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Rewarding properties of the stereoisomers of 4-methylaminorex: Involvement of the dopamine system

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Abstract

4-Methylaminorex is a potential psychostimulant drug of abuse that exists as four stereoisomers: *cis*-4*R*,5S, *cis*-4*S*,5*R*, *trans*-4*S*,5*S*, and *trans*-4*R*,5*R*. The racemic mixture of the *cis*-isomers has been encountered in illicit samples, but previous animal studies suggest that also the *trans*-isomers could have similar stimulant-like properties. We tested whether the stereoisomers possess rewarding properties and compared their potency using the conditioned place preference method in rats. Furthermore, the involvement of the brain dopaminergic system in the 4-methylaminorex reward was tested with the dopamine D1- and D2-receptor antagonists SCH 23390 and raclopride administered systemically, or with the neurotoxin 6-hydroxydopamine injected into the nucleus accumbens. All the four isomers induced place preference, with no apparent differences in their potency. SCH 23990 and raclopride attenuated 4-methylaminorex-induced increase in place preference, and 6-hydroxydopamine also tended to be efficacious. These findings indicate that all the four stereoisomers of 4-methylaminorex possess rewarding properties and thus abuse potential; the *trans*-isomers are at least as potent as the *cis*-isomers. Furthermore, the brain dopaminergic system appears to be involved in the 4-methylaminorex-reward.

Keywords: Accumbens; Dopamine; 6-Hydroxydopamine; Isomer; 4-Methylaminorex; Place preference; Psychostimulant; Raclopride; Rat; Reward; SCH 23390

1. Introduction

The phenylisopropylamine derivative 4-methylaminorex (2-amino-4-methyl-5-phenyl-Δ^2-oxazoline) is a sympathomimetic agent that exists as four stereoisomers: *cis*- 4*R*,5*S*, *cis*-4*S*,5*R*, *trans*-4*R*,5*R*, and *trans*-4*S*,5*S*. 4-Methylaminorex has been found on the clandestine market with the street names "U4Euh" and "Ice", with the racemic mixture of the *cis*-isomers being reportedly the most often encountered form (Davis and Brewster, 1988; Klein et al., 1989; Gaine et al., 2000). The involvement of 4-methylaminorex abuse has been described in an overdose fatality (Davis and Brewster, 1988) and more recently in a case series of patients suffering from pulmonary hypertension (Gaine et al., 2000). Although the current extent of 4-methylaminorex abuse is not known, users' recent experiences, opinions and recommendations regarding its effects, dosage, intake manners and synthesis methods can be found on drug culture-related Web sites (e.g., http://www.erowid.org or https://www.the-hive.ws), which illustrates that the drug is a subject of ongoing interest and further highlights its abuse potential. These manifestations of recreational abuse are in line with the abuse potential suggested by animal studies. After training with cocaine, racemic mixture of *cis*-4-methylaminorex has showed similar discriminative properties in rats (Young and Glennon, 1993) and maintained self-administration in nonhuman primates (Mansbach et al., 1990).

In reports concerning with the stereoisomers of 4-methylaminorex, both the *cis*- and *trans*-forms have

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possessed properties typical for a psychostimulant drug: each isomer shared the discriminative stimulus with *S*-amphetamine (Glennon and Misenheimer, 1990), induced locomotor activity and stereotyped behavior (Batsche et al., 1994), suppressed the basal firing rate of A10 cells (Ashby et al., 1995) and in our recent study elevated the dopamine and serotonin levels in the nucleus accumbens along with behavioral activation (Kankaanpää et al., 2002). In all the aforementioned studies, the rank order of potency between the isomers was as the following: *trans*-4*S*,5*S* > *cis*-4*R*,5*S* ≈ *cis*-4*S*,5*R* > *trans*-4*R*,5*R*. Thus, these findings imply that not only the *cis*-isomers but also the *trans*-isomers may possess rewarding properties, with the *trans*-4*S*,5*S*-isomer being possibly even more potent than the others.

Given that 4-methylaminorex possesses amphetamine-like psychostimulant properties, and that it can be readily synthesized according to instructions freely available, the compound can be considered a potential alternative among the drugs of abuse. Although the racemic *cis*-4-methylaminorex appears to be the most often encountered form in illicit samples, previous animal studies suggest that also the *trans*-isomers could possess abuse potential. The aim of this study was to evaluate whether all the stereoisomers of 4-methylaminorex possess rewarding properties and to compare their potency with each other in the conditioned place preference method. In addition, because the brain dopaminergic system is considered important in the drug-reward (Bardo, 1998), we tested the effects of the dopamine receptor blockade with the antagonists SCH 23390 (dopamine D1-like receptors; Bourne, 2001) and raclopride (D2-like receptors; Hall et al., 1988), or the depletion of the dopaminergic innervation in the nucleus accumbens with the neurotoxin 6-hydroxydopamine (6-OHDA; Breese and Taylor, 1971) on the 4-methylaminorex-reward.

