PHARMACOKINETICS AND TISSUE DISTRIBUTION OF THE STEREOISOMERS OF
4-METHYLAMINOREX IN THE RAT

Esa Meririnne, Satu Ellermaa, Aino Kankaanpää, Ali Bardy, Timo Seppälä

Drug Research Unit, Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland (E.M., S.E., A.K., T.S.); National Agency for Medicines, Helsinki, Finland (A.B.).
Pharmacokinetics and Tissue Distribution of 4-Methylaminorex

Esa Meririnne MD
Drug Research Unit, Department of Mental Health and Alcohol Research, National Public Health Institute, Mannerheimintie 166, FIN-00300 Helsinki, Finland
P.: +358-9-47441
F.: +358-9-4744 8553
esa.meririnne@ktl.fi

22 text pages
2 tables
2 figures
24 refs.

250 words in Abstract
750 words in Introduction
1124 words in Discussion

AUC, area under the curve; AUMC, area under the first moment curve; Cl, total clearance; Cmax, peak concentration; Fbioav, bioavailability; GC/MS, gas chromatography/mass spectrometry; k, rate constant; kab, kdi, and kel, absorption, distribution, and elimination rate constants, respectively; MRT, mean residence time; Vdss, volume of distribution at steady state; Tmax, time to reach the peak concentration; T½, half-life; T½ab, T½di, and T½el, absorption, distribution, and elimination half-lives, respectively.

Absorption, Distribution, Metabolism, & Excretion
Abstract

4-Methylaminorex, a potential psychostimulant drug of abuse, exists as 4 stereoisomers: cis-4R,5S, cis-4S,5R, trans-4S,5S, and trans-4R,5R, which are shown previously to possess stereospecific effects. This study characterized their pharmacokinetic and tissue distribution profiles, and metabolic turnover to norephedrine and norpseudoephedrine, in male Wistar rats. The rats received each isomer intravenously, intraperitoneally or orally, followed by blood sample collection via cannula (pharmacokinetic study), or tissue sample collection at predetermined time points (tissue distribution study). The samples were analyzed for cis- and trans-isomers, and when appropriate for norephedrine and norpseudoephedrine, with gas chromatography/mass spectrometry. Trans-4S,5S-, cis-4R,5S-, and cis-4S,5R-isomers behaved kinetically comparably (volume of distribution 1.7-2.3 l/kg, distribution half-life 3.8-7.0 min, elimination half-life 35-42 min, and bioavailability 32-57% intraperitoneally or 4-16% orally), whereas trans-4R,5R-isomer differed from the others, with a longer elimination half-life (118-169 min) and higher bioavailability (100% intraperitoneally or 83% orally). The highest isomer concentrations were observed in the kidney followed most frequently by the liver, brain, muscle, and lastly by fat and blood. The elimination half-lives of the stereoisomers from the tissues were generally similar to those in blood. No pharmacologically significant amounts of norephedrine or norpseudoephedrine were detected in blood or the brain. In conclusion, differences between the stereoisomers of 4-methylaminorex in the pharmacokinetics and tissue distribution are described. However, these differences are not compatible with, and thus may not account for, the distinct behavioral and neurochemical effects of the stereoisomers demonstrated previously. Furthermore, metabolic turnover to norephedrine and norpseudoephedrine does not appear to contribute significantly to 4-
JPET #60053
methylaminorex pharmacology.
The phenylisopropylamine derivative 4-methylaminorex (2-amino-4-methyl-5-phenyl-\(\Delta^2\)-oxazoline) is a sympathomimetic agent that has been found on the clandestine market with street names such as ‘U4Euh’ and ‘Ice’ (Davis and Brewster, 1988; Klein et al., 1989; Gaine et al., 2000). The drug has been misrepresented by dealers as cocaine or methamphetamine (Davis and Brewster, 1988), and anecdotal evidence describes it as inducing stimulant-like effects such as euphoria, vigor, restlessness, tremor, tachycardia, increase in blood pressure etc. Also an overdose fatality has been attributed to 4-methylaminorex abuse (Davis and Brewster, 1988). More recently, Gaine et al. (2000) describe a case series of patients suffering from pulmonary hypertension, a disease with poor prognosis, caused by 4-methylaminorex. 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.

4-Methylaminorex exists as 4 stereoisomers: these are cis-4R,5S, cis-4S,5R, trans-4R,5R, trans-4S,5S, which can be synthesized from pseudoephedrine (cis-isomers) and norpseudoephedrine (trans-isomers) (Poos et al., 1963; Klein et al., 1989). The racemic cis-4-methylaminorex has been earlier reported to be the most frequently encountered form in illicit samples (Klein et al. 1989, Gaine et al. 2000), and subsequently the cis-isomers were classified in schedule I in the United States (Federal Register, Schedules of Controlled Substances, 1989). While being non-scheduled, however, the trans-isomers possess similar properties in animal studies with the cis-isomers and other psychostimulant drugs of abuse, trans-4S,5S-
isomer being even more potent than the other isomers (Glennon and Misenheimer, 1989; Batsche et al., 1994; Ashby et al., 1995; Kankaanpaa et al., 2002), and instructions for their synthesis are readily available (Poos et al., 1963; Klein et al., 1989), which altogether render them a potential alternative among drugs of abuse. Accordingly, experiences of alleged trans-4-methylaminorex intake can be found in the Internet.

