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Assessment of mazindane, a pro-drug form of mazindol, in assays used to define cocaine treatment agents

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Abstract

The current studies compared mazindane (5-(4-chlorophenyl)-2,3-dihydro-5H-imidazo [2,1a] isoindole) hydrogen sulfate, a water soluble pro-drug of mazindol (5-(4-chlorophenyl-2,3-dihydro-5H-imidazo [2,1-a] isoindol-5-ol), with mazindol in assays used to define cocaine treatment agents. Both compounds enhanced motor activity (LMA) in Swiss Webster mice with ED_{50} values of 2.5 mg/kg i.p. for mazindane and 3.9 mg/kg i.p. for mazindol. At 25 mg/kg mazindane displayed toxic effects and death while mazindol was effect/death free at 50 mg/kg. In Sprague–Dawley rats trained to discriminate cocaine from saline both compounds fully substituted for cocaine with mazindane being fourfold more potent in the total session (0.33 vs. 1.3 mg/kg i.p.) and first reinforcer (0.29 vs. 1.2 mg/kg i.p). Complete substitution was observed in rhesus monkeys trained to discriminate cocaine from saline with ED_{50} values for mazindane (0.134 mg/kg i.m.) and mazindol (0.119 mg/kg i.m.). Mazindol exhibited little or no activity at 10^{-5} M in inhibiting radioligand binding at 14 neurotransmitter sites while mazindane gave weak activity at the histamine H_1 and 5-hydroxytryptamine 5-HT_3 sites. These results demonstrate that mazindane could be a useful alternative to mazindol as a pharmacological tool because of its similar profile of activity and enhanced water solubility.

Keywords: Cocaine discrimination; (Mouse); (Rat); (Rhesus monkey); Mazindane; Mazindol; Motor activity, mouse; Neurotransmitter binding

1. Introduction

Mazindol (Fig. 1: 5-(4-chlorophenyl)-2, 3-dihydro-5H-imidazo [2, 1-a] isoindol-5-ol, SaH 42-548, AN-448) is a potent inhibitor of uptake and binding at the transporter sites for dopamine (DA), norepinephrine (NE) and serotonin (5-HT) (Gogerty et al., 1975; Engstrom et al., 1975; Heikkila et al., 1981) and is marketed (Sanorex, Teronac, Mazanor) for the management of exogenous obesity (de Felice et al., 1973; Inoue, 1998; Bray, 2000) and as an orphan drug for the treatment of Duchenne muscular dystrophy (Coakley et al., 1988; Griggs et al., 1990). It has also been found to be useful in curtailing daytime sleep attacks in narcolepsy (Alvarez et al., 1991; Parkes and Schacter, 1979; Vespignani et al., 1979) and is claimed to be useful in urinary incontinence (Woodhouse, 1983), gastric ulcers (Shriver and Gluckman, 1976) and negative symptoms in schizophrenia patients (Seibyl et al., 1993). In addition, [^{3}H] mazindol has also been used as a pharmacological tool in numerous (312 citations in Medline to July 2002) in vitro and in vivo studies involving the dopamine and norepinephrine transporters (DAT and NET).

The finding (Javitch et al., 1984) that mazindol was a potent inhibitor (low nanomolar) of [^{3}H]-cocaine binding at the dopamine transporter (DAT), the binding site proposed to be implicated in the reinforcing properties of cocaine ((1R-(exo-exo)]-3-(benzoyloxy)-8-azabicyco [3.2.1] octane-2-carboxylic acid methyl ester) (Ritz et al., 1987), generated interest in the compound as a potential agent for the treatment of cocaine abuse. Studies designed to address the potential of mazindol abuse showed it was mildly dysphoric and appears to be free of abuse when given blindly to healthy patients (Chait et al., 1987). In normal healthy patients trained to distinguish D-amphetamine (D-α-methylphenethylamine) from placebo it substituted in terms of discrimination responding, but not amphetamine-like subjective effects (Chait et al., 1986) and in amphetamine dependent subjects it expressed a low-degree of amphetamine-like effects (Götestam and Gunne, 1972).

Initial open clinical studies with mazindol in cocaine abusers showed significant reduction of craving and euphoria without significant side effects (Berger et al., 1984). Subsequent studies failed to support the earlier findings that

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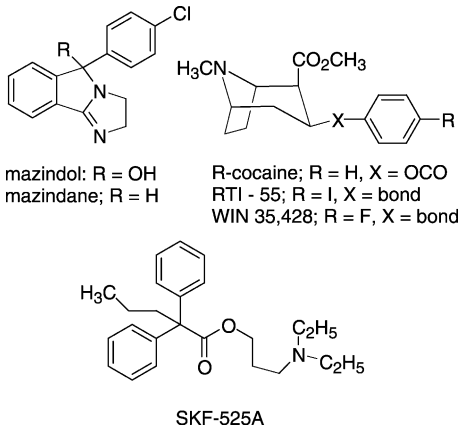

Fig. 1. Structures of mazindol, mazindane, R-cocaine, RTI-55 and SKF-525A.

mazindol substantially altered the magnitude or profile of subjective effects of cocaine, including craving (Preston et al., 1993; Stine et al., 1995). In a double-blind randomized study with methadone-maintained cocaine abusers, treatment with mazindol was suggestive in preventing relapse to cocaine (Margolin et al., 1995, 1997).

In our laboratory we have carried out extensive structure–activity relationship (SAR) studies on mazindol (Houlihan et al., 1996, 1998, 2002a,b; Kulkarni et al., 2002). One of the compounds from this study, referred to as mazindane (Fig. 1; 5-(4-chlorophenyl)-2, 3-dihydro-5H-imidazo [2, 1-a] isoindole; Metlesics and Sternbach, 1977), where the hydroxyl group (OH) in mazindol is replaced by a hydrogen atom (H), was found to possess similar in vitro activity blocking the uptake of dopamine (DA) and inhibiting the binding of 1R-(exo-exo)-3-(4′-iodophenyl)-8-methyl-8-azabicyclo [3.2.1] octane-2-carboxylic acid methyl ester ([^{125}I]RTI-55, Fig. 1) HEK cells expressing human dopamine transporter at HEK-hDAT cells (Table 1). Examination of extracts from the rat membranes and HEK-hDAT cells used in the assay of mazindane by LC-UV-MS analysis disclosed only the presence of mazindol. These findings suggest that mazindane acts as a pro-drug, being converted in vitro to mazindol (Houlihan et al., 2002a).

