Acute Neurochemical and Behavioral Effects of Stereoisomers of 4-Methylaminorex in Relation to Brain Drug Concentrations

AINO KANKAANPÄÄ, SATU ELLERMAA, ESA MERIRINNE, PAULA HIRSJÄRVI, and TIMO SEPPÄLÄ

Drug Research Unit, Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland

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ABSTRACT

4-Methylaminorex is a stimulant drug of abuse that exists as four stereoisomers: cis-4R,5S, cis-4S,5R, trans-4S,5S, and trans-4R,5R. These isomers have previously been shown to differ markedly in various respects. In the present study we assessed the effects of the isomers of 4-methylaminorex (2.5, 5.0, and 10 mg/kg i.p.) on extracellular dopamine and 5-hydroxytryptamine (5-HT) levels in the nucleus accumbens, as well as behavior in the rats simultaneously. The relative concentrations of the isomers in the brain were also measured. The samples were collected by in vivo microdialysis and then analyzed for neurotransmitters with high-performance liquid chromatography/electrochemical detection and for cis- and trans-4-methylaminorex with gas chromatography/mass spectrometry. The behavioral effects of the isomers were assessed from videotapes recorded during the microdialysis experiments. All isomers elevated the extracellular levels of both dopamine and 5-HT, with the exception of trans-4R,5R. The rank order of potency for elevating dopamine was trans-4S,5S > cis-4S,5R > cis-4R,5S > trans-4S,5S, and for elevating 5-HT cis-4S,5R > trans-4S,5S > cis-4R,5S > trans-4R,5R. Analysis of the behavioral data, together with the neurochemical data, suggests that behavioral effects of the isomers of 4-methylaminorex are related to drug-induced dopamine release and, in the case of higher doses of the most efficacious isomers, to 5-HT as well. The brain concentrations of the isomers did not reflect their neurochemical efficacy, which implies that their differences are pharmacodynamic rather than pharmacokinetic.

The phenylisopropylamine derivative 4-methylaminorex (2-amino-4-methyl-5-phenyl-3-oxazoline) is a sympathomimetic agent that exists as four stereoisomers: cis-4R,5S, cis-4S,5R, trans-4R,5R, and trans-4S,5S (Poos et al., 1963; Roszkowski and Kelley, 1963; Yelnosky and Katz, 1963). This compound was originally developed by the pharmaceutical industry, and more recently the mixture of cis-isomers has turned up in the clandestine market with street names “U4Euh” and “Ice” (Davis and Brewster, 1987; Klein et al., 1989). Since the advent of the Internet it has also been promoted worldwide on drug culture-related web sites. The abuse potential of racemic cis-(±)-4-methylaminorex has also been demonstrated in animal models: it is self-administered by nonhuman primates (Mansbach et al., 1990) and it substitutes for cocaine (Mansbach et al., 1990; Young and Glennon, 1993) in drug discrimination paradigms. In addition, the effects of cis-(±)-4-methylaminorex on brain monoaminergic systems are reported to resemble those of other amphetamine-related drugs: it decreases tissue dopamine and 5-hydroxytryptamine (5-HT) content and decreases tryptophan hydroxylase activity in neostriatum (Bunker et al., 1990). Due to the high abuse potential of cis-(±)-4-methylaminorex it has been classified in schedule I in the United States (Federal Register, Schedules of Controlled Substances, 1989).

Published reports concerning the effects of the 4-methylaminorex stereoisomers include evaluation of the isomers’ ability to substitute for S-amphetamine in drug discrimination paradigms (Glennon and Misenheimer, 1990), to induce locomotor activity or stereotyped behavior (Batsche et al., 1994), and to suppress the basal firing rate of A10 dopamine cells in an extracellular single unit study (Ashby et al., 1995). In all these studies, the rank order of potencies of the isomers was trans-4S,5S > cis-4S,5R > cis-4R,5S > trans-4R,5R.

The aim of this study was to evaluate the neurochemical and behavioral effects of the isomers of 4-methylaminorex. We used in vivo microdialysis in fully conscious rats to monitor the effects of systemically administered isomers of 4-methylaminorex (2.5, 5.0, and 10 mg/kg i.p.) on extracellular levels of dopamine and 5-HT and their metabolites 3,4-

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; AUC, area under the curve; MDMA, 3,4-methylenedioxymethamphetamine; NMDA, N-methyl-D-aspartate.
samples were collected at 20-min intervals. After four basal samples and 10 mg/kg; 2.5, 5.0, and 10 mg/kg) of 4-methylaminorex were prepared in the Laboratory of Organic Chemistry, University of Helsinki, Helsinki, Finland, by using 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 1H NMR and 13C NMR spectra.

