance was obtained (typically in 3—5 days), another dose of 4-methylaminorex was substituted. This procedure of replacing cocaine with 4-methylaminorex was continued through the study of 2 doses of 4-methylaminorex (0.1 and 0.32 mg/kg per injection) and its vehicle. The order of exposure to different doses was an ascending sequence. The drug vehicle was examined immediately after the series of doses. Data were recorded between 0800 h and 0900 h daily and drug changes were performed at this time, if appropriate.

Food was available 24 h per day under an FR 30 response schedule on the leaf lever; i.e. every thirtieth response delivered a 1-g banana-flavored food pellet (Bio-Serv, Frenchtown NJ) and produced a brief flash of the hopper light.

Rhesus monkey experiment

Subjects and apparatus. Two adult male (subject 315 and 572) and one female (subject 1100) rhesus monkeys (*Macaca mulatta*), weighing between 7.0—10 kg, were used as subjects. All of the monkeys had previously been trained to self-administer cocaine intravenously. Animals were maintained in ventilated enclosures (1 × 1 × 1 m) with free access to water. Purina Monkey Chow was fed to subjects twice per day in an amount sufficient to maintain constant or slowly increasing body weights. Intravenous silastic silicone cathethers (I.D. 0.08 cm; Ronsil Rubber Products, Belle Mead, NJ) were implanted in jugular or femoral veins under general anesthesia as described by Beardsley et al. (1986).

A response lever assembly was mounted on either side of the chamber's clear Plexiglas front door. Above each lever were three jeweled 28-v lights, one red and two white. A coiled stainless steel spring arm and harness assembly tethered the subjects to the rear of the chamber and protected the indwelling catheters from damage. Intravenous solutions were delivered by peristaltic pumps (Masterflex, Cole-Parmer, Chicago, IL). A PDP8A-based computer system programmed in SUPERSKED, located in an adjacent room, recorded the data and delivered injections.

Procedure. Subjects were tested in daily (7 days per week) 1-h sessions. Illumination of the white lights over the left lever signaled drug or saline availability; every 10 responses (FR 10) on the left lever produced a 10-s, 1-ml injection. During the injection, the associated white lights were extinguished and the red light illuminated; responses on the lever were neither recorded nor reinforced during this period. Responses on the right lever had no programmed consequences.

Monkeys had been trained to lever press for a fixed dose of cocaine prior to testing. For monkeys 315 and 572, this dose was 0.033 mg/kg per injection, and for monkey 1100 the dose was 0.01 mg/kg per injection. The cocaine dose selected was one that reliably maintained

higher response rates than did saline. Before testing the first dose of 4-methylaminorex, saline was substituted for cocaine on at least two consecutive sessions. Doses of 4-methylaminorex, presented in a mixed order, were then made available in blocks of 4 consecutive test days, separated by at least 3 days of cocaine self-administration at the training dose. In order to be tested with the next dose of 4-methylaminorex, the daily intake of cocaine must have had varied less than 20% for the last 3 days preceding drug substitution. Following the final dose of 4-methylaminorex, saline and cocaine were again substituted for 4 days each.

In analyzing the data, only the last 3 days of 4-day 4-methylaminorex substitutions were used, and only the last 3 days of the final 4-day saline series. Cocaine data on the 3 days preceding substitution tests were also included in the analysis, as well as cocaine data preceding and following the dose curve for 4-methylaminorex. The number and temporal distribution of all injections were collected for each session. 4-Methylaminorex was considered to serve as a reinforcer if the mean injection rate exceeded the rate when saline was made available and the ranges did not overlap.

Drugs. Cocaine HCl (obtained from the National Institute on Drug Abuse and from the Sigma Chemical Co., St. Louis, MO) and 4-methylaminorex HCl, obtained from the National Institute on Drug Abuse, were dissolved in physiological saline (0.9% sodium chloride) and then filter sterilized (Millipore, Bedford MA).

Results

Baboon experiment

Figure 2 presents the daily self-injection data across sequential experimental days for cocaine, two doses of 4-methylaminorex and vehicle using the cocaine substitution procedure in four individual baboons. As shown in this figure, cocaine maintained high daily rates of self-injection behavior in all four baboons: mean rates were 7.0—8.0 injections per day (Phase A). The substitution of 0.1 mg/kg per injection of 4-methylaminorex (Phase B) resulted in a progressive reduction in self-injection behavior over 1-4 days. This low dose of 4-methylaminorex produced variability in daily self-injection performance, but at the end of this phase the number of injections generally ranged between 0 and 4 injections per day. Cocaine was reintroduced and high rates of self-injection behavior were re-established within 1—3 days (Phase C).