Marked differences in potency, as mentioned briefly above, have been observed between the isomers in neurochemical and behavioral animal studies. Trans-4S,5S-isomer is the most potent isomer followed by the equally effective cis-isomers, while trans-4R,5R-isomer is relatively ineffective (Glennon and Misenheimer, 1989; Batsche et al., 1994; Ashby et al., 1995; Kankaanpaa et al., 2002). The behavioral response profile of the isomers may also differ; for instance, trans-4R,5R-isomer induced behavioral activation with slower onset than the other 3 isomers, and cis-4S,5R-isomer elicited locomotor activity while the equipotent cis-4R,5S-isomer caused mainly stereotyped behavior at a similar dose (Glennon and Misenheimer, 1989; Ashby et al., 1995; Kankaanpaa et al., 2002).

Given that the effects of individual stereoisomers are clearly distinct, the question also arises whether there are differences in the pharmacokinetics of the isomers. There are, however, only limited data available on the kinetic and metabolic properties of the stereoisomers of 4-methylaminorex. Somewhat surprisingly, our previous studies show that the concentrations of the least effective isomer trans-4R,5R might be higher than those of the other isomers in rat brain dialysate or tissue samples at given time points (Kankaanpaa, et al. 2001; Kankaanpaa et al., 2002). The published data on the metabolism of 4-methylaminorex are limited to a single
JPET #60053

study. Henderson et al. (1995) showed that a mixture containing mainly the cis-isomers was excreted in rat urine much as unchanged drug (60 % of total excretion) with 3 metabolites present: 2 pharmacologically poorly characterized oxazoline-derivatives, and the synthetic precursor norephedrine. It is not known whether norpseudoephedrine or other metabolites are formed from the trans-isomers.

In general, the pharmacokinetics of a drug is essential in comprehensive understanding of the characteristics of its abuse. Harmful patterns of consumption can be predicted by means of pharmacokinetic parameters such as bioavailability, volume of distribution, and half-life (Quinn et al., 1997). The stereospecific pharmacokinetics of a drug has also an impact on analyses and evaluations of clinical and forensic interest, and in particular, it may account for the drug’s stereospecific actions. Consequently, the aim of this study was to characterize the stereospecific pharmacokinetics and tissue distribution patterns of the isomers of 4-methylaminorex, a potential psychostimulant drug of abuse. Pharmacokinetic data were used to evaluate kinetic differences between the isomers, and to understand the time course of blood concentrations in the overall pharmacological effects of the isomers. This was supplemented with a tissue distribution study to gain further insight into kinetic behavior and its differences between the isomers. Finally, concentrations of norephedrine and norpseudoephedrine in blood and the brain were measured to evaluate their contribution to 4-methylaminorex pharmacology.
Methods

Drugs and Chemicals

The four optical stereoisomers (\textit{trans}-4R,5R, \textit{trans}-4S,5S, \textit{cis}-4R,5S, and \textit{cis}-4S,5R) of 4-methylaminorex were prepared at the Laboratory of Organic Chemistry, University of Helsinki, Helsinki, Finland, using the synthesis methods described previously (Poos et al., 1963; Klein et al., 1989). The identity of the isomers was confirmed by determining their melting points and rotation angles, and the $^1$H NMR and $^{13}$C NMR spectra. For animal experiments, the isomers were dissolved in a small volume of 2 M HCl, the pH was adjusted to physiological level with 2 M NaOH, and the solution was then brought to volume with purified water. The amounts of HCl and NaOH were calculated so that the final drug solution was roughly isotonic physiologically. Doses were calculated as free base, and the drugs were administered at a volume of 1 ml/kg (intravenous and intraperitoneal injections) or 5 ml/kg (oral administration).

Norpseudoephedrine hydrochloride was donated by the National Institute on Drug Abuse (NIDA; Bethesda, MD, USA) and norephedrine hydrochloride by the Orion Corporation (Espoo, Finland). Methylmexiletine and carbamazepine were obtained from Boehringer Ingelheim GmbH (Ingelheim am Rhein, Germany), and J.R. Geigy A.G. (Basel, Switzerland), respectively. \textit{N}-(\textit{tert}-butyldimethylsilyl)-\textit{N}methyltrifluoroacetamide (\textit{N}-methyl-\textit{N}-\textit{t}-butyldimethylsilyl trifluoroacetamide, MTBSTFA) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA), and heptafluorobutyric anhydride (HFBA) from Fluka Chemie GmbH (Buchs, Switzerland). The other common reagents used were of the highest
Animals

Adult male Han:Wistar rats weighing 250-350 g (pharmacokinetic study) or 300-400 g (tissue distribution study) were used. The rats were obtained from Harlan Nederland B.V., Horst, the Netherlands, at least 1 week prior to the experiments, and they were housed in a temperature-controlled room (22 ± 2 °C) with a light cycle of 12 h. The lights were on from 6:00 a.m. to 6:00 p.m., during which time all the experiments were conducted. The animals had free access to standard laboratory chow (Altromin® Nr. 1314, Chr. Petersen A/S, Ringsted, Danmark) and tap water, unless otherwise stated. The local Institutional Animals Care and Use Committee, and the chief veterinarian of the county administrative board approved the experiments, which were conducted according to the ‘European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes’.