2. Materials and methods

2.1. Animals

Adult male Han:Wistar rats weighing 200–300 g were used in the study. The rats were obtained from Harlan Nederland B.V., Horst, the Netherlands, at least 1 week prior to the experiments, and they were housed two per Macrolon III-type cage (18 × 33 × 15 cm) in a temperature-controlled room (22 ± 2 °C) with a light cycle of 12 h. The lights were on from 8:00 a.m. to 8:00 p.m., during which time all the experiments were conducted. The animals had free access to standard laboratory chow and tap water. The local institutional animals care and use committee, or the chief veterinarian of the county administrative board approved the experiments, and they were conducted in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Drugs

The four optical stereoisomers (*trans*-4*R*,5*R*, *trans*-4*S*,5*S*, *cis*-4*R*,5*S*, and *cis*-4*S*,5*R*) of 4-methylaminorex were prepared as free bases in the Laboratory of Organic Chemistry, University of Helsinki, Helsinki, Finland, by using the synthesis methods described by Poos et al. (1963) and Klein et al. (1989). Identity of the isomers was confirmed by determining their melting points and rotation angles, as well as ^{1}H NMR and ^{13}C NMR spectra. The rotation angles [α] were as follows: −238.2 for *cis*-4*S*,5*R*, +246.2 for *cis*-4*R*,5*S*, +6.2 for *trans*-4*S*,5*S*, and −7.6 for *trans*-4*R*,5*R*, which are in line with those reported by Klein et al. (1989). The isomers were dissolved in a small volume of saline (0.9% NaCl) acidified with a drop of glacial acetic acid. The pH was then adjusted to physiological level with 2 M NaOH, and the solution was brought to volume with saline.

SCH 23390 hydrochloride (RBI, D 054, Natick, MA, USA), raclopride L-tartrate (RBI, R 121), pargyline hydrochloride (RBI, D 026), desipramine hydrochloride (Sigma-Aldrich, D-3900, Steinheim, Germany) were dissolved in saline. Buprenorphine used was manufactured for medical purposes (Temgesic, Reckitt and Colman, Hull, UK). Drug doses were calculated as free bases, and the drugs were administered intraperitoneally at a volume of 1 ml/kg, except for buprenorphine that was given subcutaneously.

The dopaminergic neurotoxin 6-OHDA hydrochloride (H-4381) for intra-accumbal injections, and the reference standards dopamine hydrochloride, 5-hydroxytryptamine (serotonin) creatinine sulfate, norepinephrine hydrochloride for chemical assay were purchased from Sigma (St. Louis, MO). Other chemicals were of analytical grade and were supplied by Merck (Darmstadt, Germany) or Sigma.

2.3. Conditioned place preference

The place preference experiments were conducted essentially using the apparatus and procedure as described previously (Meririnne et al., 2001). In this method, animals are trained to associate a distinct environment with effects of a drug; if they show preference to the environment after the training, the drug is generally considered to possess rewarding properties and, thus, abuse potential (Carr et al., 1989).

2.3.1. Apparatus

The conditioned place preference test was conducted in eight identical rectangular boxes (60 × 30 × 45 cm) made of polyvinyl chloride. Each box was divided into two compartments of equal size by a separating wall with a guillotine door (8 × 6 cm). Both compartments were covered with loose-fitting transparent plastic lids. One compartment was black with a smooth floor and small drops (approximately 7.5 μl) of 3% acetic acid added in both back corners, and the other was white with wire mesh on the floor and no acetic acid added. Thus, the compartments differed in three modalities: visual, tactile, and olfactory. The boxes were placed in a quiet dim

room (approximately 0.05 lx above the boxes) with white noise present for masking external sounds.

2.3.2. Place preference procedure

The place preference procedure consisted of three phases:

(1) Preconditioning phase (days 1–3): The guillotine door of the box was open, and the rats were equitably placed in the black or white compartment for exploring freely both compartments for 15 min (900 s). On the third day the times spent in both compartments were measured with a stopwatch. According to this preconditioning time, the rats were assigned to treatment groups with the less-preferred compartment serving as the drug-paired compartment. If the preconditioning time for a rat was less than 180 s in either compartment, the rat was excluded from further testing. In each treatment group, approximately half of the rats were assigned to the black compartment as the drug-paired compartment, while the other half were assigned to the white compartment as the drug-paired compartment (±1 rat when $n \leq 10$; ±2 rats when $n > 10$).

(2) Conditioning phase (days 4–6): During the conditioning phase the guillotine door was closed. The rats experienced two conditioning sessions each day. In the first session, the rats received one or two injections of vehicle, after which they were immediately confined to the vehicle-paired compartment (opposite the drug-paired compartment) for 40 min. After an interval of at least 90 min, the second session of the day began; the rats received one or two injections of drugs or vehicle, after which they were immediately confined to the drug-paired compartment for 40 min.

(3) Postconditioning phase (day 7): The guillotine door was opened, the rats were placed in the drug-paired compartment, and they were allowed to move freely between the compartments. The time the rats spent in