Table 1
Effect of mazindol and mazindane on inhibition of in vitro binding and uptake at monoamine transporters[a]

Transporter	Mazindol		Mazindane	
	Binding[b] K_i, nM	Uptake[c] IC_{50} nM	Binding[b] K_i, nM	Uptake[c] IC_{50} nM
Dopamine[d]	45 ± 1	42 ± 2.0	1.7 ± 0.8	3.7 ± 0.4
Norepinephrine[e]	18 ± 2	4.9 ± 0.5	170 ± 75	6.9 ± 1.5
Serotonin[f]	50 ± 15	94 ± 32	39 ± 11	53 ± 7

[a] See (Houlihan et al., 2002a) for details on procedures.
[b] Inhibition of [^{3}H] RTI-55.
[c] Inhibition of [^{3}H] DA, [^{3}H] NE or [^{3}H] 5-HT.
[d] HEK-hDAT cells.
[e] HEK-hNET cells.
[f] HEK-hSERT cells.

The present work compares the in vivo activity of mazindol and mazindane H_2SO_4 salt (referred to as mazindane) in mouse, rat and monkey assays used to detect compounds with cocaine-like properties that might be useful in the treatment of cocaine abuse. In addition the inhibition of ligand binding at selected monamine transporters and neurotransmitter sites is reported.

2. Materials and methods

2.1. Drugs

Cocaine·HCl and SKF-525A (2,2-diphenylpentanoic acid [3-(diethylaminopropyl)] ester) were obtained in crystalline form from the National Institute on Drug Abuse (NIDA), National Institutes of Health, Bethesda, MD. Mazindol was obtained from Sandoz (Novartis) Pharmaceuticals, East Hanover, NJ and mazindane·H_2SO_4 was prepared by a

Table 2
Effect of mazindol and mazindane on inhibition of binding at neurotransmitter sites[a]

Neurotransmitter[b]	% Inhibition at 10^{-5} M or (IC_{50}, nM)		Receptor membranes
	Mazindol	Mazindane	
Alpha α_1	15.4	56.6	rat forebrain
Alpha α_2	10.4	29.7	rat cortical
Beta β	− 10.4	10.0	rat cortical
Dopamine D_1	5.5	− 0.5	rat striatal
Dopamine D_2	23.5	36.1	rat striatal
Histamine H_1	38.3	(708)	bovine cerebellar
Histamine H_2	1.4	50.2	g. pig striatal
Muscarinic M_1	58.9	(1670)	bovine striatal
Muscarinic M_2	59.7	73.0	rat cardiac
Muscarinic M_3	22.4	26.2	g. pig ileum
Serotonin 5-HT_1	22.0	32.4	rat cortical
Serotonin 5-HT_{1A}	35.5	(2050)	bovine hippocampal
Serotonin 5-HT_2	18.7	37.5	rat cortical
Serotonin 5-HT_3	28.9	(493)	NIE-115 cells

[a] See Section 2.2 for procedure.
[b] Radioligands, references and positive control compounds, media, time (min) and temperature (°C) for each assay: α_1, [^{3}H] prazosin and prazosin 50 mM TRIS–HCl (pH 7.7), 60, 25°; α_2, [^{3}H] RX 821002 and RX 821002 50 mM TRIS–HCl (pH 7.4), 90, 0°; β, [^{3}H] DHA and alprenolol, 50 mM TRIS–HCl (pH 7.4), 30, 37°; D_1, [^{3}H] SCH 23390 and SCH 23390, 50 mM HEPES (pH 7.4) containing 1.0 mM EDTA, 4.0 mM $MgSO_4$ and 10 mM ketanserin, 60, 37°; D_2, [^{3}H] sulpiride and sulpuride, 50 mM TRIS–HCl (pH 7.5) containing 100 mM NaCl, 60, 25°; H_1, [^{3}H] pyrilamine and triprolidine, 50 mM $NaKPO_4$ (pH 7.5), 30, 25°; H_2, [^{3}H] tiotidine and cimetidine, media as H_1, 20, 25°; M_1, [^{3}H] pirenzepine and atropine, media as H_1, 60, 25°; M_2, [^{3}H] AF-DX384 and methoctramine, 10 mM $NaKPO_4$ (pH 7.4), 30, 25°; M_3, [^{3}H] N-methylscopolamine and 4-DAMP methiodide, 30 mM HEPES (pH 7.4) containing NaCl, KCl, $CaCl_2$, Na_2CO_3, $MgCl_2$, glucose, 60, 37°; 5-HT, [^{3}H] hydroxytryptamine binoxalate and serotonin, media as α_2, 45, 37°; 5-HT_{1A} [^{3}H] 8-OH-DPAT and 8-OH-DPAT for reference and serotonin for control, media as α_2, 10, 37°; 5-HT_2 [^{3}H] ketanserin and methysergide, 50 mM TRIS–HCl (pH 7.6), 15, 37°; 5-HT_3, [^{3}H] GR 65630 and MDL-72222, 20 mM HEPES (pH 7.4) containing 150 mM NaCl, 30, 25°.

reported procedure from this laboratory (Houlihan et al., 2002a).

2.1.1. Solubility assays

Mazindol or mazindane was added portionwise to a stoppered vial containing 1.00 ml of distilled H_2O or 0.9% saline maintained at ambient temperature (ca. 22 °C), shaken for 2 min after each addition and then observed for solubility.

The solubility of mazindol and mazindane was <2 and >25 mg/ml in H_2O and <2 and >30 mg/ml in 0.9% saline.

2.2. Neurotransmitter receptor binding assays

A solution containing 0.005 mol of mazindol or mazindane solubilized in 0.2 ml 100% dimethyl sulfoxide (DMSO) was diluted in distilled H_2O (4.81 ml) to yield a 1 mM solution. This stock solution was stored at 4 °C for up to 2 weeks during which time samples were taken as needed and diluted 10-fold in 4% DMSO at room temperature. A 1:10 dilution into the assay tubes yields a 10^{-5} M testing concentration.

The competitive binding assays were performed in either 250 or 500 μl volumes containing, by volume, 80% receptor preparations, 10% radioligand and 10% of test compound/cold ligand (nonspecific binding determination)/4% DMSO (total binding determinant). The receptor source, radioligands, positive controls, media, time and temperature for all assays is given in Table 2.

Assays were terminated by rapid vacuum filtration onto Whatman glass fiber filters. Radioactivity trapped onto the filters was determined by either liquid scintillation or ∝ spectrometry and compared to control values to ascertain any interaction of mazindol or mazindane with the binding site.