Pharmacokinetic Experiments

At least 6 days prior to the experiments, the rats underwent surgery for the implantation of vascular cannulas. During the surgery, the rats were anesthetized using halothane gas (Halothane Liquid®, Rhodia Ltd., Bristol, UK). The right jugular vein was exposed and cannulated with a heparinized (Heparin Leo® 5000 IU/ml, Leo Pharma A/S, Ballerup, Denmark, diluted with saline to 31 IU/ml) polyethylene tube (PE50) for blood sampling. The tip of the cannula was advanced to the right atrium of the heart. When intravenous
administration so required, the left femoral vein was also exposed and cannulated with a heparinized polyethylene tube (PE10), which was advanced to the inferior vena cava. With other routes of administration, the femoral vein was left uncannulated. The cannulas were then passed under the skin and fixed near the base of the neck. After surgery, the rats received a subcutaneous injection of buprenorphine (0.05 mg/kg; Temgesic® 0.3 mg/ml, Reckitt & Colman Ltd., Hull, UK), and a intravenous bolus of a mixture of sulphadiazine and trimethoprim (50 mg/kg and 10 mg/kg, respectively; Tribrissen® mite 200 mg/ml and 40 mg/ml, Schering-Plough A/S, Farum, Danmark, diluted with saline to 50 mg/ml and 10 mg/ml). The antibiotic mixture was also applied to the wounds. The cannulas were flushed with heparinized saline every other day.

On the test day, the rats received intravenously, orally, or intraperitoneally a single bolus of 1 isomer at the dose of 2 mg/kg. This dose was chosen because some animals had died after intravenous administration of trans-4S,5S-isomer at higher doses in preliminary experiments. The intravenous injection was given slowly (30 sec) via the femoral vein-cannula. For oral administration, an oral gavage with a syringe was used in rats that had fasted for at least 4 hours. The intraperitoneal injection was given into the left lower quadrant of the abdomen. Blood samples (150 µl) were drawn into a heparinized syringe via the jugular vein-cannula as follows: 0 (2-10 min prior to drug administration), 2, 10, 20, 30, 40, 60, 80, 150, 180, 210, and 240 min after the drug administration. The collected blood volume was replaced with 150 µl of saline after each sample. The blood samples were stored in vials at +5 °C and analyzed for 4-methylaminorex concentrations within 1-5 days. According to our preliminary experiments blood sample concentrations would remain sufficiently stable during this period of storage.
When measured in the same sample at 1-week interval, the relative isomer concentrations on day 7 were as follows (as percentage of day 1 concentrations ± 95% probability limits; n=4-6):

94 ± 11% for cis-4R,5S, 109 ± 9% for cis-4S,5R, 107 ± 15% for trans-4S,5S, and 106 ± 11% for trans-4R,5R.

Tissue Distribution Experiments

On the test day, the rats received a single bolus of 1 isomer at the dose of 5 mg/kg intraperitoneally, into left lower quadrant of the abdomen. At time points of 30, 60, 100, 150, 210, or 280 min after the injection, the rats were lightly sedated with carbon dioxide and decapitated. In our preliminary tests this light exposure to carbon dioxide did not affect the 4-methylaminorex concentrations in tissues. Immediately after the decapitation, trunk blood was collected in a heparinized tube. The brain, liver, left kidney, and samples of pectoral muscle and subcutaneous fat were then dissected out and placed in vials. The blood and tissue samples were stored at +5 °C until analyzed within a few days.

Chemical Analyses

The concentrations of the stereoisomers of 4-methylaminorex were determined from blood and the tissues with gas chromatography/mass spectrometry (GC/MS). In addition, blood and brain tissue samples were analyzed for the presence of the 4-methylaminorex active metabolites, norephedrine and norpseudoephedrine.
Tissue samples required pre-treatment prior to extraction. They were weighed and homogenized in a 4-fold quantity of freshly made 0.1 M HClO$_4$. After centrifugation the supernatants of brain tissue and fat samples were washed with 5 ml of $n$-hexane, while the other tissues needed no further processing before extraction.

The quantitative determination of cis- and trans-4-methylaminorex concentrations in blood and the tissue samples was performed essentially as described previously (Kankaanpaa et al., 2001). The method used distinguishes the cis- and trans-isomers by their retention times, but not the 2 cis-isomers from each other, or the 2 trans-isomers from each other. Briefly, a 100-µl (pharmacokinetic study) or 1-ml (tissue distribution study) aliquot of blood, or a 1-ml aliquot of pre-treated tissue sample was mixed with 1 ml of 0.5 M NaOH and 5 ml of toluene containing carbamazepine (8 µg/100 ml) as an internal standard. After centrifugation, the toluene layer was evaporated to dryness, and the derivatization reagent, 120 µl of MTBSTFA/acetonitrile (1:6), was added to the residue. After a 30-min incubation at 80°C, a 1-µl aliquot of the mixture was injected into the GC/MS apparatus consisting of an Agilent 5890 Series GC System and Agilent 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, CA, USA). The system was operated in the splitless injector mode. The GC column was a DB-35MS 30 m long, with an internal diameter of 0.32 mm and a film thickness of 0.25 µm (J&W Scientific Inc., Folsom, CA, USA). Helium was used as the 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.
The extraction recovery was determined from spiked tissue samples as follows: an aqueous solution of 4-methylaminorex (\textit{cis}-4S,5R; 0.010 mg/ml, 50 µl) was injected into several loci in the intact tissue sample with a hypodermic needle. After over-night incubation at +5°C, the tissue samples were pre-treated and extracted as described above. The blood samples were spiked and extracted as usual. The extraction recovery (n=7-10) from blood, brain, liver and kidney ranged from 77% to 81% compared to plasma samples, while the corresponding values for muscle and fat were 89% and 107%, respectively. Based on these results it was concluded that standard curves constructed from spiked rat plasma samples could be used to determine 4-methylaminorex in all the matrices studied, with tissue-specific correction made according to the extraction recovery. The rest of the validation data correspond to that presented earlier (Kankaanpaa et al., 2001), except that the limit of quantification was set at 0.020 µg/ml.