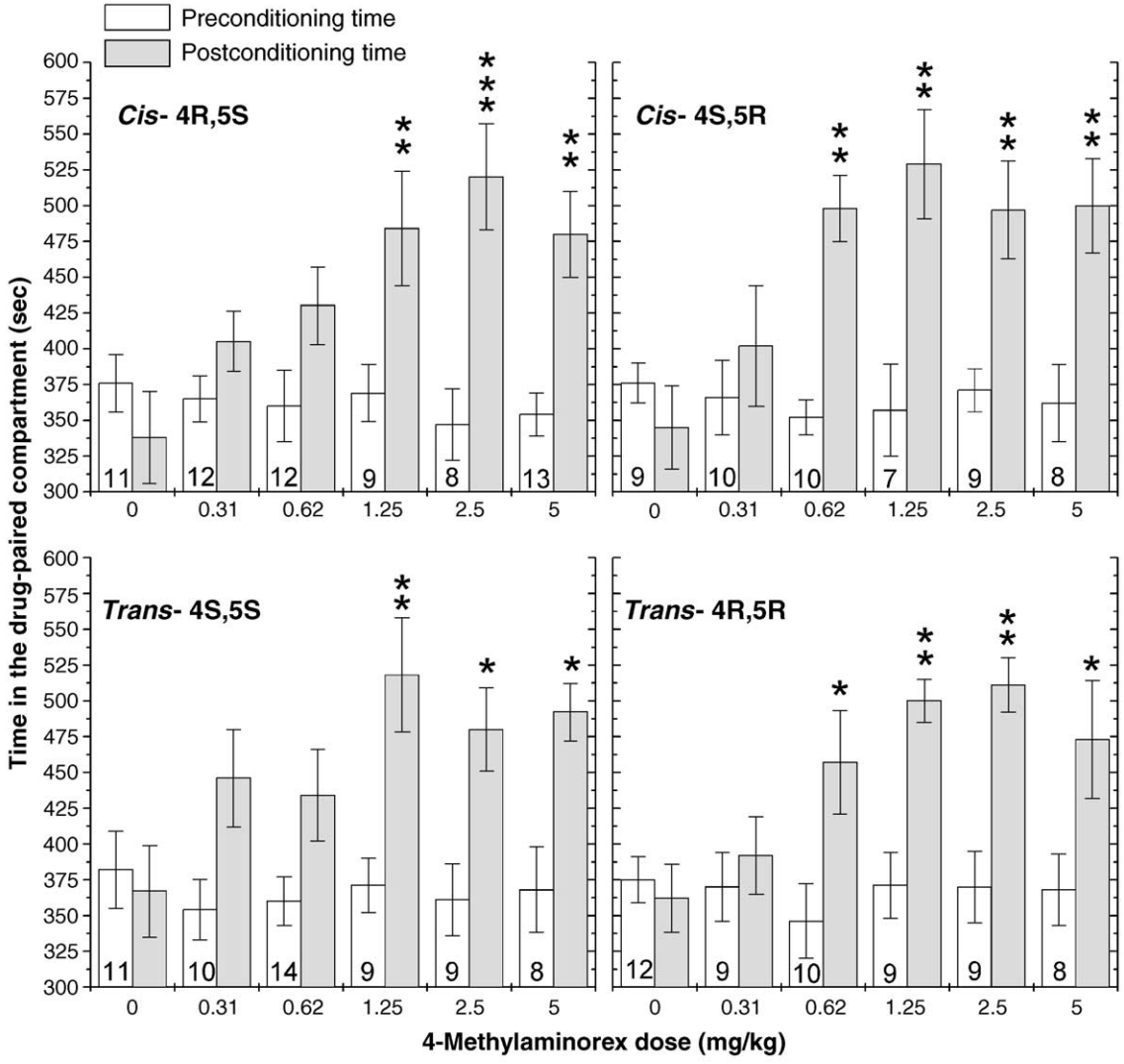

Fig. 1. Increased place preference induced by the stereoisomers of 4-methylaminorex. The numbers within the 'preconditioning' columns refer to the number of animals in the test group. *$p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ when compared to the corresponding vehicle group, Bonferroni's test.

the drug-paired compartment (postconditioning time) was measured for 15 min (900 s). Shift in preference for the drug-paired compartment induced by conditioning (postconditioning time − preconditioning time) served as the measure of reward.

2.3.3. Treatments

2.3.3.1. Place preference induced by the stereoisomers of 4-methylaminorex. The isomers were injected at doses of 0.31, 0.62, 1.25, 2.5 and 5 mg/kg. Higher doses were considered inappropriate for the place preference test, because in our previous study, the 10-mg/kg dose of *trans*-4*S*,5*S*- or the *cis*-isomers produced severe dysfunction in behavior, such as catatonia (Kankaanpää et al., 2002), and some animals even died. The isomers were normally injected just prior to placement in the drug-paired compartment. Some previous studies, however, have shown that the onset of behavioral activation by *trans*-4*R*,5*R*-isomer may be more delayed (45–60 min) than that of the other isomers (15 min) (Glennon and Misenheimer, 1990; Batsche et al., 1994). This could attenuate the ability of *trans*-4*R*,5*R*-isomer to induce place preference. We ran therefore a separate test with the 60-min interval between the injection of *trans*-4*R*,5*R*-isomer and placement in the compartment. The test dose was the highest *trans*-4*R*,5*R*-isomer dose that was ineffective when administered without an interval. If this test dose is still ineffective after the 60-min interval, it appears unlikely that the use of the interval would markedly enhance the effect of *trans*-4*R*,5*R*-isomer.

2.3.3.2. Effects of the dopamine receptor antagonists. During the conditioning phase, dopamine receptor antagonist injections preceded the injection of 4-methylaminorex in the second session. *Trans*-4*S*,5*S*- and *cis*-4*S*,5*R*-isomers at the dose of 1.25 mg/kg were chosen because they reliably induced increase in place preference. Dopamine D1-like receptor antagonist SCH 23390 (0.2 or 0.4 mg/kg) or D2-like receptor antagonist raclopride (0.8 mg/kg) were administered 40 min or 10 min prior to 4-methylaminorex, respectively. According to previous studies, these doses and intervals should be behaviorally relevant (Ågmo et al., 1993; Hoffman and Donovan, 1995; Meririnne et al., 2001). For example, 0.2 mg/kg of SCH 23390 with a 40-min interval, and 0.5 mg/kg of raclopride with a 15-min interval have attenuated place preference induced by the psychostimulants amphetamine or methylphenidate (Hoffman and Donovan, 1995; Meririnne et al., 2001). In particular, the possible affinity of SCH 23390 for serotonin 2 receptors appears to be of no significance at these doses (Bischoff et al., 1986).

2.3.3.3. Effects of the dopaminergic lesion in the nucleus accumbens. First, the rats underwent surgery and received bilateral intra-accumbal injections of 6-OHDA or corresponding vehicle (see below). At least 1 week from the surgery, after the rats had gained full recovery with normal behavior and body weight, the place preference test was conducted as described above: during the conditioning phase, *cis*-4*S*,5*R*-isomer (1.25 mg/kg) or vehicle was administered.