2.2.1. [^{3}H]WIN 35, 428 binding assays with SKF-525A

Brains from male Sprague–Dawley rats weighing 200–225 g (Taconic Labs) were removed, striatum dissected and placed on ice. Membranes were prepared by homogenizing tissues in 20 volumes (w/v) of ice cold modified Krebs–HEPES buffer (15 mM HEPES, 127 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.3 mM NaH_2PO_4, 10 mM D-glucose, pH adjusted to 7.4) using a Brinkman Polytron (setting 6 for 20 s) and centrifuged at 20,000 × *g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer, recentrifuged and resuspended in buffer to a concentration of 10 mg/ml. Ligand binding experiments were conducted in assay tubes containing 0.5 ml modified Krebs–HEPES buffer for 60 min on ice. Each tube contained 1.5 nM [^{3}H] WIN 35,428 (specific activity 84 Ci/mmol) and 2.5 mg striatal tissue (original wet weight), mazindane (5 nM) or mazindol (5 nM) and 0, 0.1 or 1.0 μM SKF-525A. Nonspecific binding was determined using 0.1 mM cocaine HCl. For determination of binding affinity, triplicate samples of membranes were preincubated for 5 min in the presence or absence of the compound being tested. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.1% BSA (bovine serum albumin), using a Brandel R48 filtering manifold (Brandel Instruments, Gaithersburg, MD). The filters were washed twice with 5 ml cold buffer and transferred to scintillation vials. Beckman Ready Safe (3.0 ml) was added and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, CA). The dpm values are given in Table 3.

2.3. Locomotor activity: 8 h study

A time course/dose response study of cocaine-induced locomotor stimulation was conducted according to the Medications Development Division (MDD) of NIDA locomotor activity studies time course protocol (Mar. 28, 1997). Mice (Sasco, Omaha, NE) were housed on hardword litter at constant temperature and humidity with a standard 12-h light/dark cycle. Animals were allowed to acclimatize for 3 days before testing.

The study was conducted using 40 Digiscan locomotor activity testing chambers (40.5 × 40.5 × 30.5 cm) housed in sets of two, within sound-attenuating chambers. A panel of infrared beams (16 beams) and corresponding photodetectors were located in the horizontal direction along the sides of each activity chamber. A 7.5-W incandescent light above each chamber provided dim illumination. Fans provided an 80-dB ambient noise level within the chamber. Separate groups of eight non-habituated male Swiss-Webster mice (Hsd:ND4, aged 2–3 months) were injected via the intraperitoneal (i.p.) route with either vehicle (0.9% saline or 0.16% tartaric acid), cocaine, mazindol or mazindane, immediately prior to locomotor activity testing. In all studies, horizontal activity (interruption of photocell beams) was measured for 8 h within 10 min periods, beginning at 08:00 h (2 h after lights on). Testing was conducted with one mouse per activity chamber. ED_{50} values (dose producing 1/2 maximal stimulant activity, where maximal stimulant activity = maximum − mean control counts/10 min) were estimated from a linear regression against $\log_{10}$ dose of the ascending portion of the dose–effect curve.

Table 3
Inhibition of [^{3}H] WIN 35,428 binding by mazindane and mazindol at rat brain membranes in the presence of SKF-525A[a]

SKF-525A μM	Specific Binding[b], dpm	% of Specific Binding[c]	
		Mazindol[d]	Mazindane[d]
0	2463	72.5	67.1
0.1	1647	91.9	77.7
1.0	770	102.0	80.3

[a] See Section 2.2.1 for procedure.

[b] Specific binding, disintegrations per minute = total dpm − blank dpm: 0 μM, 2643-180; 0.1 μM, 1827-180; 1.0 μM, 981-211.

[c] % of specific binding = mazindol or mazindane and SKF-525A dpm/specific binding dpm.

[d] Mazindol and mazindane concentration is 5 nM in all assays. [^{3}H] WIN 35,428 IC_{50} values: mazindol 8.1 ± 1.2 nM and mazindane 29.3 ± 3.1 nM (Houlihan et al., 2002a).

2.4. Rat cocaine discrimination study

Experiments were conducted according to the standard operating protocol of the Cocaine Treatment Development Program (CTDP) of NIDA for drug discrimination testing in rats standard operating procedure (SOP) of March 28, 1997, as modified March 30, 1998. The rats (Sasco, Wilmington, MA) were housed in standard, commercially available cages and maintained as the mice (cf. Section 2.3).

Six male Sprague–Dawley rats were trained to discriminate cocaine (10 mg/kg i.p.) from saline using a two-lever choice methodology. Food was available as a reinforcer under a fixed ratio 10 schedule when responding occurred on the injection appropriate lever. All tests occurred in standard, commercially available chambers (Coulbourn Instruments), using 45 mg food pellets (Bioserve) as reinforcers.

Training sessions occurred in a double alternating fashion, and tests were conducted between pairs of identical training sessions (i.e., between either two saline or two cocaine training sessions). Tests occurred only if in the two preceding training sessions, subjects met the criteria of emitting 85% of responses on the injection correct lever for both the first reinforcer (first fixed ratio) and the total session. Test sessions lasted for 20 min, or until 20 reinforcers had been obtained.

Intraperitoneal (i.p.) injections of the test compound (1 ml/kg), or its vehicle (0.16% tartaric acid for mazindol and 0.9% saline for mazindane), occurred 30 min prior to the start of the test session. A dose range of 0.1–5.0 mg/kg for mazindol and 0.1–3.0 mg/kg of mazindane was examined. An ED_{50} value using linear regression analysis was calculated if a $\geq 80\%$ drug-appropriate responding was observed. Results are given in Figs. 4 and 5.

2.5. Monkey cocaine discrimination study

2.5.1. Subjects

The subjects, obtained from the New England Regional Primate Center (Southborough, MA), were four male rhesus monkeys (*Macaca mulatta*) each weighing 5.7–8.1 kg. Each monkey was maintained on a diet of three to four monkey biscuits (Purina Monkey Chow Jumbo #5037) and one piece of fresh fruit per day. During the week, all food was delivered after the experimental session, whereas on weekends, food was delivered between 9 a.m. and noon. Water was freely available for all monkeys at all times. The room in which the monkeys were housed was maintained on a 12-h light/dark cycle, with lights on from 7 a.m. to 7 p.m.

Animal maintenance and research was conducted in accordance with the guidelines provided by the Committee on Laboratory Animal Resources. The facility was licensed by the US Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee. The health of the monkeys was periodically monitored by consulting veterinarians. Monkeys had visual, auditory and olfactory contact with other monkeys throughout the study. Operant food self-administration procedures provide an opportunity for environmental manipulation and enrichment.

2.5.2. Apparatus

Each monkey was housed individually in a well-ventilated, stainless steel chamber (56 × 71 × 69 cm). The home cages of all monkeys were modified to include an operant panel (28 × 28 cm) mounted on the front wall. Three square translucent response keys (6.4 × 6.4 cm) were arranged 2.54 cm apart in a horizontal row 3.2 cm from the top of the operant panel. Each key could be transilluminated by red or green stimulus lights (Superbright LED's). In addition, the operant panel supported an externally mounted pellet dispenser (Gerbrands, Model G5310, Arlington, MA) that delivered 1 g fruit-flavored food pellets (Precision Primate Pellets Formula L/I Banana Flavor, P.J. Noyes, Lancaster, NH) to a food receptacle mounted on the cage beneath the operant response panel. Operation of the operant panels and data collection were accomplished with Apple IIGS computers located in a separate room.