The following treatments (n = 6 rats per treatment) were carried out: vehicle; cis-4R,5S (2.5, 5.0, and 10 mg/kg); cis-4S,5R, and cis-4S,5S; and trans-4S,5R (2.5, 5.0, and 10 mg/kg); trans-4R,5S (2.5, 5.0, and 10 mg/kg); and trans-4S,5S (2.5, 5.0, and 10 mg/kg). The 4-methylaminorex 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. The concentrations of the drugs in the solutions, all expressed as free base, were adjusted so that they could be injected in a volume of 1 ml/kg. The drugs and their corresponding vehicles were injected i.p.

Microdialysis Experiments. The rats were anesthetized using 5% halothane gas (Halothane Liquid; Rhodia Limited, Bristol, UK). A guide cannula (CMA/12; CMA Microdialysis, Solna, Sweden) was implanted 2 mm above the nucleus accumbens [A, +2.0; L, −1.2; V, −6.0 as calculated relative to the bregma according to Paxinos and Watson (1986)] and secured with dental cement (Aqualox; VOCO, Cuxhaven, Germany) and two small screws. During surgery halothane was administered at a concentration of 2% and body temperature was maintained at 38°C by using a temperature-controller system. The rats were allowed to recover from the surgery for 6 days, and then 1 day before the experiment a microdialysis probe (CMA/12, membrane length 2 mm) was inserted through the guide cannula into the nucleus accumbens. The next day, the rat was placed in a test cage and the probe was connected to a CMA/100 microinjection pump and perfused with modified Ringer’s solution (147 mM NaCl, 1.2 mM CaCl2, 2.7 mM KCl, 1.0 mM MgCl2, pH 6) at a flow rate of 2 μl/min. A 6.5-μl aliquot of antioxidant solution (1.0 mM oxalic acid, 3.0 mM L-cysteine, 0.1 M acetic acid) was added to the vials before collection of the dialysate samples (Kankaanpää et al., 2001a). The perfusate was discarded during the first 60 min, after which samples were collected at 20-min intervals. After four basal samples (2 h 20 min from the beginning of the perfusion) the 4-methylaminorex isomers were administered. Control rats received vehicle injections at the corresponding time. Video recording of the animals’ behavior was begun 20 min before drug injection. Aliquots (15 μl) of the samples were assayed for neurotransmitters and their metabolites by using high-performance liquid chromatography, and the remaining dialysate was deep-frozen (−70°C) to be later assayed for cis- and trans-4-methylaminorex. At the end of the experiment the animal was exposed to halothane and decapitated, after which the brain was dissected out and immersed in 10% buffered formalin solution. Correct placement of the microdialysis probe was verified, and data were included only from animals with accurate placements.

Determination of the Concentrations of Dopamine, 5-HT, and Their Metabolites in Dialysate Samples. Dopamine, 5-HT, DOPAC, HVA, and 5-HIAA were quantified using high-performance liquid chromatography with electrochemical detection (ANTEC Analytical Technology, Leyden B.V., Leyden, The Netherlands) and an Inertsil ODS-3V 5-μm (250×4.6-mm i.d.) reversed phase column (GL-Sciences Inc., Tokyo, Japan), as described previously (Kankaanpää et al., 2001a). Briefly, the mobile phase was a buffer containing 50 mM NaH2PO4, 0.1 mM EDTA, 2.3 mM octanesulfonic acid, and acetoniure (21% v/v in the final solution), with the pH adjusted to 3.0 with phosphoric acid. The mobile phase was filtered through a 0.22-μm GH-Polypro filter (Gelman Sciences, Ann Arbor, MI) and degassed under vacuum. The flow rate was 1.2 ml/min and the detector potential +780 mV versus a Ag/AgCl reference electrode.

Characterization of Behavioral Changes after Administration of 4-Methylaminorex Isomers. Behavior of the rats during the microdialysis experiments was characterized from video tapes by an observer blind to drug conditions. Recording was begun 20 min before drug injection and continued for 220 min after the injection. Locomotor activity of the animals receiving the isomers at doses of 0, 2.5, 5.0, and 10 mg/kg was estimated from the number of complete passes (all 4 ft) across midline of the test cage during intervals of 20 min (corresponding to the sampling interval in the microdialysis experiments).

A more detailed behavioral analysis was carried out with the rats receiving 5.0- and 10-mg/kg doses of the isomers: the onset, frequency, and/or duration of different behaviors were monitored visually for 1 min every 5th min by an observer blind to the drug conditions. This behavioral analysis was begun 20 min before drug injection, after which monitoring was discontinued for 20 min to exclude the effect of injections on the behavior, and then continued for 200 min at 20-min intervals.