2.4. Surgery and assay of monoamines in brain tissue

The surgery and assay were conducted essentially as described previously (Koistinen et al., 2001). The rats were anesthetized with halothane (4% for 5 min, and then 1.5–2.5% during the operation) and attached to the stereotactic frame. Bilateral injections of 6-OHDA (8 μg/2 μl, calculated as free base), dissolved in 0.1% ascorbic acid in saline, were injected into the nucleus accumbens with a 10-μl microsyringe (Hamilton, No. 701, Reno, NV) attached to a microinjector (Model 5000 Microinjection Unit, David Kopf, Tujunga, CA). The injection was given over 4 min at the rate of 0.5 μl/min, and the needle was left in place for a further 5 min for diffusion before being withdrawn from the brain. The following coordinates were used: AP=+1.7, L=±1.5, DV=−7.0 (Paxinos and Watson, 1998). The controls received the vehicle only. All the rats were pretreated with the norepinephrine uptake inhibitor desipramine (25 mg/kg) and the monoamine oxidase inhibitor pargyline (50 mg/kg) 30 min before the application of 6-OHDA. These compounds have been reported to minimize the destruction of norepinephrine neurons and augment the neurotoxic action of 6-OHDA,

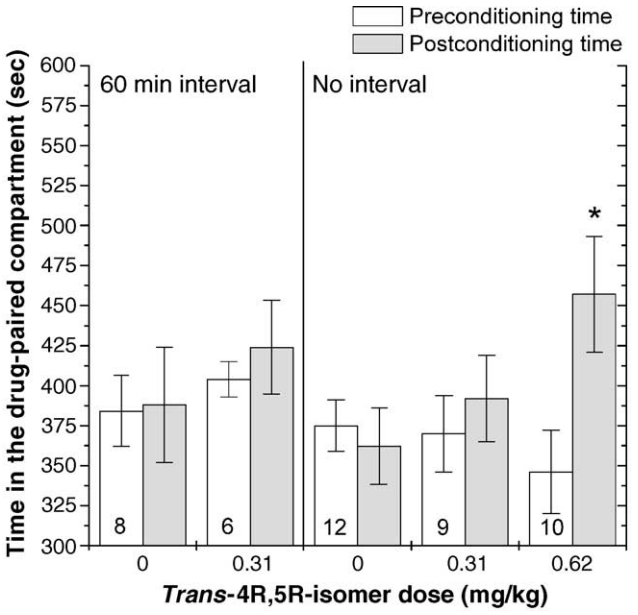

Fig. 2. *Trans*-4*R*,5*R*-induced increase in place preference is not enhanced after the interval of 60 min. The interval was kept between the injection and placement in the compartment. *Trans*-4*R*,5*R*-induced increase in preference without the use of interval (Fig. 1) is re-shown for comparison. The numbers within the 'preconditioning' columns refer to the number of animals in the test group. * $p<0.05$ when compared to the group receiving only vehicle, Bonferroni's test.

respectively (Breese and Taylor, 1971). The rats were administered buprenorphine (0.15 mg/kg) immediately after the surgery and during the next days if normal behavior was impaired. If body weight was lost considerably after the surgery, the rats were given food and water by an oral tube to aid recovery.

After completion of the place preference experiment, the rats were decapitated, and brains were removed and stored at −75 °C. The frozen coronal sections (1000 μm thick) were cut out approximately from AP+2.2 to +1.2, and the area of the nucleus accumbens and striatum were punched out with stainless steel punches, with a diameter of 1.2 mm for the nucleus accumbens and 2.0 mm for the striatum. The samples were sonicated for 2× 3 s in 200 volumes of 0.1 M perchloric acid containing 0.019% ethylenediaminetetraacetic acid (EDTA) (Branson Sonic Power Company, Danbury, CT). After centrifugation for 5 min at 4 °C and 6500×*g* (Heraeus, Osterode, Germany), the samples were filtered with a PVDF syringe filter with 0.45-μm pores (Gelman Sciences, Ann Arbor, MI).

The concentrations of dopamine, norepinephrine and serotonin in the tissue samples were analyzed with high-performance liquid chromatography with electrochemical detection. The system consisted of a Hewlett-Packard 1100 isocratic pump (Palo Alto, CA) with degasser unit, an