2.5.3. Discrimination training

Discrimination training was conducted 5 days per week during daily sessions composed of multiple cycles. Each cycle consisted of a 15-min time-out period followed by a 5-min response period. During the time-out, all stimulus lights were off, and responding had no scheduled consequences. During the response period, the right and left response keys were transilluminated red or green, and monkeys could earn up to 10 food pellets by responding under a FR 30 schedule of food presentation. For all monkeys in this study, the left key was illuminated green, and the right key was illuminated red. The center key was not illuminated at any time, and responding on the center key had no scheduled consequences. If all available food pellets were delivered before the end of the 5 min response period, the stimulus lights transilluminating the response keys were turned off, and responding had no scheduled consequences for the remainder of the 5 min period.

On training days, monkeys were given an i.m. injection of either saline or 0.40 mg/kg cocaine hydrochloride dissolved in sterile saline 5-min after the beginning of each time-out period (i.e., 10 min before the response period). Following the administration of saline, responding on only the green key (the saline-appropriate key) produced food, whereas following administration of 0.40 mg/kg cocaine, only responding on the red key (the drug appropriate key) produced food. Responses on the inappropriate key reset the FR requirement on the appropriate key. Sessions consisted of one to five cycles, and if the training dose of cocaine was administered, it was administered only during the last cycle. Thus, training days consisted of zero to five saline cycles followed by zero to one drug cycle.

During the response period of each cycle, three dependent variables were determined: (1) percent injection-appropriate

responding before delivery of the first reinforcer [(injection-appropriate responses emitted before first reinforcer/total responses emitted before delivery of first reinforcer) × 100]; 100]; (2) percent injection-appropriate responding for the entire response period [(injection-appropriate responses emitted during response period/total responses emitted during response period) × 100]; and (3) response rate (total responses emitted during response period/total time response keys were transilluminated).

Monkeys were considered to have acquired cocaine discrimination when the following three criteria were met for seven of eight consecutive training sessions: (1) the percent injection-appropriate responding prior to delivery of the first reinforcer was greater than or equal to 80% for all cycles; (2) the percent injection-appropriate responding for the entire cycle was greater than or equal to 90% of all cycles; (3) response rates during saline training cycles were greater than 0.5 responses per second.

2.5.4. Discrimination testing

Once monkeys met criterion levels of cocaine discrimination, testing began. Test sessions were identical to training sessions except that responding on either key produced food, and mazindol (0.0032–0.32 mg/kg dissolved in 1% lactic acid in sterile saline) or mazindane (0.01–1.0 mg/kg dissolved in distilled water) was administered using a substitution protocol. In this substitution protocol, mazindol or mazindane was administered alone, instead of either saline or cocaine, using a cumulative dosing procedure. Monkeys received an i.m. injection of mazindol or mazindane 5 min after the beginning of each cycle of a multiple cycle session, and each dose increased the total dose by 1/2 log units.

Test sessions were conducted only if the three criteria listed above were met during the training day immediately preceding the test day. Mean data from saline and drug cycles during the training day immediately preceding the initial test day served as the control data for the subsequent test day. If responding did not meet criterion levels of discrimination performance, then training was continued until criterion levels of performance were obtained for at least two consecutive days.

2.5.5. Data analysis

Individual subject graphs of the Percent Cocaine-Appropriate Responding (for the entire response period) and the Response Rate were plotted as a function of mazindane or mazindol (log scale). Mazindane and mazindol were considered to have generalized to cocaine if some dose of mazindane or mazindol produced at least 90% cocaine-appropriate responding. In those monkeys in which mazindane or mazindol produced greater than 50% cocaine-appropriate responding, the ED_{50} value of the dose–effect curve was determined by drawing a line between the points above and below 50% cocaine-appropriate responding, and then using linear regression to interpolate the dose that would produce 50% cocaine-appropriate responding.

3. Results

3.1. Neurotransmitter binding assays

Mazindol, at 10^{-5} M, showed very weak or no displacement of the [^{3}H] ligands at all the neurotransmitter binding sites. Mazindane displayed moderate activity at the 5-hydroxytryptamine, 5-HT_3 and histamine H_2 sites, weak activity at the muscarinic M_1 and 5-HT_{1A} sites and very weak or no displacement at the other sites (Table 2). At all sites, except for dopamine D_1, mazindane showed better selectivity than mazindol, with maximum differences of 50-fold at H_2 and >50-fold at the H_1, 5-HT_{1A}, 5-HT_3 and M_1 binding sites.

SKF-525A could not be used to block the oxidation (hydroxylation) of mazindane in the [^{3}H] WIN 35,428 binding assay at rat membranes because it interacts at the WIN binding site in a dose–response manner (Table 3).

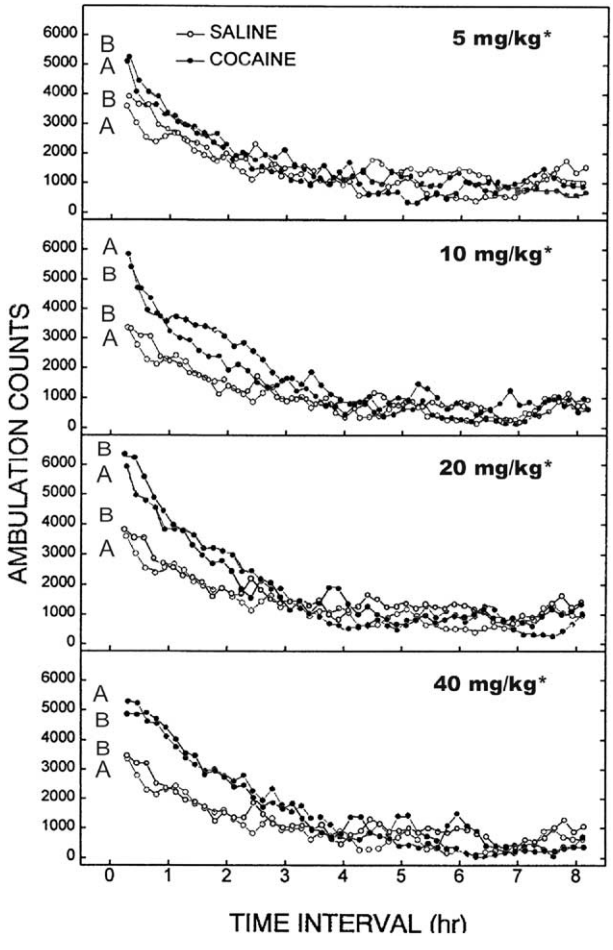

Fig. 2. Effect of cocaine on horizontal activity counts/10 min on separate groups of eight non-habituated Swiss–Webster mice as a function of dose (top to bottom panels) and time interval during an 8 h session. The panel shows two groups compared with vehicle (0.9% saline) control for the mazindol study (A) and mazindane study (B). *$P < 0.05$ compared with (0–30 min) for both studies.