The behavioral patterns characteristic for each isomer were then scored according to a rating scale modified from scales based on observations of rats exposed to increasing doses of amphetamine (Creese and Iversen, 1973) and phencyclidine (Sturgeon et al., 1979), the behavioral effects of which resemble those induced by the most potent isomer of 4-methylaminorex. The rats were given a single behavioral score per each 20-min sampling interval as follows: 0, passive or long-lasting (>10 min) motionlessness; 1, active motionlessness; 2, active motionlessness with occasional rearings; 3, locomotor activity with bursts of rearings; 4, stereotyped behavior; 5, intense stereotyped behavior; 6, ataxia; and 7, catatonia.

When rats performed passive motionless the animal was stationary, lying or sleeping, or sniffing peacefully, whereas active motionlessness included alertness, movements of the head, and active sniffing in various directions. Locomotor activity was defined as movement of the animal over the surface of the observation cage floor. Stereotyped behavior was defined as compulsive-like, rapid, and repetitive purposeless behavior that occurred at an abnormally high frequency. According to our preliminary experiments with increasing doses of the presumably most potent isomer of 4-methylaminorex, trans-4S,5S, we considered behavioral patterns such as intensive sniffing (while on the cage floor or during a prolonged rearing), head bobbing, or head weaving performed at a moderate rate to be milder forms of
stereotyped behavior, in comparison with vigorous backward walking, circling, and body weaving, which were considered intense stereotyped behavior. This kind of classification of stereotyped behavior is further supported by findings with phencyclidine by Sturgeon et al. (1979). Ataxia was defined accordingly to Sturgeon et al. (1979) as impairment in the ability of the animal to execute coordinated motor responses leading, in the extreme, to incapacitation of the animal. Typically, ataxic animals exhibited uncoordinated motor movements with some falling while attempting to move, or “swimming-like” movements with splaying of fore- and hindlimbs. Catatonia was the extreme form of ataxia: the rats were lying flat and were unresponsive.

**Determination of Concentrations of cis- and trans-4-Methylaminorex in Dialysate Samples.** The cis- and trans-4-methylaminorex were quantified with GC/mass spectrometry as tert-butyldimethylsilyl derivatives by using a previously described method (Kankaanpää et al., 2001b) adapted for dialysate samples. The derivatives were formed using N-(tert-butyldimethylsilyl)-N-methyltrimfluoracetamide obtained from Aldrich Chemical (Milwaukee, WI). The procedure was begun with extraction of 4-methylaminorex from the pooled dialysate samples (two consecutive 16-μl samples pooled) by mixing with 1 ml of 0.5 M NaOH and 5 ml of toluene containing carbamazepine (10 μg/100 ml) as internal standard. After centrifugation, the toluene layer was transferred into a clean test tube and evaporated to dryness. The derivatization reagent, 120 μl of N-(tert-butyldimethylsilyl)-N-methyltrimfluoracetamide/acetonitrile (1:6), was added to the dry evaporation residue. After incubation for 2 h at 55°C, the mixture was transferred to autosampler vials with septa and injected in a volume of 1 μl into GC/mass spectrometry apparatus.

Quantification of 4-methylaminorex was carried out with a Hewlett Packard G1800A GCD System (Hewlett Packard, Palo Alto, CA), which consists of a gas chromatograph, an electron ionization detector (positive ions, 70 eV), and a data system. The system was operated in the splitless injector mode. The GC column was a HP-5 film thickness. Helium was used as a carrier gas. The inlet and detector temperatures were maintained at 250 and 280°C, respectively. The column temperature was initially 150°C with a hold time of 2.0 min and was increased 15°C/min to 320°C with a final hold time of 3.0 min.

**Statistical Analyses.** In the microdialysis experiments the mean of the four samples before the drug treatments was considered to represent basal release (100%) of neurotransmitters and their metabolites according to which relative changes after the injections were calculated. Concentrations of cis- and trans-4-methylaminorex were calculated as picomoles per dialysate sample. In the locomotor activity test the absolute number of passes across midline was used. The behaviors of the animals were scored as described above. For statistical evaluations of both neurochemical and behavioral data, as well as concentrations of the isomers, areas under the curves (AUCs) during the indicated intervals were calculated with the trapezoidal method, and peak effect or minimum value where appropriate were taken directly from the data (Matthews et al., 1990). The microdialysis and locomotor activity data were then subjected to one-way analysis of variance (ANOVA) followed by Tukey’s test. The behavioral scores after administration of the isomers were analyzed with Kruskall-Wallis nonparametric ANOVA, and multiple comparisons were made using the Mann-Whitney U test with Bonferroni protection.