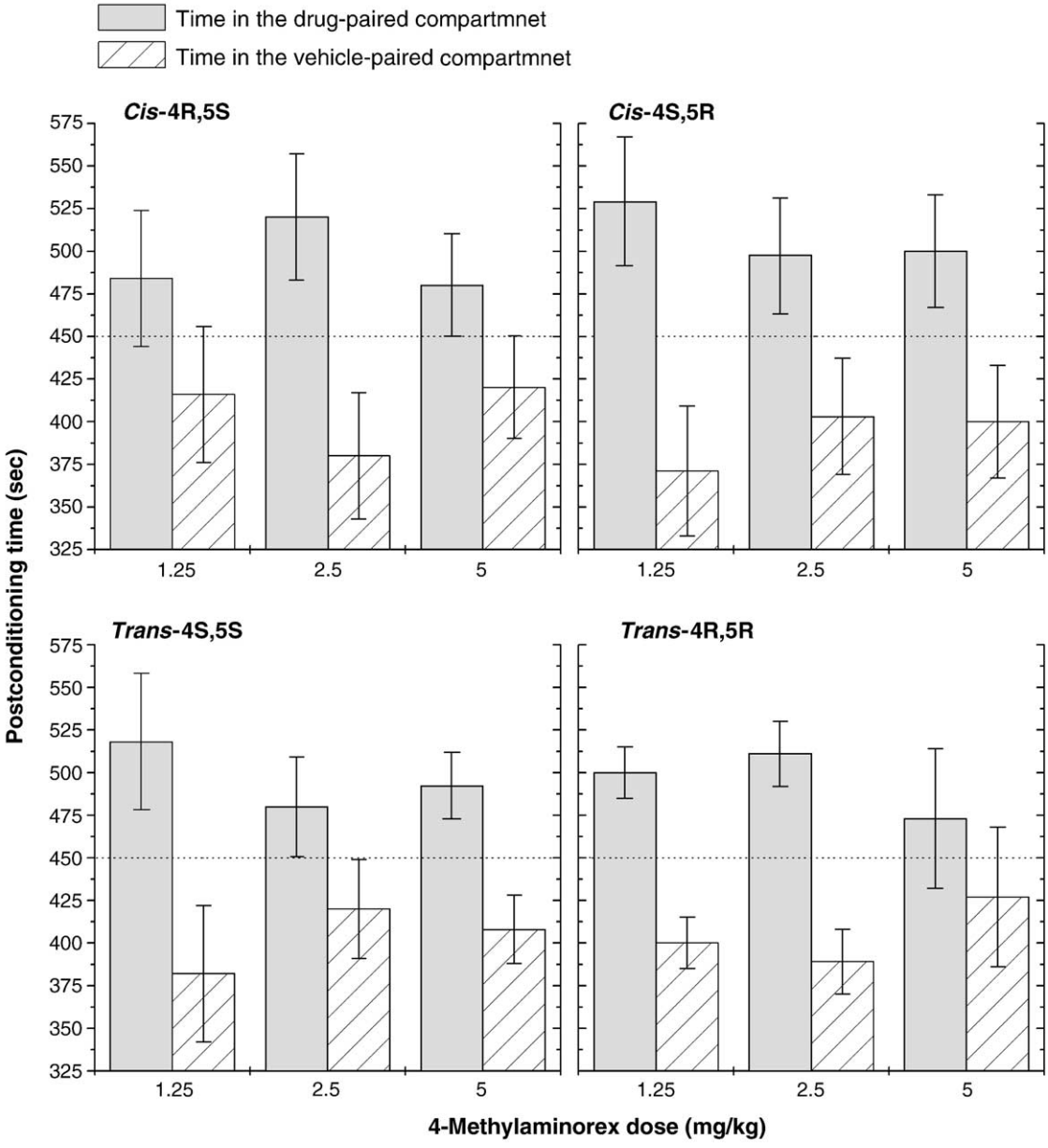

Fig. 3. Place preference induced by the stereoisomers of 4-methylaminorex. ANOVA for repeated measure showed that within these doses, the postconditioning time was greater in the drug-paired compartment than in the vehicle-paired compartment: $p=0.041$ for *cis*-4*R*,5*S*; $p=0.009$ for *cis*-4*S*,5*R*; $p=0.018$ for *trans*-4*S*,5*S*; $p=0.008$ for *trans*-4*R*,5*R*. For numbers of animals in the groups, see Fig. 1.

Agilent Technologies 1100 Series thermostatic autosampler (Palo Alto, CA) and an amperometric detector INTRO (Antec Leyden B.V., Laiden, the Netherlands) with a glassy carbon VT-03 cell. The glassy carbon working electrode was set to 700 mV vs. the Ag/AgCl reference electrode. The column was MIC 15-3-C18 with particle size of 3 μm (Inertsil, 150 × 1.0 mm inner diameter, LC-Packings, Amsterdam, the Netherlands). The mobile phase was 10% (v/v) methanol in 0.05 M phosphate/citrate, 0.15 mM EDTA, and 0.17 mM octyl sulfonate at pH 4.7. The solution was filtered through a PVDF filter with 0.45-μm pores (Millipore Corporation, Bedford, MA). The flow rate of the mobile phase was 40 μl/min, and the injection volume was 10 μl. The chromatograms were acquired and processed