3.2. Locomotor activity

The effects of cocaine, mazindol and mazindane on mice ambulatory activity during an 8 h period are shown in Figs. 2 and 3. Cocaine treatment in both control groups resulted in a time- and dose-dependent stimulation of LMA in doses from 5 to 40 mg/kg via the intraperitoneal (i.p.) route. Onset of stimulation was rapid (10 min) and lasted 100–130 min (Fig. 2, A) or 100–200 min (Fig. 2, B). Analysis of the dose–response data occurring at the time period (0–30 min) where cocaine produced maximum stimulation. Using Table Curve 2D v 2.03 software (Jandel Scientific), the mean horizontal activity counts for this 30-min period were fit to a three-parameter logistic peak function of $\log_{10}$ dose (with the constants set to 2847 and 3306, the means of the saline-treated groups) and the maximum was estimated from the resulting curve (maximum = 5151 counts/10 min at 26.2 mg/kg in A and 5651 counts/10 min at 19.0 mg/kg in B). The ED_{50} (dose producing 1/2 maximal stimulant activity, where maximal stimulant activity = maximum − mean control counts/10 min) was estimated at 4.8 mg/kg i.p. for the mazindol control (Fig. 2, A) and 6.6 mg/kg i.p. for the mazindane control (Fig. 2, B) from a linear regression against $\log_{10}$ dose of the ascending portion of the dose effect curves (5–20 mg for A, and 5–10 mg for B).

A two-way analysis of variance conducted on horizontal activity counts/10 min indicated significant effects of treatment $F(4, 35) = 3.7$, $P = 0.013$, 10 min periods $F(47, 1645) = 94.6$, $P < 0.001$, and in the interaction of those factors $F(188, 1645) = 2.3$, $P < 0.001$ for the mazindol control. Two-way analysis of the mazindane control indicated a significant analysis of treatment with 10-min periods, as well as a main effect of 10-min periods (P's < 0.001). The main effect of treatment was not significant in the two-way analysis, $F(4, 35) = 1.4$, $P = 0.252$. A one-way analysis of variance conducted on $\log_{10}$ horizontal activity counts for the 0–30 min time period (maximal stimulant effect) indicated a significant effect of treatment $F(4, 35) = 7.2$, $P < 0.001$ and $F(4, 35) = 7.1$, $P < 0.001$, and planned comparisons (a priori contrast) against the vehicle group showed significant difference for 5, 10, 20 and 40 mg/kg in both studies (all P's < 0.05 denoted by an asterisk in Fig. 2).

Treatment of mice with mazindol (i.p. in 0.16% tartaric acid) at doses of 1, 2.5, 5, 10, 25, and 50 mg/kg resulted in a time-dependent stimulation of locomotor activity at the 10, 25, and 50 mg/kg doses. Maximum stimulation (6155 counts/10 min at 7.1 mg/kg) and duration of action (180

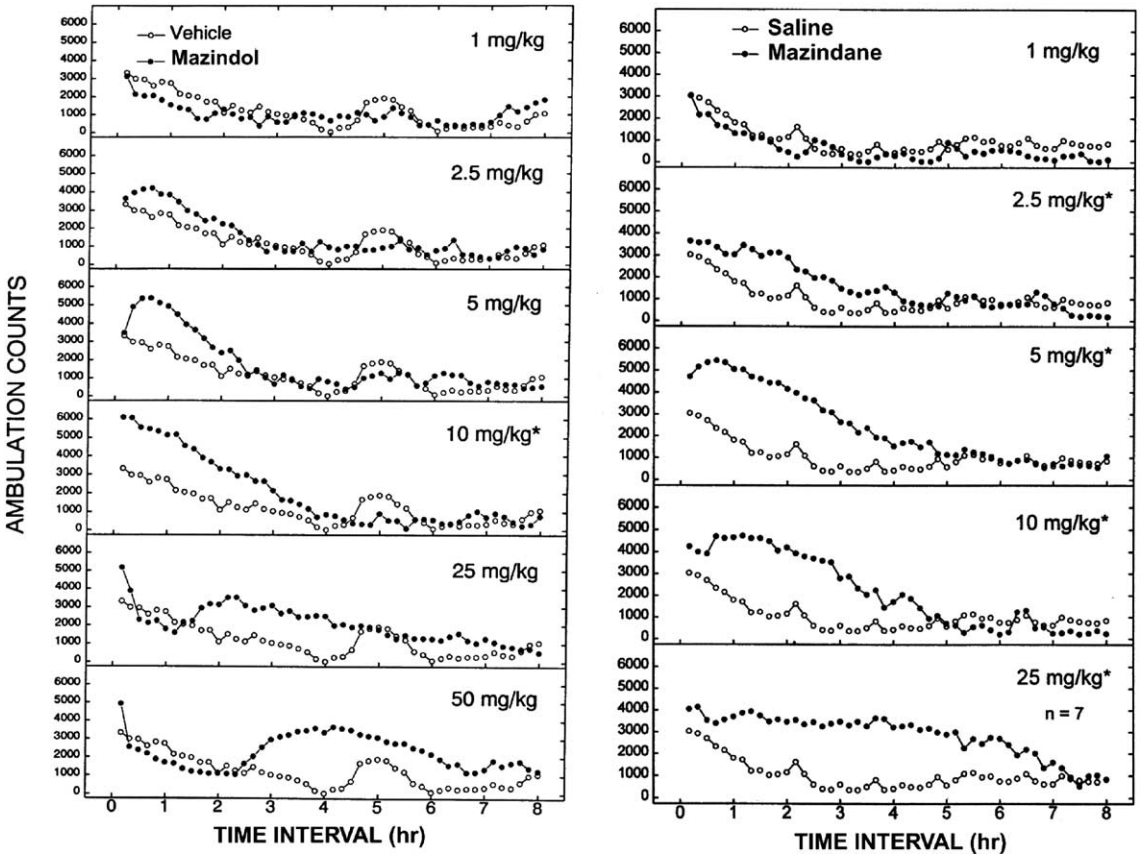

Fig. 3. Effect of mazindol (left panels) and mazindane (right panels) on horizontal activity counts/10 min on separate groups of eight non-habituated Swiss–Webster mice as a function of dose (top to bottom panels) and time interval during an 8 h session. Each panel shows one group compared with 0.16% tartaric vehicle for mazindol and 0.9% saline vehicle for mazindane. *$P < 0.05$ compared with 0.16% tartaric acid (10–40 min) and 0.9% saline (50–80 min).