**Results**

**Effects of Isomers on Extracellular Levels of Dopamine, 5-HT, and Their Metabolites.** The absolute basal values of the different treatment groups did not differ significantly. The means (± S.E.M.; n = 30) of the basal concentration in the nucleus accumbens dialysate were as follows: dopamine, 93 ± 10 fmol/40 μl; 5-HT, 18.0 ± 2.0 fmol/40 μl; DOPAC, 13.5 ± 0.75 pmol/40 μl; HVA, 6.9 ± 0.6 pmol/40 μl; and 5-HIAA, 6.5 ± 0.4 pmol/40 μl. The basal levels of the neurotransmitters and their metabolites remained unaltered in the vehicle-treated rats.

Figure 1 summarizes the effects of the 4-methylaminorex isomers (2.5, 5.0, or 10 mg/kg) on extracellular dopamine levels in the nucleus accumbens. Compared with the vehicle-treated rats, one-way ANOVA revealed statistically significant elevations of extracellular dopamine levels after administration of the isomers trans-4S,5S (p = 0.014, AUC; p = 0.007, peak effect), cis-4S,5R (p = 0.006, AUC; p = 0.009, peak effect), and cis-4R,5S (p = 0.002, AUC; p = 0.002, peak effect). As seen in Fig. 1, although the increases of dopamine induced by these isomers at a dose of 10 mg/kg were about
equal, the differences between the isomers were evident when comparing their effects at the lower doses: only trans-4S,5S, but neither of the cis-isomers, elevated extracellular dopamine levels significantly even at an intermediate dose of 5.0 mg/kg, as evaluated by the Tukey's post hoc test. In contrast, the isomer trans-4R,5R failed to elevate extracellular dopamine levels significantly, although a slight elevation of dopamine was observed.

As seen in Fig. 2, the isomers trans-4S,5S, cis-4S,5R, and cis-4R,5S had a marked effect on 5-HT as well. One-way ANOVA revealed statistically significant elevations of extracellular 5-HT levels after administration of the isomers trans-4S,5S (p < 0.001, AUC; p < 0.001, peak effect), cis-4S,5R (p = 0.001, AUC; p = 0.002, peak effect), and cis-4R,5S (p < 0.001, AUC; p < 0.001, peak effect). All of these had a strong effect on 5-HT at the highest dose, but only cis-4S,5R induced a statistically significant effect even at the intermediate dose (Tukey's test). The isomer trans-4R,5R had no effect on the 5-HT levels.

The effects of the 4-methylaminorex isomers on the dopamine metabolites DOPAC and HVA are presented in Figs. 3 and 4. In contrast to the results concerning dopamine itself, all of the isomers decreased the extracellular concentrations of DOPAC significantly (all isomers: p < 0.001, AUC; p < 0.001, minimum value; ANOVA). Extracellular concentrations of HVA were also decreased significantly as revealed by one-way ANOVA: trans-4S,5S (p = 0.012, AUC; p = 0.025, minimum value), cis-4S,5R (p = 0.002, AUC; p = 0.076, minimum value), cis-4R,5S (p < 0.001, AUC; p < 0.001, minimum value), and trans-4R,5R (p < 0.001, AUC; p = 0.001, minimum value). Regarding HVA however, although a net decrease followed the administration of all the isomers, a pattern with initial decrease followed by an increase and a subsequent decrease was observed when the isomers trans-4S,5S, cis-4S,5R, and cis-4R,5S were administered at the highest dose. This effect was most pronounced with the isomer cis-4S,5R, the administration of which resulted in a net increase in extracellular HVA concentration.

The effects of the isomers on the 5-HT metabolite 5-HIAA are presented in Fig. 5. All isomers decreased the extracellular 5-HIAA levels significantly, as revealed by one-way ANOVA: trans-4S,5S (p = 0.001, AUC; p < 0.001, minimum value), cis-4S,5R (p = 0.074, AUC; p = 0.034, minimum value), cis-4R,5S (p = 0.001, AUC; p < 0.001, minimum value), and trans-4R,5R (p = 0.006, AUC; p = 0.083, minimum value). The decreases induced by the isomers trans-4S,5S and cis-4R,5S were most pronounced. After reaching minimum levels the concentrations began to increase again, reaching a level of 150% of baseline in rats treated with the isomers trans-4S,5S and cis-4R,5S.