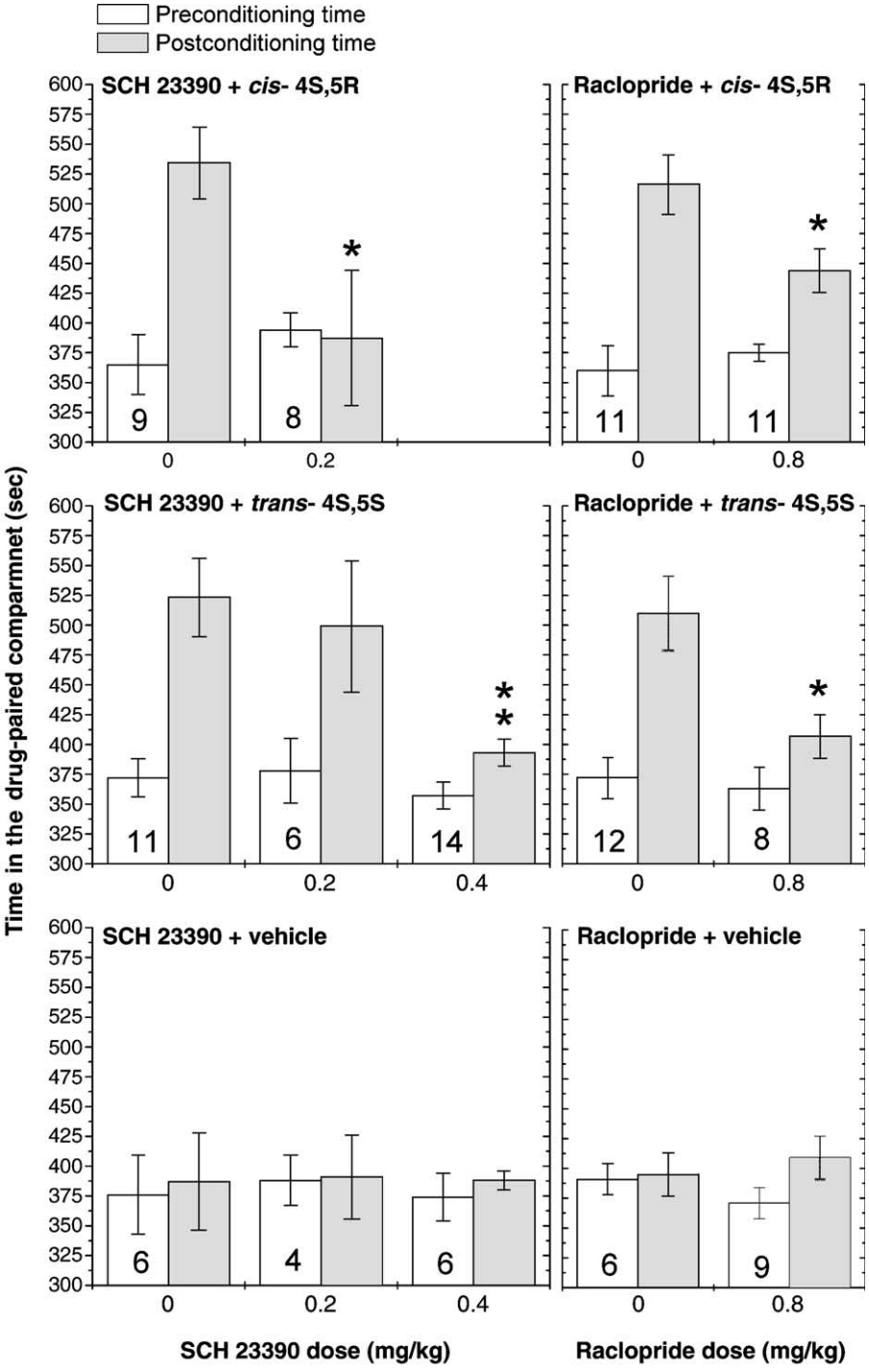

Fig. 4. 4-Methylaminorex-induced (1.25 mg/kg of *cis*-4*S*,5*R*- or *trans*-4*S*,5*S*-isomer) increase in place preference is attenuated by the dopamine D1- or D2-receptor blockade (SCH 23390 and raclopride, respectively). The numbers within the 'preconditioning' columns refer to the number of animals in the test group. * $p<0.05$ and ** $p<0.01$ when compared to the group receiving the isomer without antagonist, Bonferroni's test.

with Waters 820 Maxima software, version 3.31 (Waters Association, Milford, MA).

2.5. Statistics

Statistical analyses were performed using ANCOVA (dependent factor: shift in preference; covariant: preconditioning time). For the overall comparison between the isomers, two-way ANCOVA (independent factors: isomers and doses) was used. In addition, a quantal dose–response curve for each isomer was calculated; the response was determined as a proportion of rats (in percentage) that show actual place preference, i.e., the postconditioning time over 450 s (see below). The ascending part of this curve was subjected to probit analysis to obtain the median effective dose (ED_{50}).

Because the isomers were paired with the less-preferred compartment, an increase in preference (postconditioning time > preconditioning time) does not necessarily reflect actual place preference (postconditioning time in the drug-paired compartment > postconditioning time in the vehicle-paired compartment; i.e., postconditioning time in the drug-paired compartment > 450 s). Increase in preference without place preference may occasionally result from drug-induced reduced aversion to the less-preferred (drug-paired) compartment, instead of truly rewarding properties. In order to assess whether the isomers are able to induce place preference, we ran a separate analysis using ANOVA for repeated measure that allows a within-subject analysis between the compartments (dependent factors: postconditioning times in the drug- and vehicle paired compartments; independent factor: dose).

Effects of the dopaminergic manipulations were tested with one-way ANCOVA (independent factor: dose of antagonist or lesion status) followed by Bonferroni's test adjusted for appropriate number of comparisons.

Finally, paired and two-sample *t*-tests were used when appropriate. Unless otherwise stated, all data are expressed as mean ± S.E.

3. Results

3.1. Place preference induced by the stereoisomers of 4-methylaminorex

When the effect of the isomers were assessed separately, all of them induced increase in place preference (Fig. 1): each isomer showed significant difference between the dose [$F(5,58)=5.4$, $p<0.001$ for *cis*-4*R*,5*S*; $F(5,46)=5.0$, $p=0.001$ for *cis*-4*S*,5*R*; $F(5,54)=3.1$, $p=0.015$ for *trans*-4*S*,5*S*; $F(5,50)=5.6$, $p<0.001$ for *trans*-4*R*,5*R*; one-way ANCOVA]. The isomers appeared to be equipotent in inducing place preference. The overall analysis between the isomers with two-way ANCOVA showed neither difference between the isomers [$p=0.751$] nor the isomer × dose interaction [$p=0.879$], although the effect of the dose was significant [$F(5,211)=33.6$, $p<0.001$]. Furthermore, ED_{50}s with 95% confidence limits were overlapping between the isomers ($n=4–6$ doses): 0.80 mg/kg (0.50–1.57 mg/kg) for *cis*-4*R*,5*S*, 0.44 mg/kg (0.13–0.74 mg/kg) for *cis*-4*S*,5*R*, 1.11 mg/kg (0–2.5 mg/kg) for *trans*-4*S*,5*S*, and 0.48 mg/kg (0.21–0.79 mg/kg) for *trans*-4*R*,5*R*.