Norephedrine and norpseudoephedrine were extracted by mixing 1 ml of the sample (blood or pre-treated brain tissue) with 1 ml 0.5M NaOH and 5 ml of toluene, with methylmexiletine as the internal standard (0.500 µg per sample). After centrifugation and separation of the phases, the derivatization reagent, 8 µl of HFBA per sample, was added to the toluene layer, vortexed, and washed with 1 ml of saturated NaHCO₃. The mixture was then centrifuged and the toluene layer evaporated to dryness, after which the dry residue was dissolved in 100 µl of toluene, and injected into the GC/MS apparatus at a volume of 3 µl. The apparatus and the analysis conditions were comparable to those described above, except that the GC column was a DB-5MS 30 m long, with an internal diameter of 0.32 mm and a film thickness of 1 µm (J&W Scientific Inc., Folsom, CA, USA). The limit of quantification was set at 0.010 µg/ml. RSD was 4.8% at a concentration level of 0.050 µg/ml, and 8.5% at the limit of quantification.
Pharmacokinetic and Statistical Analyses

The data from the pharmacokinetic study were analyzed using model-independent methods. When appropriate, compartmental analyses were also used to obtain rate constants for absorption, distribution, and elimination (k_{ab}, k_{di}, and k_{el}, respectively). The data were fitted using the non-linear least squares fitting method of the Systat® version 10 program (SPSS Inc., Chicago, IL, USA). The goodness of fit and the most appropriate model were determined by assessing the randomness of the scatter of actual data points around the fitted function, and by using Akaike’s information criterion (Akaike 1976). The intravenous and intraperitoneal data were best described with a two-compartmental model and a one-compartmental model with the absorption phase, respectively. No model could be fitted with the oral data of the isomers, with the exception for trans-4R5R. The peak concentration (C_{max}) and time to reach it (T_{max}) were taken directly from the data. The area under the curve from 0 min to 240 min (AUC_{0-240}) or to infinity (AUC_{0-\infty}), and the area under the first moment curve from 0 min to infinity (AUMC_{0-\infty}) were calculated using the trapezoidal method. The terminal area from the last sampling point to infinity was extrapolated using the k_{el} value. The mean residence time (MRT), bioavailability (F_{bioav}; using the average AUC_{0-240} of the intravenous data as the reference value), total clearance (Cl), volume of distribution at steady state (V_{dss}), absorption half-life (T_{1/2ab}), distribution half-life (T_{1/2di}), and elimination half-life (T_{1/2el}) were calculated using standard formulas (Rowland and Tozer, 1995).

In the tissue distribution study, pharmacokinetic parameters for blood and the tissues were
calculated with average 4-methylaminorex concentrations at each time point. The tissue-
specific rate constant k for the declining phase was calculated using the non-linear least
squares fitting method as described above. The best fit in all the tissues was obtained with the
weighting factor 1/(observed concentration)². T½ and AUC₃₀⁻₁₅₀ were likewise calculated as
described above.

Statistical analyses were performed using the one-way ANOVA or Kruskall-Wallis tests. One-
way ANOVA was followed by Bonferroni’s test, adjusted for the appropriate number of
comparisons. The two-sample t-test, Mann-Whitney test, and Pearson correlation were
calculated when appropriate. Data are expressed as mean ± SEM or median (min-max).
Results

Pharmacokinetics of the 4-Methylaminorex Isomers

The blood concentration-time profiles of the stereoisomers of 4-methylaminorex after intravenous, intraperitoneal and oral routes of administration are illustrated in Figure 1. The pharmacokinetic parameters calculated from the data are summarized in Table 1.