Behavioral Changes after Administration of 4-Methylaminorex Isomers. Administration of all the 4-methylaminorex isomers increased locomotor activity of the animals significantly as revealed by one-way ANOVA: trans-4S,5S (p = 0.015, AUC; p = 0.026, peak effect), cis-4S,5R (p = 0.004, AUC; p = 0.007, peak effect), cis-4R,5S (p = 0.040, AUC; p < 0.014, peak effect), and trans-4R,5R (p = 0.004, AUC; p = 0.001, peak effect). The temporal profiles of the locomotor activity induced by administration of the isomers at doses of 0, 2.5, 5.0, and 10 mg/kg are presented in Fig. 6. At a dose of 10 mg/kg, all isomers except trans-4R,5R produced a biphasic pattern with a rapid initial rise in locomotor activity followed by a rapid decline when the rats began to engage in stereotyped activity maintained in one location or to show signs of ataxia or even catatonia. At the lower doses both cis-isomers produced dose-dependent increases in locomotor activity, unlike trans-4S,5S, which also produced the biphasic effect at a dose of 5.0 mg/kg and only with the lowest dose increased locomotor activity. The trans-4R,5R isomer failed to increase locomotor activity significantly at the two lower doses.

As shown in Fig. 7, at a dose of 10 mg/kg all isomers induced behavioral patterns clearly distinguishable from those of the vehicle-treated rats (p < 0.001, AUC; p < 0.001, peak effect; Kruskall-Wallis). Behavioral effects of trans-4S,5S-4-methylaminorex were the most dramatic; after the initial peak in locomotor activity, the behavior of the rats
changed to stereotypic head movements, followed by intense backward walking, which peaked 40 to 60 min after the injection (individual frequencies: 44, 28, 8, 4, 3, 1 per monitoring interval). Shortly after the period of backward walking, a majority of the animals began to exhibit ataxia or catatonia, which typically lasted until the end of the recording time, with more attempts to move and some unsteady walking seen toward the end of the experiments. Interestingly, two rats did not produce such states, but rather exhibited a unique behavioral pattern consisting of increased locomotor activity with intensive backward walking (with individual frequencies up to 44 and 33 during one interval) and circling (with individual frequencies up to 69 and 12 during one interval).

Similarly to trans-4S,5S-4-methylaminorex, at a dose of 10 mg/kg both cis-isomers produced an episode of increased locomotor activity and rearings, after which the behavioral pattern began to change to stereotyped behavior maintained at one position. The stereotypes consisted of intensive sniffing, head bobbing, and weaving, and also some forepaw treading, performed at one position while on the cage floor or during a prolonged rearing. This lasted until approximately 140 min after injection, after which the behavior began to normalize. However, one cis-4R,5S-treated rat showed a period of very intense backward walking and circling for 60 to 140 min between periods of stereotyped behavior maintained at one position. Nevertheless, the behavior of the rats receiving the two cis-isomers was more uniform than the behavior of the rats receiving the trans-isomers.

Fig. 3. Temporal profiles of the effects of the 4-methylaminorex isomers on extracellular DOPAC in the nucleus accumbens. cis-4R,5S-, cis-4S,5R-, trans-4S,5S-, and trans-4R,5R-4-methylaminorex (2.5, 5.0, and 10 mg/kg i.p.) were administered at 0 min, as indicated by arrows. Data expressed as percentages of basal release are given as means ± S.E.M. (n = 6). Histograms represent AUC 0 to 240 min or the minimum (MIN) values after injections of the drugs. *p < 0.05, **p < 0.01, ***p < 0.001, compared with control group, Tukey’s test.

Fig. 4. Temporal profiles of the effects of the 4-methylaminorex isomers on extracellular HVA in the nucleus accumbens. cis-4R,5S-, cis-4S,5R-, trans-4S,5S-, and trans-4R,5R-4-methylaminorex (2.5, 5.0, and 10 mg/kg i.p.) were administered at 0 min, as indicated by arrows. Data expressed as percentages of basal release are given as means ± S.E.M. (n = 6). Histograms represent AUC 0 to 240 min or the minimum (MIN) values after injections of the drugs. *p < 0.05, **p < 0.01, ***p < 0.001, compared with control group, Tukey’s test.
of the rats receiving trans-4S,5S, and the two cis-isomers were equally potent in their behavioral effects.

The changes after administration of the isomer trans-4R,5R at a dose of 10 mg/kg were different from the other isomers regarding both onset and pattern of drug-induced behavior. The brief episode of increased locomotor activity shortly after drug injection observed with all other isomers was absent, but after nearly 1 h incidences of rearing, walking, and sniffing began to gradually increase, eventually leading to compulsive-like behavior consisting of a pattern of locomotor activity, rearing, and abrupt stops. This behavior lasted until the end of the recording time but diminished toward the end of the experiment.