The 60-min interval did not enhance *trans*-4*R*,5*R*-induced increase in place preference (Fig. 2). When tested without the interval, the dose of 0.31 mg/kg was the highest ineffective dose. After the use of the interval, this test dose was still ineffective [$F(1,11)=0.109$, $p=0.748$, ANCOVA], which indicates that no marked enhancement resulted from the use of the 60-min interval.

In addition to increased preference, all the isomers were also able to induce actual place preference (Fig. 3). When each isomer was tested statistically at the doses of 1.25, 2.5 and 5 mg/kg, the postconditioning time in the drug-paired compartment was greater than the corresponding time in the vehicle-paired compartment [$F(1,27)=4.6$, $p=0.041$ for *cis*-4*R*,5*S*; $F(1,21)=8.4$, $p=0.009$ for *cis*-4*S*,5*R*; $F(1,23)=6.5$, $p=0.018$ for *trans*-4*S*,5*S*; $F(1,23)=8.6$, $p=0.008$ for trans-4*R*,5*R*; ANOVA for repeated measure]. There were neither differences between the doses nor the dose × compartment interaction.

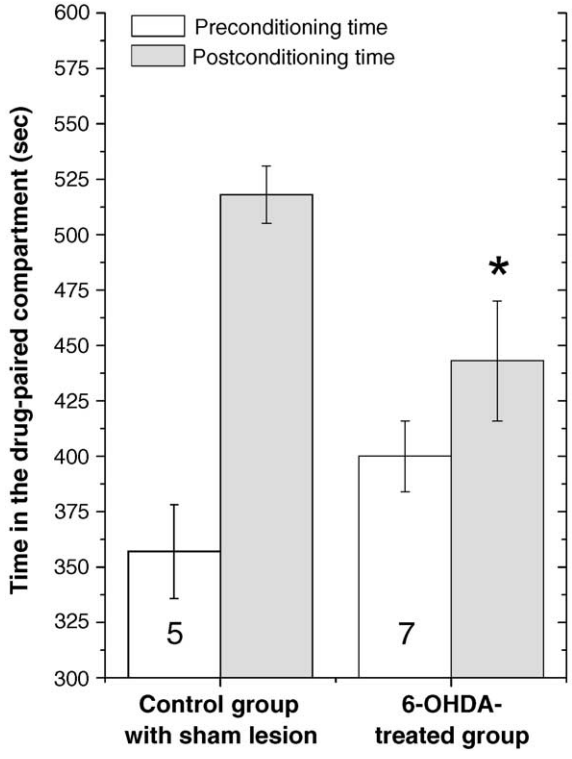

Fig. 5. Dopaminergic lesion in the nucleus accumbens by 6-OHDA attenuates 4-methylaminorex-induced (1.25 mg/kg of *cis*-4*S*,5*R*-isomer) increase in place preference. The numbers within the 'preconditioning' columns refer to the number of animals in the test group. * $p=0.053$ when compared to the control group with sham lesion, Bonferroni's test.

3.2. Effects of the dopamine receptor antagonists

SCH 23390 and raclopride attenuated increase in place preference induced by the isomers *cis*-4*S*,5*R* [$F(1,14)=7.042$, $p=0.0189$ for SCH 23390; $F(1,19)=7.218$, $p=0.0146$ for raclopride; ANCOVA] or *trans*-4*S*,5*S* [$F(2,27)=7.075$, $p=0.003$ for SCH 23390; $F(1,17)=14.148$, $p=0.002$ for raclopride] (Fig. 4). A higher dose of SCH 23390 was needed with *trans*-4*S*,5*S* than with *cis*-4*S*,5*R* (0.4 vs. 0.2 mg/kg, respectively).

Neither SCH 23390 nor raclopride intraperitoneally induced place preference or aversion [$F(2,12)=0.08$, $p=0.924$ for SCH 23390; $F(1,12)=0.405$, $p=0.537$ for raclopride; ANCOVA] (Fig. 4). We have previously shown that raclopride at the similar dose of 0.8 mg/kg can, somewhat unexpectedly, induce increase in preference (Meririnne et al., 2001). In that study, however, the antagonist was given subcutaneously, which is a more potent route of administration than the intraperitoneal one, presumably due to a rapid first-pass metabolism of raclopride in the rat (Wadenberg and Ahlenius, 1991).

3.3. Effects of the dopaminergic lesion in the nucleus accumbens

Dopaminergic lesion in the nucleus accumbens by 6-OHDA seemed to attenuate the increase in place preference induced by *cis*-4*S*,5*R*-isomer [$F(1,9)=4.95$, $p=0.053$; ANCOVA] (Fig. 5). In support of this, *cis*-4*S*,5*R* induced increase in place preference in the control group [$t(4)=8.60$, $p=0.001$; paired *t*-test], but not in the 6-OHDA-treated group [$t(6)=1.49$, $p=0.19$].

Approximately 80% of dopamine was depleted by 6-OHDA in the nucleus accumbens [$t(10)=11.64$, $p<0.001$ between the control and 6-OHDA-treated groups, two-sample *t*-test] (Table 1). Accumbal norepinephrine and serotonin concentrations also seemed to decrease, but clearly to a lesser extent (35–40%) [statistical significance was reached only with serotonin, $t(10)=2.98$, $p=0.014$]. The lesion was not totally restricted to the nucleus accumbens; in the striatum, 35% of dopamine was depleted [$t(10)=3.33$, $p=0.008$]. Instead, striatal norepinephrine and serotonin concentrations were not affected.