The kinetic behavior of trans-4S,5S-, cis-4R,5S-, and cis-4S,5R-isomers in blood was virtually the same, whereas that of trans-4R,5R-isomer differed markedly from the others. The most marked differences were observed in the elimination kinetics. The elimination of trans-4R,5R-isomer was approximately 3 times slower than that of the others (T½el 119-129 min vs. 35-42 min; MRT 162 min vs. 41-52 min). It needs to be emphasized that, although the most optimal sampling period for trans-4R,5R-isomer may had been longer than the used 240 min, appropriate and valid fit was obtained with all the isomers. One-way ANOVA showed statistical differences between the isomers in the following parameters: Cl [F(3.17)=5.0, p=0.011 (intravenous data)], kₐ [F(3.17)=12.7, p<0.001 (intravenous data); F(3.11)=3.9, p=0.040 (intraperitoneal data)], T½ₑ [F(3.17)=41.8, p<0.001 (intravenous data); F(3.11)=15.1, p<0.001 (intraperitoneal data)], and MRT [F(3.17)=60.4, p<0.001 (intravenous data)]. According to post hoc comparisons, most often trans-4R,5R-isomer differed from all the others. The elimination kinetics remained the same, regardless of whether the isomers were administered intravenously or intraperitoneally.
The absorption and distribution of the isomers after intraperitoneal and intravenous administration, respectively, occurred with half-lives of a few minutes ($T_{1/2}$ ab 1-5 min; $T_{2di}$ 4-7 min). No distribution phase could be distinguished from the intraperitoneal data, as it was probably partly masked by the absorption phase. Nevertheless, the intravenous data suggest relatively extensive distribution ($V_{dss}$ approximately 2 l/kg). There were no differences between the isomers in the absorption or distribution kinetics after intraperitoneal and intravenous administration. After intraperitoneal and oral administrations, one-way ANOVA showed differences between the isomers as follows: $F_{bioav}$ [$F(3.11)=7.4$, $p=0.006$ (intraperitoneal data); $F(3.14)=87.3$, $p<0.001$ (oral data)], and $C_{max}$ [$F(3.11)=4.3$, $p=0.031$ (intraperitoneal data); $F(3.14)=58.8$, $p<0.001$ (oral data)]. Again, post hoc comparisons revealed that trans-4R,5R-isomers differed from the others. After oral administration, in particular, the $F_{bioav}$ values for trans-4S,5S-, cis-4R,5S-, and cis-4S,5R-isomers were low (4-23%). The corresponding values were significantly higher, but not maximal, after oral administration (32-57%) [$t(6)=3.4$, $p=0.015$ (cis-4R,5S); $t(6)=6.2$, $p=0.0008$ (cis-4S,5R); $t(7)=3.4$, $p=0.012$ (trans-4S,5S); two-sample t-test]. By contrast, the $F_{bioav}$ values of trans-4S,5S-isomer were high after both routes of administration (83-100%), reflecting relatively negligible presystemic metabolism of the isomer.

Tissue Distribution of the 4-Methylaminorex Isomers

The blood and tissue concentrations of the stereoisomers of 4-methylaminorex after intraperitoneal administration are illustrated in Figure 2. The tissue-specific pharmacokinetic parameters are presented in Table 2.
Analogously with the blood concentrations, the tissue concentrations of the *trans*-4R,5R-isomer were markedly higher than those of the other isomers, which in turn were mostly similar to each other. The highest concentrations were found typically in the kidney followed by the liver, brain and muscle. The lowest concentrations were in the fat and blood. One exception was the liver concentration of *cis*-4R,5S-isomer, which was lower than the brain concentration of the same isomer, and at least 4-fold lower than the liver concentrations of the other isomers. Penetrability into the tissues, expressed as AUC$_{30-150\text{tissue}}$/AUC$_{30-150\text{blood}}$-ratio, was almost identical between the isomers, although the ratios for the *trans*-4R,5R-isomer appeared to be somewhat lower. As suggested by the low liver concentration, the penetrability of the *cis*-4R,5S-isomer into this organ was lower than that of the other isomers. The elimination rate of the isomers from the tissues, expressed as T½ of the declining phase, paralleled that in blood closely. An exception was T½ of the *trans*-4R,5R-isomer in the kidney, which was over twice as long as that in blood. Finally, the blood concentrations of an isomer correlated well with the concentrations in a given tissue [R=0.73-0.99, p≤0.004-0.001, depending on the isomer and tissue].

Blood and brain tissue samples from 1-3 rats per isomer were analyzed for the presence of the active 4-methylaminorex metabolites norephedrine and norpseudoephedrine. Norephedrine was found occasionally only in the samples taken from the rats treated with the *cis*-isomers, while norpseudoephedrine was likewise found only in the rats treated with the *trans*-isomers. However, the concentrations of these metabolites were almost always below the limit of quantification (0.01 μg/ml or μg/g). The measured concentrations were as follows:
norephedrine in the brain 0.016 µg/g (30 min) after *cis*-4R,5S, and 0.035 µg/g (30 min) and 0.031 µg/g (60 min) after *cis*-4S,5R; norpseudoephedrine in blood 0.014 µg/g (120 min) and 0.011 µg/g (150 min) after *trans*-4S,5S; norpseudoephedrine in the brain 0.039 µg/g (30 min) after *trans*-4R,5R. Furthermore, concentrations below the quantification limit were observed as follows: norephedrine in blood (30 min and 60 min) after *cis*-4S,5R-isomer; norpseudoephedrine in blood (30 min) after *trans*-4S,5S. Because the highest concentrations measured were generally lower than those observed after the administration of norephedrine or norpseudoephedrine at pharmacologically relevant doses (Frosch, 1977; Meyer and Portmann, 1982; Coutts et al., 1984; Henderson and Fuller, 1992; Kamakura and Satake, 1998), the samples from the rest of the rats were not analyzed.
The present paper describes differences between the stereoisomers of 4-methylaminorex in the pharmacokinetics and tissue distribution. \textit{Trans}-4S,5S-, \textit{cis}-4R,5S-, and \textit{cis}-4S,5R-isomers behaved somewhat comparably, each demonstrating low oral bioavailability, but extensive distribution with good penetrability into the brain, and relatively short elimination half-life, whereas \textit{trans}-4S,5S-isomer differed from the others, with high oral bioavailability and over 3-fold longer elimination half-life. The typical rank order of the isomers’ tissue concentrations was kidney>liver>brain>muscle>fat≈blood. The elimination rates in these tissues usually paralleled those in blood. Finally, the metabolites norephedrine and norpseudoephedrine were detectable, but only at low concentrations, in blood and the brain.