The substitution of 0.32 mg/kg per injection of 4-methylaminorex (Phase D) produced a cyclic pattern of self-injection performance, with days of high rates of self-injection alternating with days of low rates of self-injection in all four baboons. In addition, this dose produced some suppression of food intake in three of the four baboons tested (Baboons AC, DI, JB; data not shown). During exposure to the high dose of 4-methylaminorex (0.32 mg/kg/injection) the erratic pattern of 4-methylaminorex self-injection behavior and suppression of food intake was accompanied by behavioral agitation (psychomotor agitation; hypersensitivity to noise; stereotypic movements; appearing to track non-existent visual objects, suggesting hallucinations) in three of the four baboons (Baboons DI, GA, JB).

When cocaine was reintroduced following 4-methylaminorex, high rates of cocaine self-injection behavior were re-established within 1—3 days in all four animals (Phase E). Subsequent vehicle substitution resulted in a reduction in self-injection behavior; at the end of this phase, the number of injections ranged between 0 and 4 injections per day (Phase F). Final reintroduction of cocaine produced high rates of self-injection behavior within 1—2 days (Phase G).

Rhesus monkey experiment

Cocaine maintained higher rates of self-administration than did saline in each of the subjects tested (Fig. 3). The mean number of cocaine injections over the course of the study per 1-h session varied from 27.8 in monkey 315 to 66.5 in monkey 1100. Substitution tests with 4-methylaminorex produced injection rates substantially greater than for saline in each