Table 1
Contents of dopamine, norepinephrine and serotonin in the nucleus accumbens and striatum of the rats treated with 6-OHDA or vehicle (mean±S.E.)

	Control group (nmol/g)	6-OHDA group (nmol/g)	Depletion (%)
Nucleus accumbens			
Dopamine	124.6±8.9	24.6±3.7***	80.2±3.0
Norepinephrine	1.7±0.1	1.1±0.3	34.2±15.3
Serotonin	6.5±0.8	4.0±0.5*	39.0±8.9
Striatum			
Dopamine	90.7±8.9	58.9±5.1**	35.0±5.6
Norepinephrine	1.5±0.3	1.3±0.2	13.1±11.3
Serotonin	4.3±0.4	4.6±0.4	−5.5±9.3

*$p<0.05$, ** $p<0.01$, and *** $p<0.001$ between the 6-OHDA and control groups, two-sample *t*-test ($n=5$ in the control group and $n=7$ in the 6-OHDA group).

4. Discussion

In the present study, all the four isomers of 4-methylaminorex induced place preference, with no significant differences in their potency. Furthermore, place preference induced by *trans*-4*S*,5*S* and *cis*-4*R*,5*S* was attenuated by the dopamine D1- or D2-receptor antagonists SCH 23390 and raclopride, respectively; and place preference induced by *cis*-4*R*,5*S* was attenuated by dopaminergic lesion in the nucleus accumbens by 6-OHDA.

It has been previously shown that a racemic mixture of the *cis*-isomers can maintain self-administration after training with cocaine in non-human primates (Mansbach et al., 1990). Our findings with drug-naïve animals indicate that not only the *cis*-isomers, but also the *trans*-isomers can induce place preference, and thus possess rewarding properties. These results are in agreement with the previous studies showing that the isomers can share the discriminative stimulus with amphetamine, induce locomotor and stereotyped behavior, or elevate dopamine levels in the nucleus accumbens (Glennon and Misenheimer, 1990; Ashby et al., 1995; Batsche et al., 1994; Kankaanpää et al., 2002).

In the previous studies, the isomers have induced neurochemical and behavioral effects quite consistently with the following order of potency: *trans*-4*S*,5*S*>*cis*-4*R*,5*S*≈*cis*-4*S*,5*R*>*trans*-4*R*,5*R* (Glennon and Misenheimer, 1990; Batsche et al., 1994; Ashby et al., 1995; Kankaanpää et al., 2002). This is somewhat different from our results, in which all the isomers appeared to be equipotent in inducing place preference. There appears not to be any straightforward interpretation for this divergence. Conditioned place preference, however, is a distinct behavioral paradigm, utilized typically to assess drug reward that is a separate phenomenon from, e.g., drug discrimination or motor activity. Not necessarily do the same neuronal mechanisms mediate them all. There are also methodological differences to be noted; for instance, in the place preference test, compounds are typically administered repeatedly and thus the development of sensitization (i.e., enhancement of drug effect by repeated drug administration) may play some role. Certainly, no such effect would be observed in the above-mentioned 4-methylaminorex studies because they employed only single injections. One explanation for the lack of differences in this study could be marked optical impurities in the isomer preparations. We have, however, made every effort to confirm the purity of the isomers (see Materials and methods). In addition, we have used the same preparations in our previous study (Kankaanpää et al., 2002), in which the neurochemical and behavioral effects of the isomers followed the similar rank order of potency also reported by others.

Thus, it seems that the present results cannot be explained by terms of optical impurities. Finally, in the previous studies that have concerned determining the effective dose for the isomers from a dose–response curve, *trans*-4*S*,5*S* has been at least 5-fold more, and *trans*-4*R*,5*R* 2.5-fold less potent than the equipotent *cis*-isomers (Glennon and Misenheimer, 1990; Ashby et al., 1995). One could expect differences of this magnitude to be detected in our experimental set-up as well. After all, nevertheless, our results suggest that all the four stereoisomers of 4-methylaminorex possess rewarding properties and, thus, abuse potential of roughly comparable potency.

Our findings indicate the involvement of the brain dopaminergic system in the 4-methylaminorex reward. Both dopamine D1- and D2-receptors appear to play a role; the involvement of dopamine in the nucleus accumbens might be suggested as well, but this is somewhat less definite because the lesion produced by 6-OHDA-treatment was subtotal, though comparable to what has been reported by other authors (Rassnick et al., 1992; Cousins et al., 1996). The depletions in the accumbal norepinephrine and serotonin concentrations, or in striatal dopamine concentrations were substantially more limited, however. These findings are in agreement with the previous view on the importance of the brain dopaminergic system in the psychostimulant reward (Bardo, 1998). More specifically, the dopaminergic mechanisms of the 4-methylaminorex-induced place preference seem to be analogous with those of amphetamine-like dopamine releasing drugs; but perhaps separable from those of cocaine-like dopamine uptake blockers, which induce place preference that is somewhat insensitive to the D2-receptor blockade or accumbal 6-OHDA-lesion (Spyraki et al., 1982a,b; Tzschentke, 1998).

In conclusion, our findings indicate that all the four stereoisomers of 4-methylaminorex possess rewarding properties, with the *trans*-isomers being at least as potent as the *cis*-isomers. Although in illicit samples the *cis*-isomers are the most frequently encountered forms (Klein et al., 1989; Gaine et al., 2000), it appears that in terms of their pharmacological actions, the *trans*-isomers are likewise subject to abuse. Furthermore, in the 4-methylaminorex-reward, the brain dopaminergic system appears to be important.

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