Pharmacokinetics and tissue distribution properties are important factors determining patterns of drug consumption. High potential for harmful use with persistent self-administration can be predicted for a drug with high F\textsubscript{bioav}, good penetrability to the brain, short T\textsubscript{1/2} and high free drug Cl (Quinn et al., 1997). This profile agrees with the kinetic properties of \textit{trans}-4S,5S-, \textit{cis}-4R,5S-, and \textit{cis}-4S,5R-isomers administered intravenously, but not orally, as this route demonstrated low F\textsubscript{bioav}. Indeed, the pharmacokinetic parameters of the 3 isomers calculated from the intravenous data correspond to those reported previously for amphetamine, methamphetamine, and MDMA (Cho et al., 1990; Hutchaleelaha et al., 1994; Riviere et al., 2000). This parallel prompts speculation on whether the consumption patterns of these isomers would resemble abuse habits characteristic of highly addictive amphetamines.
Unlike the other isomers, trans-4R,5R-isomer demonstrated over 3-fold longer \( T_{\frac{1}{2}} \) and markedly better \( F_{\text{bioav}} \) after intraperitoneal and oral administrations. These findings confirm and expand upon the results of our earlier studies, in which the concentrations of trans-4R,5R-isomer appeared to be higher than those of the other isomers in rat brain microdialysis samples, or in rat serum and brain at a single time point (Kankaanpaa et al., 2001; Kankaanpää et al., 2002). Thus, the rank order of blood and brain concentrations of the stereoisomers was trans-4R,5R- > cis- 4R,5S- ≈ cis-4S,5R- ≈ trans-4S,5S-isomer. This is clearly incompatible with the rank order of potency trans-4S,5S- > cis- 4R,5S- ≈ cis-4S,5R- > trans-4R,5R-isomer observed in previous behavioral and neurochemical studies (Glennon and Misenheimer, 1989; Batsche et al., 1994; Ashby et al., 1995; Kankaanpaa et al., 2002). The dissociation of in vivo drug effects from pharmacokinetics is further evidenced by the observation that trans-4R,5R-isomer administered intraperitoneally or subcutaneously induced behavioral activation more slowly than the other isomers (Glennon and Misenheimer, 1989; Batsche et al., 1994; Kankaanpaa et al., 2002), although their \( k_{\text{abs}} \)s and \( T_{\frac{1}{2}} \)s are equal, indicating a similar rate of absorption. Furthermore, we found no differences between the concentrations of the cis-isomers in blood, brain or virtually any other tissue, despite the finding that they yielded qualitatively a different behavioral response at equipotent doses: locomotor activity suffused after cis-4S,5R-isomer, contrasted with stereotyped behavior after cis-4R,5S-isomer (Batsche et al., 1994). Thus, if our findings are considered jointly with the previous data, it appears that the differences between the stereoisomers in both potency and behavioral response profile are not due to pharmacokinetic factors.

There were marked differences in the distribution of the stereoisomers among the tissues. The
highest concentrations with all the stereoisomers were found in the kidney, which is compatible with the previous suggestion that the 4-methylaminorex elimination occurs primarily via this organ, with roughly 60% of total excretion as unchanged drug (Henderson et al., 1995). The next highest concentrations were mostly found in the liver, except for cis-4R,5S-isomer, which showed very low liver concentrations, followed by brain, muscle and then equal concentrations in fat and blood. In almost all the tissues the elimination rate of the isomer paralleled rather closely that in blood, which indicates that no unexpected accumulation should occur in these tissues with repeated administration. An exception was the kidney concentration of trans-4R,5R-isomer, which fell over twice as slow as that in blood. As this isomer also showed the slowest elimination rate from blood and the other tissues, it would be tempting to speculate that trans-4R,5R-isomer may penetrate more poorly than the others into urine, thereby resulting in the slower elimination.

Little is currently known about the metabolic fate of 4-methylaminorex. After the administration of a mixture containing mainly the cis-isomers, 3 metabolites were recognized in rat urine: the synthetic precursor norephedrine, and 2 pharmacologically poorly characterized oxazoline-derivatives, 5-phenyl-4-methyl-2-oxazolidinone and 2-amino-5-(p-hydroxyphenyl)-4-methyl-2-oxazoline (Henderson et al., 1995). It was not possible to analyze these oxazolines because of the lack of reference standards, but norephedrine was found as a metabolite in blood and the brain after the cis-isomers, as against norpseudoephedrine that was present only after the trans-isomers. Their concentrations, however, remained below the limit of quantification in most samples. Even when quantified, the concentrations appeared to be low; for instance, when administered at behaviorally relevant doses, norephedrine
concentrations were at least 40-fold higher in rat brain, and 20-fold higher in blood than in our study (Coutts et al., 1984; Henderson and Fuller, 1992; Kamakura and Satake, 1998).