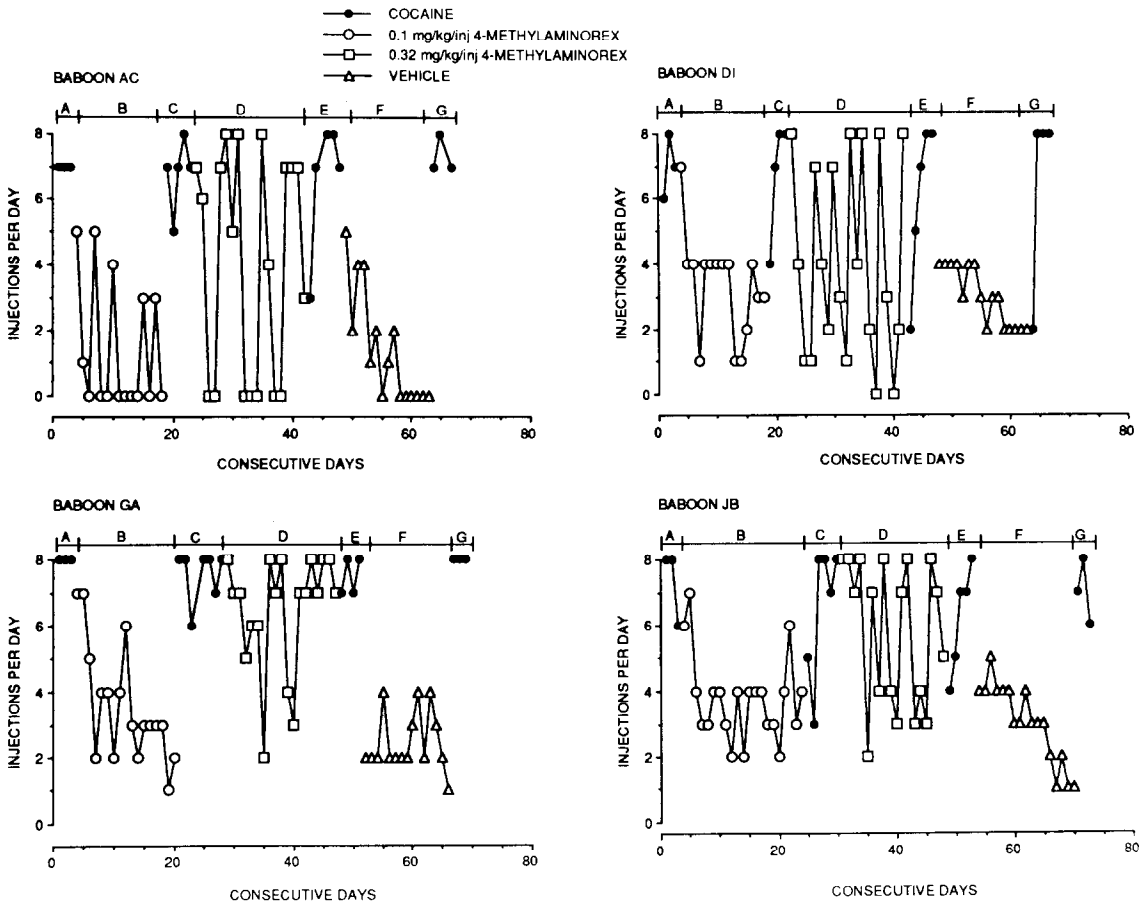


Fig. 2. Intravenous self-administration of 4-methylaminorex in baboons. Shown are the daily number of self-injections of 0.32 mg/kg per injection cocaine, 4-methylaminorex (0.1 mg/kg and 0.32 mg/kg per injection) or saline in each of 4 subjects. Vertical axes represent the number of injections per day; horizontal axes represent consecutive days. Phases A-G represent the sequential substitution of cocaine, 4-methylaminorex doses or vehicle over consecutive experimental days.

subject at two or more doses, where the ranges of rates did not overlap the range of saline rates. Considerable intersubject variability was evident in the dose of 4-methylaminorex that maintained maximal rates of responding. For subject 315, maximum rates of 4-methylaminorex self-administration occurred at 0.003 mg/kg/injection, while maximum rates for subjects 572 and 1100 occurred at 0.01 and 0.03 mg/kg/injection, respectively. In subjects 315 and 572, 4-methylaminorex maintained higher maximal rates of self-administration (79.0 and 91.7 injections/session, respectively) than did cocaine training dose.

When saline was the available substance, most injections occurred in the first 15 min of the session, with comparatively few in successive 15-min periods (Fig. 4). Cocaine self-administration, however, was more evenly distributed throughout the session than was saline-maintained behavior in most cases, though the greatest number of injections were still typically administered in the first 15-min period. The self-administration of 4-methylami-

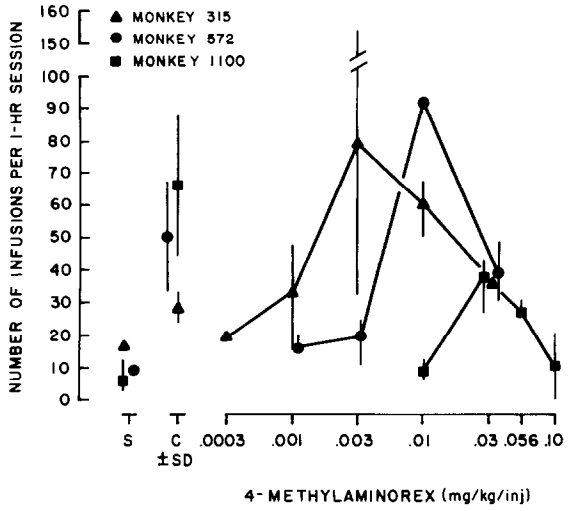


Fig. 3. Intravenous self-administration of 4-methylaminorex in three rhesus monkeys. Illustrated on the left side of the panel are the mean numbers of saline (S) or cocaine (C) injections. Vertical lines through the cocaine points represent the standard deviation (SD) of all performances in the 3-day periods preceding 4-methylaminorex substitution and also before and after the dose-response curve. Lines through 4-methylaminorex points represent the range of observations during the last 3 days of substitution tests for each dose. Lines through saline points represent the range of observations during the last 3 days of substitution which followed the completion of 4-methylaminorex testing.