As evident from Fig. 7, administration of the isomers trans-4S,5S, cis-4S,5R, and cis-4R,5S at a dose of 5.0 mg/kg resulted in behaviors markedly different from those of the vehicle-treated animals (p = 0.002, AUC; p = 0.001, peak effect; Kruskall-Wallis), although the behavioral patterns were, as expected, less dramatic than those observed after treatment with 10-mg/kg doses of the isomers. The behaviors induced by the 5.0-mg/kg dose of the isomer trans-4R,5R, which had only negligible neurochemical effects, were not subjected to detailed analysis. When comparing the effects of the isomers at a dose of 5.0 mg/kg the most pronounced behavioral changes were observed, similar to the experiments with 10-mg/kg doses, in trans-4S,5S-4-methylamin-
orex-treated rats. Specifically, these behaviors resembled the intense stereotyped behaviors that occurred transiently between the periods of locomotor activity and ataxia induced by the highest dose of the same isomer. At a 5.0-mg/kg dose of the isomer trans-4S,5S ataxia was seen only rarely, and catatonia, the most severe disruption of behavior, was absent.

In general terms, the behavior induced by both cis-isomers at a dose of 5.0 mg/kg can be described as a compulsive-like pattern of locomotor activity, rearing, and stops that resembles the effects of the 10-mg/kg dose of the isomer trans-4R,5R. However, more detailed analysis revealed that administration of the isomer cis-4S,5R, but not cis-4R,5S, produces apractic movements and falling, indicative of ataxia, as well as circling and coprophagy, in addition to the common behavior described above. Due to these behaviors, cis-4S,5R-induced changes were scored higher than those induced by the isomer cis-4R,5S (Fig. 7).

Concentrations of cis- and trans-4-Methylaminorex in Dialysate Samples. The cis- and trans-isomers of 4-methylaminorex were separated by the analysis method used, but not the two cis-isomers or the two trans-isomers from each other. The concentrations of the isomers were thus measured as cis- and trans-4-methylaminorex.

As evidenced by one-way ANOVA, dialysate concentrations of 4-methylaminorex differed significantly from each other both at the dose of 5.0 mg/kg (p = 0.003, AUC; p = 0.010, peak effect) and 10 mg/kg (p = 0.017, AUC; p = 0.040, peak effect). Specifically, pairwise comparisons with the Tukey’s post hoc test revealed that administration of the isomer trans-4R,5R resulted in significantly higher dialysate concentrations than treatment with the cis-isomers, both at doses of 5.0 mg/kg (trans-4R,5R versus cis-4R,5S (p = 0.009 AUC; p = 0.036, peak effect), trans-4R,5R versus cis-4S,5R (p = 0.011, AUC; p = 0.027, peak effect)) and 10 mg/kg (trans-4R,5R versus cis-4R,5S (p = 0.020, AUC; p = 0.066, peak effect), trans-4R,5R versus cis-4S,5R (p = 0.047, AUC; p = 0.128, peak effect)). Concerning the dose of 10 mg/kg, the lack of significant difference in peak effects is likely to result from the excessive variation in the measured concentrations of the isomer trans-4R,5R. The means of the concentrations of cis-and trans-4-methylaminorex in the dialysate samples after administration of the isomers at doses of 10 and 5.0 mg/kg are presented in Fig. 8, a and b, together with the neurochemical and behavioral changes they induced.

Discussion

One of the main findings of the present study was that i.p. administration of the isomers trans-4S,5S-, cis-4S,5R-, and cis-4R,5S 4-methylaminorex elevated extracellular levels of 5-HT and dopamine in rat nucleus accumbens. The magnitude of changes in both transmitters depended on the isomer administered. The rank order for elevating 5-HT was cis-4S,5R > trans-4S,5S = cis-4R,5S > trans-4R,5R, whereas the corresponding order for elevating extracellular dopamine was trans-4S,5S > cis-4S,5R = cis-4R,5S > trans-4R,5R. The latter is consistent with the preliminary microdialysis report by Eberle et al. (1992) and also with observations concerning the potential of the isomers in substituting for 8-amphetamine (Glennon and Misenheimer, 1990), inducing locomotor activity and stereotypes (Batsche et al., 1994) and suppressing the basal firing rate of A10 dopamine cells (Ashby et al., 1995). As remarked by Glennon and Misenheimer (1990), similar rank order can be predicted from the established structure-activity relationships for central stimulant and discriminative stimulus properties of phenylisopropylamines. Because activation of the dopaminergic neuronal system in mesolimbic brain areas, including the nucleus accumbens, is thought to play a major role in the rewarding properties of drugs of abuse (Wise and Bozarth, 1987), it seems that the isomer trans-4S,5S may also possess stronger potential for abuse than the cis-isomers and thus should also be classified in schedule I.