Although no such precise data on norpseudoephedrine are available, it seems quite unlikely that its effective concentrations are dozens of times smaller than those of norephedrine. Certainly concentrations that release dopamine from rat brain tissue in vitro (Kalix, 1983; Kalix et al., 1987), or concentrations considered therapeutic in human plasma after a single drug dose (Meyer and Portmann, 1982) are several times higher than the highest concentrations in our study. Taken together, it appears that the cis- and trans-isomers can be metabolized into norephedrine and norpseudoephedrine, respectively, but their contribution to 4-methylaminorex pharmacology is probably of minor significance.

It should be noted that the analytical method used in the present study distinguishes the cis- and trans-isomers by their retention times, but makes no distinction between the enantiomers (2 cis- or 2 trans-isomers). The current method was considered applicable to our purposes for 2 reasons, however. Firstly, each stereoisomer was administered separately in our study, and secondly, no signs of cis-isomer were found after the administration of a trans-isomer, or vice versa, thereby ruling out the conversion of 1 isomer into another.

In summary, the present paper describes the differences in pharmacokinetics and tissue distribution between the stereoisomers of 4-methylaminorex, a potential psychostimulant drug of abuse. Trans-4S,5S-, cis- 4R,5S-, and cis-4S,5R-isomers behaved kinetically somewhat comparably, whereas trans-4R,5R-isomer differed from the others in having high oral Fbioav, longer T½ el, and higher tissue concentrations. However, these differences are not compatible...
JPET #60053

with, and thus may not account for, the distinct pharmacodynamic profiles of the isomers observed in previous research. Furthermore, although the metabolic turnover of the isomers to norephedrine and norpseudoephedrine was evident, due to the low concentrations this may not significantly contribute to 4-methylaminorex pharmacology. Taken together, the spatial structure of 4-methylaminorex appears to have a distinct impact on its kinetic, metabolic, and dynamic properties, and these need to be considered in pharmacological and analytical studies.
Acknowledgments

We thank Ms Riitta Husso, Ms Tuuli Kosola, and Ms Satu Klefström for their skillful technical assistance.
References


Federal Register, Schedules of Controlled Substances (1989) Placement of (±) cis-4-
methylaminorex into schedule I. 54: 14799-14800.


This work was supported by the Yrjö Jahnsson Foundation, Helsinki, Finland.

Esa Meririnne MD
Drug Research Unit
Department of Mental Health and Alcohol Research
National Public Health Institute
Mannerheimintie 166
FIN-00300 Helsinki
Finland
Legends for figures

Figure 1. Blood concentration-time profiles of the stereoisomers of 4-methylaminorex after intravenous, intraperitoneal, and oral routes of administration at the dose of 2 mg/kg (N=3-6). Error bars covered by symbol are not shown.