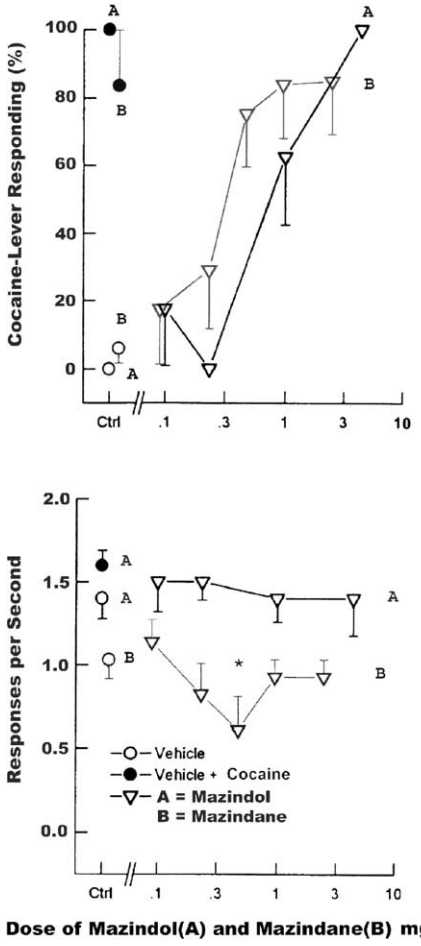

Fig. 4. Effects of mazindol and mazindane in male Sprague–Dawley rats trained to discriminate injections of cocaine (10 mg/kg) from saline. The upper panel shows the percent of response emitted on the cocaine-lever during the total session as a function of dose for mazindol (A) and mazindane (B). The lower panel shows the response rate for mazindol (A) and mazindane (B) for all subjects tested. Each point represents the effect in six rats. To the left of the break, control (Ctrl) data are shown for vehicle alone (0.16% tartaric acid for mazindol (A) and 0.9% saline for mazindane (B)) and the training doses of cocaine is shown to the right of the axis break. *$P < 0.05$ compared to vehicle control.

min) occurred with the 10 mg/kg dose (Fig. 3, left panels). Both the 25 and 50 mg/kg doses showed a short duration of maximal stimulation at onset that lasted for ca. 0.5 h, followed by a second longer lasting (ca. 3 h for 25 mg/kg and 4 h for 50 mg/kg doses), but weaker period of stimulation. The period 10–40 min at the 10 mg/kg dose was selected for analysis of dose–response data as in cocaine. The mean average horizontal activity counts/10 min was fit to a three-point parameter logistic peak function of $\log_{10}$ dose (constant set to 2843, the mean of the 0.16% tartaric acid vehicle group) and the maximum effect estimated from the resulting curve (maximum = 6155 counts/10 min at 7.1 mg/kg). The ED_{50} was estimated at 3.9 mg/kg. The maximal effect/cocaine maximal effect (ME/CME) was equal to 1.2 based on the maximum effect of mazindol and cocaine reference in Fig. 2, A.

A two-way analysis of variance conducted on horizontal activity counts/10 min indicated significant effects of treatment $F(6, 49) = 5.9$, $P < 0.001$, 10-min periods $F(47, 2303) = 51.1$, $P < 0.001$, and the interaction of those factors $F(282, 2303) = 7.2$, $P < 0.001$. A one-way analysis of variance conducted on $\log_{10}$ horizontal activity counts for the 10–40 min time period (maximal stimulant effect) indicated a significant effect of treatment $F(6, 49) = 4.7$, $P = 0.001$, and planned comparisons (a priori contrast) against the vehicle group showed a significant difference for 10 mg/kg (all P's < 0.05 denoted on Fig. 2 with an asterisk).

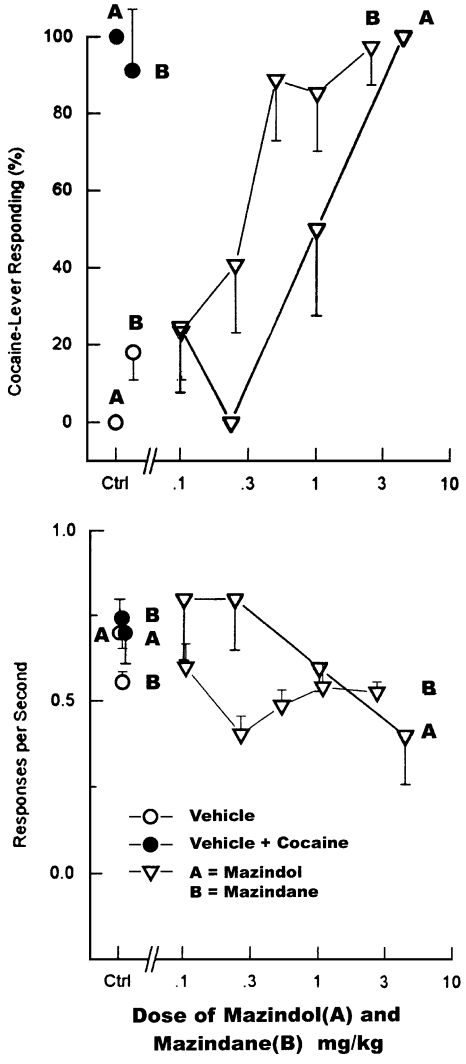

Fig. 5. The first reinforcer data show the substitution of mazindol and mazindane in male Sprague–Dawley rats trained to discriminate injection of cocaine (10 mg/kg) from saline. The upper panel shows the percent of responses emitted on the cocaine-lever for mazindol (A) and mazindane (B) as a function of dose for those subjects that received at least one reinforcer. The lower panel shows the response rates caused by mazindol (A) and mazindane (B) as a function of dose for all subjects tested. To the left of the axis break, control (Ctrl) data for vehicle alone (0.16% tartaric acid for mazindol (A) and 0.9% saline for mazindane (B)) and the training dose of cocaine (10 mg/kg) dissolved in these vehicles. Data for the substitution studies of mazindol and mazindane for the training dose of cocaine are shown to the right of the break.

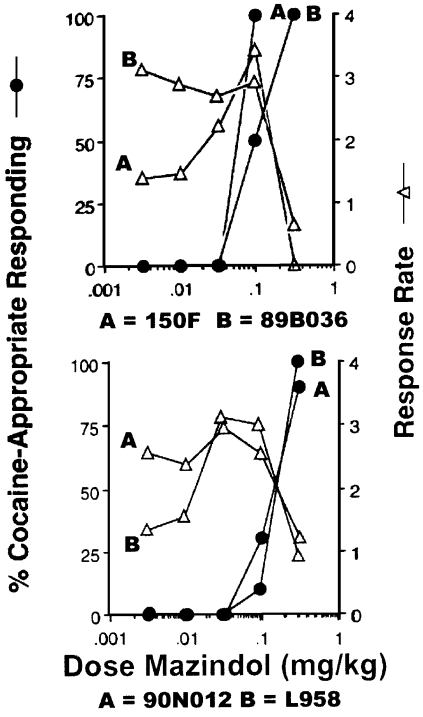

Fig. 6. Effects of mazindol in individual rhesus monkeys trained to discriminate 0.4 mg/kg cocaine from saline. Abscissae: dose of mazindol in mg/kg (log scale). Left ordinates: percent cocaine-appropriate responding for the entire cycle (filled circles). Right ordinates: response rate in responses/sec (open triangles). All dose–effect curves show data from a single determination.