In the present study, administration of the trans-4S,5S-, cis-4S,5R-, and cis-4R,5S 4-methylaminorex induced a strong elevation in extracellular levels of dopamine and a decrease in DOPAC and HVA in the nucleus accumbens, which very closely resemble the dopaminergic effects of amphetamine as assessed by the in vivo microdialysis technique (Zetterström et al., 1983, 1986). Because the predominant fraction of DOPAC is likely to derive from the metabolism of intraneuronal dopamine, the partial depletion of cytoplasmic...
largely responsible for the amphetamine-induced decline in dopamine resulting from its release was suggested to be to those of Figs. 1 and 2.

Fig. 8. Concentrations of the 4-methylaminorex (4-MAX) isomers in dialysate samples (columns) plotted together with their neurochemical and rated behavioral effects in rats treated with 10- (a) and 5.0-mg/kg (b) doses of the isomers. The scalings of the neurochemical data correspond to those of Figs. 1 and 2.

dopamine resulting from its release was suggested to be largely responsible for the amphetamine-induced decline in DOPAC (Kuczenski, 1980; Zetterström et al., 1986). The changes in HVA, the other major metabolite of dopamine, can be expected to follow the changes in DOPAC, because HVA is its secondary metabolite (Westerink, 1985). In the present study, the effects of the isomers on HVA clearly paralleled their effects on DOPAC at low doses but diverged at high doses. Similarly, high doses of amphetamine were shown to increase HVA as a result of dopamine metabolism shifting to the extraneuronal pathway, which was suggested to reflect dopamine uptake inhibition (Kuczenski, 1980). A similar phenomenon may also underlie the changes observed in both cis-isomers and the isomer trans-4S,5S. Such dual mechanisms are supported by observations of Ashby et al. (1995) who showed that the suppressant effects of trans-4S,5S-4-methylaminorex on A10 dopamine cells are attenuated by pretreating animals with α-methyl-p-tyrosine or reserpine, which depletes newly synthesized cytoplasmic dopamine and vesicular stores of monoamines, respectively (Garattini and Samanin, 1981). Taken together, it seems that both cytoplasmic and vesicular dopamine mediate the effects of trans-4S,5S-, cis-4S,5R-, and cis-4R,5S-4-methylaminorex, which implies an action mechanism involving both dopamine release and uptake inhibition.

The nearly equal elevation of accumbal dopamine and 5-HT induced by the 4-methylaminorex isomers cis-4S,5R, trans-4S,5S, and cis-4R,5S resembles the effects of 3,4-methylenedioxymethamphetamine (MDMA; “Ecstasy”) in the same brain structure (Kankaanpää et al., 1998). Because drug-induced release of endogenous dopamine was implicated in mediating the development of MDMA-induced degeneration of serotonergic neurons (for review, see Green et al., 1995), it can be speculated whether administration of the 4-methylaminorex isomers would similarly induce neurotoxic effects. In fact, there is evidence that administration of multiple doses of racemic cis-(-)-4-methylaminorex causes long-term (7-day) declines in striatal tryptophan hydroxylase activity, which may be associated with neurotoxic action on the serotoninergic system (Hanson et al., 1992).

The behavioral pattern induced by all isomers of 4-methylaminorex included locomotor activity, which changed to stereotypic behavior maintained at one location after administration of higher doses of the cis-isomers and the isomer trans-4S,5S, and even ataxia after the highest dose of trans-4S,5S. When scored according to the rating scale used, the rank order of potential for inducing behavioral changes matches that observed by Batsche et al. (1994), namely, trans-4S,5S > cis-4S,5R ≈ cis-4R,5S > trans-4R,5R. This rank order is also identical to that observed when measuring dopaminergic parameters (Eberle et al., 1992; Ashby et al., 1995; this study), which suggests that the behavioral effects of moderate doses of 4-methylaminorex are mediated by its effect on dopamine. Furthermore, dopaminergic manipulations were shown to attenuate locomotor activity induced by trans-4S,5S-4-methylaminorex (Batsche et al., 1994) and the ability of the same isomer to suppress basal firing rates of A10 dopamine cells (Ashby et al., 1995).