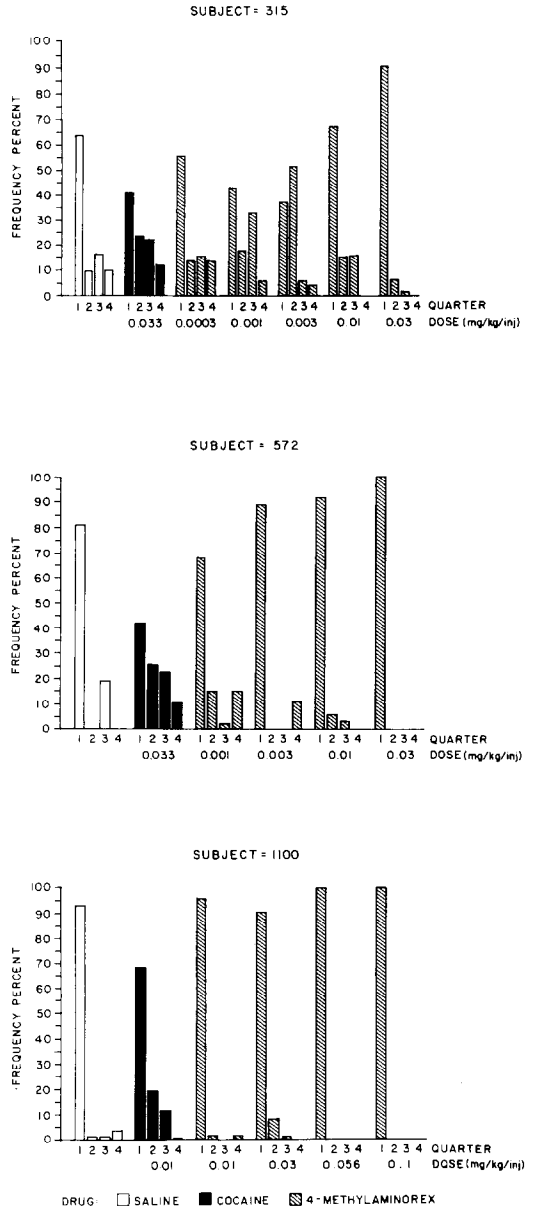


Fig. 4. Within-session distributions of saline, cocaine and 4-methylaminorex self-administration in three rhesus monkeys. The vertical bars represent the percent of total injections (frequency percent) occurring in each of the four 15-min segments (quarters) of the 1-h session. Data included are from the last 3 substitution days for 4-methylaminorex, and the mean performances for all of the 3-day cocaine baseline sessions preceding 4-methylaminorex substitution. Included in the saline data are the sessions preceding and following 4-methylaminorex dose curves.

norex also appeared to occur predominantly in the early portions the session. Even at doses of 4-methylaminorex that maintained response rates higher than those maintained by cocaine (0.003 mg/kg per injection in monkey 315 and 0.01 mg/kg per injection in monkey 572), injections nevertheless occurred predominantly in these early portions of the session.

Discussion

The results of this study clearly show that 4-methylaminorex serves as a positive reinforcer in nonhuman primates. In baboons, 4-methylaminorex at a dose of 0.32 mg/kg per injection maintained high, though erratic rates of responding, and decreased concurrent food-

maintained behavior. The profile of cyclic self-injection behavior, disruption of food intake and behavioral agitation produced by this dose of 4-methylaminorex suggests classic psychomotor stimulant toxicity.

In rhesus monkeys, 4-methylaminorex maintained rates of responding comparable to or greater than cocaine in many instances. In each monkey, the ranges of saline injection rates did not overlap with those maintained by two or more doses of 4-methylaminorex, establishing the drug as a reinforcer under these conditions. Although rates of cocaine- and 4-methylaminorex-maintained responding were often comparable, the pattern of responding within session differed considerably. Cocaine maintained more constant rates over the 1-h session than did 4-methylaminorex, where the large majority of injections were taken early in the session. Such contrasting effects have also been observed between cocaine and amphetamine when tested under similar circumstances (Balster and Schuster, 1973).

The tendency for 4-methylaminorex self-administration to be negatively accelerated in the rhesus monkey suggests that drug accumulation may account for the cyclical nature of self-administration behavior in the baboon. For example, in a study by Griffiths et al. (1979a), responding was maintained by cocaine under an FR 160 schedule with 3-h time-outs. At high doses (between 3.2 and 4.0 mg/kg) response rates were decreased relative to those maintained by intermediate doses. However, when the time-out period was increased to 12 h, these highest doses were not associated with a downturn in the dose-effect curve. Therefore, it is possible that the disruptive effects of the high 4-methylaminorex dose in baboons on some days was due to a high number of injections during previous drug availability periods. The relationship between temporal scheduling of injections and reinforcing doses of 4-methylaminorex may support this explanation. In the baboon, where 3-h time-outs were scheduled between successive injections, maximal rates were maintained by 0.32 mg/kg per injection. In the rhesus monkey, where animals had unlimited access for 1 h and self-administered as many as 50–100 injections, maximal rates of responding were maintained at 0.003 to 0.03 mg/kg per injection, suggesting that reinforcing efficacy is affected by frequency and spacing of drug availability.

In summary, 4-methylaminorex served as a positive reinforcer in baboons and in rhesus monkeys responding under different procedures of response-contingent intravenous delivery. The finding that 4-methylaminorex is a reinforcer and will produce psychomotor stimulant toxicity at higher doses is similar to that previously reported with several other central nervous system stimulants: *l*-methylenedioxyamphetamine, *d*-amphetamine and methylenedioxymethamphetamine (Balster and Schuster, 1973; Griffiths et al., 1976; Griffiths et al., 1979b; Beardsley et al., 1986; Lamb and Griffiths, 1987). These results suggest that 4-methylaminorex has a clear potential for abuse.

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