Mazindane administered i.p. in 0.9% saline to mice at doses of 1, 2.5, 5, 10 and 25 mg/kg also resulted in a time-and dose-dependent stimulation at all doses except 1.0 mg/kg. Lethality occurred in 1/8 mice and convulsions lasting 10 min in 3/8 mice immediately following 25 mg/kg. Because of this, the 50 mg/kg dose, as given in mazindol, was not administered. Stimulant effects occurred within 10 min following injection, with maximum stimulation occurring at 5 and 10 mg/kg that lasted for ca. 280 min. The longest duration of stimulation (ca. 360 min), but weaker intensity, occurred at 25 mg/kg (Fig. 3). The period 50–80 min, where maximal stimulant effects first appeared as a function of dose, was selected for analysis of dose–response data as in cocaine. The mean average horizontal activity counts/10 min for this period were fit to a three-parameter logistic peak function of $\log_{10}$ dose (constant set to 1579, the mean of the 0.9% saline group) and the maximum effect estimated from the curve (maximum = 5127 counts/10 min at 8.8 mg/kg). The ED_{50} was estimated at 2.5 mg/kg. The maximal effect/maximal cocaine effect was equal to 1.5 based upon the maximum effect of mazindane and cocaine reference in Fig. 2 (B).

A two-way analysis of variance conducted on horizontal activity counts/10 min indicated significant effects of treatment $F(5, 41) = 14.3$, $P < 0.001$, 10-min periods $F(47, 1927) = 48.7$, $P < 0.001$, and the interaction of those factors $F(235, 1927) = 3.9$, $P < 0.001$. A one-way analysis of variance conducted on $\log_{10}$ horizontal activity counts for the 50–80 min time period (maximal stimulant effect) indicated a significant effect of treatment $F(5, 41) = 8.4$, $P < 0.001$, and planned comparisons (a priori contrast) against the vehicle group showed a significant difference for 2.5, 5, 10, and 25 mg/kg (all P's < 0.05 denoted in Fig. 3 with an asterisk).

3.3. Cocaine discrimination: rats

Mazindol and mazindane fully substituted for the discriminative stimulus produced by cocaine (10 mg/kg) in male Sprague–Dawley rats over the total session studies when administered at doses of 0.1–5.0 mg/kg for mazindol and 0.1–3.0 mg/kg for mazindane (Fig. 4A and B in upper panel). An ED_{50} of 1.3 mg/kg i.p. for mazindol and 0.33 mg/kg i.p. for mazindane was determined based on a linear regression of $\log_{10}$ doses of 0.25–5.0 mg/kg for mazindol and 0.1–1.0 mg/kg for mazindane. Maximum average response rate was decreased to 85% of vehicle (0.16% tartaric acid) control at 5.0 mg/kg for mazindol and to 63% vehicle (0.9% saline) control at 0.5 mg/kg for mazindane (Fig. 4C and D in lower panel). The results of the first reinforcer were in general accordance with the total session

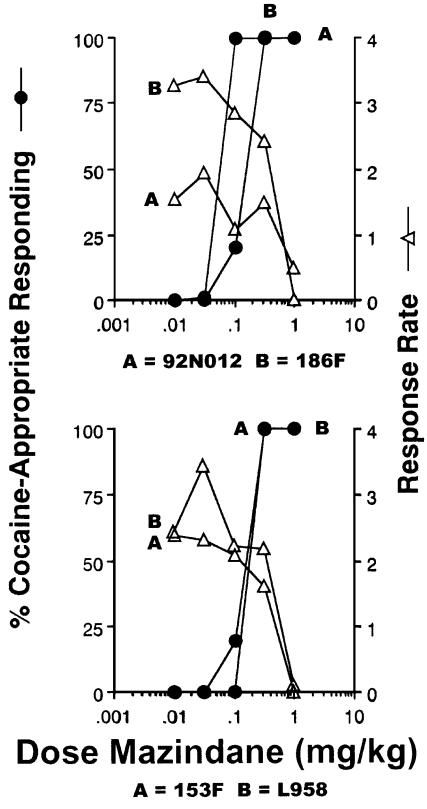

Fig. 7. Effects of mazindane in individual rhesus monkeys trained to discriminate 0.4 mg/kg cocaine from saline. Abscissae: dose of mazindane in mg/kg (log scale). Left ordinates: percent cocaine-appropriate responding for the entire cycle (filled circles). Right ordinates: response rate in responses per second (open triangles). Dose–effect curves show data from one determination in each monkey.

Table 4
Effects of mazindol and mazindane administered intramuscular (i.m.) in producing cocaine-appropriate responding in individual cocaine discriminating male rhesus monkeys

Monkey	ED_{50} mg/kg	
	Mazindol	Mazindane
150F	0.056	n.d.
89B036	0.10	n.d.
92N012	0.15	0.056
L 958	0.17	0.18
153F	n.d.	0.15
186F	n.d.	0.15

n.d. = not determined.

data with an ED_{50} of 1.2 mg/kg i.p. for mazindol and 0.29 mg/kg i.p. for mazindane (Fig. 5A and B in upper panel). Average response rate was decreased to 74% of vehicle maximum control by mazindol at 5.0 mg/kg and 61% for mazindane at 0.25 mg/kg (Fig. 5C and D in lower panel).

3.4. Cocaine discrimination: monkeys

The effects of mazindol (0.0032–0.32 mg/kg i.m.) and mazindane (0.01–1.0 mg/kg i.m.) on cocaine-appropriate responding and response rate for each of four male rhesus monkeys trained to discriminate cocaine (0.40 mg/kg) from saline is given in Figs. 6 and 7. Both compounds produced complete generalization to cocaine in all subjects. The ED_{50} values for producing cocaine appropriate responding in each monkey is given in Table 4.

Complete generalization to cocaine was seen at 0.1 mg/kg in one mazindol and one mazindane treated monkey. The remaining monkeys showed complete generalization at 0.32 mg/kg.

Mazindol had no effects on response rate at 0.1 mg/kg in two monkeys. Increased response rate occurred at intermediate doses of 0.032–0.1 mg/kg in the other two monkeys and all subjects showed a marked decrease at 0.32 mg/kg. Small reduction in response rate was seen at 0.1–0.32 mg/kg doses of mazindane, while the 1.0 mg/kg dose resulted in substantial rate decreasing effects in all four monkeys. Both mazindol and mazindane did not produce noticeable behavioral effects in any monkey over the tested dose ranges.