The stereotyped behaviors induced by the isomers trans-4S,5S, cis-4S,5R, and cis-4R,5S at a dose of 10 mg/kg, however, included repetitive head movements and vigorous sniffing behavior, but not oral stereotypies such as gnawing or licking characteristic for treatment with high doses of amphetamine (Creese and Iversen, 1973). Instead, behaviors such as forepaw treading, head-weaving, ataxia, and/or catalepsy were observed; this is consistent with administration of the three most potent isomers at a dose of 10 mg/kg. These resemble behaviors characteristic of the so-called 5-HT syndrome, which in animals consists of a complex series of behaviors including forepaw treading, head-weaving, hindlimb abduction, ataxia, and unawareness, leading finally to convulsions and death (Graham-Smith, 1971; Green and Heal, 1985). Because the present results showed that the three most potent isomers of 4-methylaminorex have a marked effect on 5-HT in addition to dopamine, there may also be serotonergic components in their behavioral effects, as is the case with MDMA (Slikker et al., 1989; Spanos and Yamamoto, 1989). In the study of Batsche et al. (1994), however, serotonergic manipulations had no effect on locomotor activation induced by the isomer trans-4S,5S at a dose of 3 mg/kg. This is consistent with minor effect of a 5.0-mg/kg dose of the same isomer on 5-HT relative to dopamine, and lack of serotonergic-like behavioral effects observed in the present study. Thus, it is possible that
5-HT effect is masked in the strong dopaminergic effect induced by the isomer trans-4S,5S at this dose, or alternatively the serotonergic effect is simply not strong enough to induce perceivable behavioral changes. Instead, at the same dose the isomer cis-4S,5R, which produced apractic movements and falling indicative of ataxia, affects 5-HT more profoundly than dopamine. Taken together, it seems that the serotonergic-like behaviors occur only after very strong elevation of synaptic 5-HT-levels, or under conditions when serotonergic stimulation is more pronounced than dopaminergic stimulation. Furthermore, regarding the study of Batsche et al. (1994), it seems that the serotonergic mechanisms may mediate behavioral effects other than locomotor activation, which seems to be of dopaminergic origin.

Surprisingly, the severe disturbances in motor coordination observed after treatment with the 10-mg/kg dose of trans-4S,5S-4-methylaminorex, resembled those reported after administration of phenylcyclo- and other NMDA glutamate receptor-channel antagonists (Sturgeon et al., 1979; for review, see Carter, 1995). Furthermore, because seizures are strongly related to abnormal glutamate activity at NMDA receptors (for review, see Upton, 1994), the seizure-promoting properties of 4-methylaminorex (Hanson et al., 1999) may provide another link to glutamatergic systems. However, some of the behavioral consequences of NMDA receptor-channel antagonists may actually be mediated via activation of dopaminergic and serotonergic pathways because, for example, systemic administration of phenylcyclohexane was shown to increase both dopamine (Steinpreis and Salamone, 1993) and 5-HT levels (Millan et al., 1999) in the nucleus accumbens, as measured by in vivo microdialysis. Nevertheless, the role of amino acid neurotransmitter systems in the neurochemical and behavioral effects of 4-methylaminorex remains to be elucidated.

As evident from Fig. 8, the concentrations of both trans-isomers, especially the isomer trans-4R,5R, were higher than those of the cis-isomers in the dialysate. The same phenomenon was also seen in brain tissue and plasma samples obtained from rats treated with i.p. injections of the 4-methylaminorex isomers in our previous study (Kankaanpää et al., 2001b). One possible explanation for the differences observed may be the rapid conversion of one isomer to another, but there were no signs of cis-isomers after administration of trans-isomers or vice versa, this seems rather unlikely. Massive conversion to an unknown metabolite is not a very plausible explanation either, because racemic 4-methylaminorex was shown to be excreted predominantly as the parent compound (Henderson et al., 1995). Nevertheless, determination of the concentration of cis- and trans-4-methylaminorex revealed that the relative inefficacitvess of trans-4R,5R was not due to pharmacokinetic factors, because its concentrations in the samples were at least as high as those of any other isomers.

In conclusion, the present results show that the isomers of 4-methylaminorex, with the exception of trans-4S,5R, elevate both extracellular 5-HT and dopamine to approximately the same extent in the nucleus accumbens. Consistent with previous studies, the isomer trans-4S,5S induced most profound dopaminergic and behavioral effects, the former of which implies strong potential for abuse, and thus classification of this isomer in schedule I. Regarding the role of 5-HT, it seems that serotonergic-like behaviors occur only after very strong elevation of synaptic 5-HT-levels, or under conditions when serotonergic effect is more profound than dopaminergic effect. Taken together, it seems that behaviors induced by the 4-methylaminorex isomers result from an interplay of at least dopaminergic and serotonergic systems, perhaps with serotonergic dominance at high doses of the most efficacious isomers. Because the brain concentrations of the isomers do not reflect their neurochemical efficacy, it seems that the differences between the isomers are pharmacodynamic rather than pharmacokinetic.

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References


Address correspondence to: Aino Kankaanpää, Drug Research Unit, Department of Mental Health and Alcohol Research, National Public Health Institute, Mannerheimintie 166, FIN-00300 Helsinki, Finland. E-mail: aino.kankaanpaa@ktl.fi