4. Discussion

The present study focused on the pharmacology of mazindane, a pro-drug form of mazindol, has shown that it exhibits a similar in vivo profile of activity in mouse, rat and monkey assays designed to detect compounds with cocaine-like properties that might be useful in the treatment of cocaine abuse. A summary of the ED_{50} values and other relevant effects seen in these assays is given in Table 5. The in vitro effect of these compounds on the inhibition of ligand binding and uptake at monoamine transporters and at a series of neurotransmitters was also determined (Tables 1 and 2).

Although i.p. treatment of mice with either mazindol or mazindane caused a time-dependent stimulation that occurred ca. 10 min post injection and resulted in similar doses needed for maximum stimulation (7.1 vs. 8.8 mg/kg) and ME/CME (1.2 vs. 1.5) values, there was significant differences in responses at comparable doses. At doses between 2.5 and 25 mg/kg, mazindane caused ca. two-fold longer duration of stimulation than mazindol (Fig. 3). However, the data for the 25 mg/kg dose of mazindane should be used with caution since 3/8 of the mice exhibited convulsions and 1/8 lethality immediately after dosing. The cause of this toxicity is not obvious. One possibility is that the higher dose results in a slower metabolic conversion to the less toxic mazindol and that mazindane reached the brain in sufficient amount to induce convulsions and lethality.

Inspection of the 2.5–10 mg/kg results for mazindol reveals an effect-time profile similar to cocaine with maximum stimulation occurring within 0.5 h after dosing followed by a gradual decrease to vehicle level for the remainder of the study. The 25 mg/kg, and particularly the 50 mg/kg dose of mazindol, exhibited a bi-phasic pattern of stimulation with a maximum occurring ca. 10 min and then rapidly dropping to vehicle level in 20–40 min. This was then followed by a second, but weaker stimulation, after ca. 1–2 h that lasted ca. 3 h. Mazindane did not display a similar pattern of stimulation at any dose studied. A possible cause of this "double maximum" seen with mazindol, especially at 50 mg/kg, is that an active metabolite is formed in sufficient concentration to be detected. Failure to detect the presence of this possible metabolite at lower doses (<25 mg/kg) suggests that it is a weaker stimulant than mazindol.

Previously reported studies (Witkin et al., 1991; Mansbach and Balster, 1993) that mazindol produced a dose-dependent increase in cocaine appropriate responding and

Table 5
Comparison of ED_{50} values for mazindol and mazindane in mouse locomotor activity and cocaine discrimination in rats and monkeys

Assay	ED_{50}, mg/kg		Ratio, mazindol/mazindane
	Mazindol	Mazindane	
Locomotor activity (8 h): mice[a], i.p.	3.9	2.5	1.56
Cocaine discrimination: rats, i.p.			
Total session[b]	1.3	0.33	3.94
First reinforcer[c]	1.2	0.29	4.14
Cocaine discrimination: monkeys, i.m.	0.119[d]	0.134[d]	0.89

[a] Maximum stimulation at 7.1 mg/kg and ME/CME = 1.2, for mazindol and 8.8 mg/kg and 1.5, for mazindane.

[b] Maximum response rate decreased to 85% vehicle (0.16% tartaric acid) at 5 mg/kg for mazindol and 63% vehicle (0.9% saline) at 0.5 mg/kg for mazindane.

[c] Maximum response rate decreased to 74% vehicle (0.16% tartaric acid) at 5.0 mg/kg for mazindol and 61% vehicle (0.9% saline) at 0.25mg/kg for mazindane.

[d] Average values of four subjects treated with mazindol (0.056–0.17 mg/kg) or mazindane (0.056–0.18 mg/kg).

fully substituted in male Sprague–Dawley rats trained to discriminate cocaine from saline was confirmed in this study. Mazindane showed similar results, but was four times more potent than mazindol in both the total session and first reinforcer assays (Table 5). The decrease in maximum response rate was also greater for mazindane and occurred at a 13-fold lower dose than mazindol.

The present drug discrimination studies confirmed the earlier findings (Kleven et al., 1990) that mazindol produced a dose-dependent response and complete substitute for cocaine in male rhesus monkeys. The potencies and profiles of mazindol and mazindane were very similar and both showed complete substitution with almost identical average ED_{50} values for producing cocaine appropriate responding and a non-linear dose–effect on response rates. No noticeable behavioral effects were produced in any of the monkeys over the tested dose ranges. This is in contrast to behavioral changes found when mazindol was self-administered to rhesus monkeys at similar dose ranges (Corwin et al., 1987) or rhesus trained to self-administer cocaine (Mansbach and Balster, 1993). The 10-fold difference in activity of mazindol in cocaine discrimination assays in rats and rhesus monkeys is similar to the anorexic ED_{50} values where it is 31 and 16 times weaker in the rat than in the squirrel or capuchin monkey, respectively (Aeberli et al., 1975).

The variation in radioligand displacement at the various transporter and transmitter sites is likely due to the ability of the membranes used in each assay to oxidize mazindane to mazindol and also to the duration and temperatures (Tables 1 and 2). It appears that rat and guinea pig membranes possess more oxidative power than the bovine membranes or NIE-115 cells. Those assays (alpha α_2, beta β, 5-HT_1 and 5-HT_2) carried out on the same membranes (rat cortical) differ only by 10–20% at 10^{-5} M suggesting that similar amounts of mazindol was produced in each assay. Other assays using rat and guinea pig membranes differ by a low 4% (M_3) to a high of 49% (H_2) at 10^{-5} M. The greatest variation in activity occurred with those assays using bovine membranes (H_1, M_1 and 5-HT_{1A}) and NIE-115 cells (5-HT_3). If the increase in activity seen in these bovine membranes and NIE-115 cells is due to the incomplete conversion to mazindol, it would appear that mazindane is a more effective inhibitor of binding at these, and possibly, other sites.

In an attempt to determine the activity of mazindane at the DAT varying amounts of SKF-525A, a substance known to cause general blockage of hydroxylation (Netter, 1980), was added to the [^{3}H] WIN 35,428 binding assay at rat brain membranes in the presence of fixed concentrations of mazindane with mazindol as a control. The findings (Table 3) indicate that SKF-525A interacts with the WIN binding site at the DAT and cannot be used as an anti-oxidant in assays involving the DAT.

In conclusion, the current results confirm that the profile of in vivo activity of mazindane is very similar, especially in monkeys, to mazindol. In addition, the previous reports on the discriminate effects of mazindol in rats and monkeys has been confirmed and extended to mice. Mazindane·H_2SO_4, because of its enhanced water solubility and stability relative to mazindol, could serve as a pharmacological tool in assays where the use of mazindol is limited because of solubility.

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