Figure 2. Concentration-time profiles of the stereoisomers of 4-methylaminorex in blood, and brain, fat, muscle, liver, and kidney tissues after intraperitoneal administration at the dose of 5 mg/kg (N=2-3). Error bars covered by symbol are not shown.
Pharmacokinetic parameters of the stereoisomers of 4-methylaminorex after intravenous, intraperitoneal, or oral administration at the dose of 2 mg/kg.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cis- 4R,5S</th>
<th>Cis-4S,5R</th>
<th>Trans-4S,5S</th>
<th>Trans-4R,5R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{di}$</td>
<td>0.19 ± 0.02</td>
<td>0.26 ± 0.09</td>
<td>0.23 ± 0.1</td>
<td>0.15 ± 0.08</td>
</tr>
<tr>
<td>$T_{1/2di}$ (min)</td>
<td>3.8 ± 0.4</td>
<td>4.3 ± 1.1</td>
<td>5.3 ± 1.5</td>
<td>7.0 ± 1.8</td>
</tr>
<tr>
<td>$V_{dss}$ (l/kg)</td>
<td>2.3 ± 0.4</td>
<td>2.0 ± 0.1</td>
<td>1.7 ± 0.4</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>$k_d$</td>
<td>0.019 ± 0.002</td>
<td>0.019 ± 0.002</td>
<td>0.021 ± 0.002</td>
<td>0.006 ± 0.001***</td>
</tr>
<tr>
<td>$T_{1/2el}$ (min)</td>
<td>38.6 ± 5.1</td>
<td>38.7 ± 3.7</td>
<td>34.5 ± 2.7</td>
<td>118.8 ± 11.4***</td>
</tr>
<tr>
<td>$Cl$ (l/min)</td>
<td>0.013 ± 0.003</td>
<td>0.011 ± 0.002</td>
<td>0.012 ± 0.002</td>
<td>0.004 ± 0.001*</td>
</tr>
<tr>
<td>$MRT$ (min)</td>
<td>51.2 ± 4.6</td>
<td>51.9 ± 3.4</td>
<td>40.6 ± 1.1</td>
<td>161.6 ± 15.6***</td>
</tr>
<tr>
<td>$AUC_{0-240}$ (µg/ml*min)</td>
<td>45.8 ± 5.0</td>
<td>51.3 ± 3.0</td>
<td>51.1 ± 7.0</td>
<td>113.3 ± 5.5***</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (µg/ml*min)</td>
<td>47.6 ± 5.1</td>
<td>52.9 ± 3.1</td>
<td>52.3 ± 7.0</td>
<td>148.0 ± 10.5***</td>
</tr>
<tr>
<td>$AUMC_{0-\infty}$ (µg/ml*min²)</td>
<td>2473 ± 330</td>
<td>2796 ± 283</td>
<td>2120 ± 284</td>
<td>24496 ± 3423***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intraperitoneal administration</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (µg/ml)</td>
<td>0.42 ± 0.08#</td>
<td>0.51 ± 0.06#</td>
<td>0.33 ± 0.05#</td>
<td>0.86 ± 0.14†</td>
</tr>
<tr>
<td>$T_{max}$ (min)</td>
<td>10 (10-10)</td>
<td>12.5 (10-20)</td>
<td>17.5 (10-40)</td>
<td>22.5 (10-30)</td>
</tr>
<tr>
<td>$F_{bioav}$ (%)</td>
<td>46.4 ± 15.7#</td>
<td>56.5 ± 4.1#</td>
<td>32.4 ± 8.7#</td>
<td>100.6 ± 14.2*</td>
</tr>
<tr>
<td>$k_{ab}$</td>
<td>0.77 ± 0.32</td>
<td>0.54 ± 0.23</td>
<td>0.26 ± 0.08</td>
<td>0.30 ± 0.09</td>
</tr>
<tr>
<td>$T_{1/2ab}$ (min)</td>
<td>1.2 ± 0.4</td>
<td>2.7 ± 1.2</td>
<td>4.8 ± 2.7</td>
<td>3.9 ± 1.9</td>
</tr>
<tr>
<td>$k_d$</td>
<td>0.018 ± 0.002</td>
<td>0.022 ± 0.004</td>
<td>0.021 ± 0.006</td>
<td>0.006 ± 0.001†</td>
</tr>
<tr>
<td>$T_{1/2el}$ (min)</td>
<td>39.8 ± 3.4</td>
<td>34.8 ± 3.7</td>
<td>41.9 ± 10.7</td>
<td>128.5 ± 18.3**</td>
</tr>
<tr>
<td>$AUC_{0-240}$ (µg/ml*min)</td>
<td>20.6 ± 5.9</td>
<td>29.0 ± 1.7</td>
<td>16.6 ± 3.5</td>
<td>113.8 ± 12.6***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oral administration</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (µg/ml)</td>
<td>0.06 ± 0.02#</td>
<td>0.14 ± 0.04#</td>
<td>0.08 ± 0.01#</td>
<td>0.63 ± 0.04***</td>
</tr>
<tr>
<td>$T_{max}$ (min)</td>
<td>20 (10-40)</td>
<td>27.5 (10-60)</td>
<td>18 (10-30)</td>
<td>50 (30-80)</td>
</tr>
<tr>
<td>$F_{bioav}$ (%)</td>
<td>4.4 ± 1.2#</td>
<td>15.5 ± 5.2#</td>
<td>6.2 ± 1.0#</td>
<td>83.1 ± 6.9***</td>
</tr>
<tr>
<td>$AUC_{0-240}$ (µg/ml*min)</td>
<td>2.0 ± 0.4</td>
<td>8.0 ± 2.1</td>
<td>3.2 ± 0.4</td>
<td>94.2 ± 6.2***</td>
</tr>
</tbody>
</table>

The data are presented as mean ± SEM or median (min-max). $k_{ab}$, $k_{di}$, and $k_d$ absorption, distribution and elimination rate constants; $T_{1/2ab}$, $T_{1/2di}$ and $T_{1/2el}$ absorption, distribution and elimination half-lives; $Cl$ total clearance; $MRT$ mean residence time; $AUC_{0-240}$ and $AUC_{0-\infty}$ area under the curve from 0 min to 240 min or infinity; $AUMC_{0-\infty}$ area under the first momentary curve from 0 min to infinity; $C_{max}$ and $T_{max}$ observed maximum concentration and
time to reach it; $F_{bioa}$, bioavailability. *p<0.05, **p<0.01, and ***p<0.001 compared to other isomers; †p<0.05 compared to trans-SS-isomer; ‡p<0.05 compared to cis-SR- and trans-SS-isomer (Bonferroni’s test). # p<0.05, # # p<0.01 between the intraperitoneal and oral routes of administration (two-sample t-test).
Table 2.
Pharmacokinetic parameters of the stereoisomers of 4-methylaminorex in blood and tissues after intraperitoneal administration at the dose of 5 mg/kg.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>Cis-4R,5S</th>
<th>Cis-4S,5R</th>
<th>Trans-4S,5S</th>
<th>Trans-4R,5R</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;30-150&lt;/sub&gt; (µg/ml<em>min or µg/g</em>min)</td>
<td>blood</td>
<td>41.7</td>
<td>46.7</td>
<td>76.7</td>
<td>327.6</td>
</tr>
<tr>
<td></td>
<td>brain</td>
<td>328.2</td>
<td>336.8</td>
<td>474.7</td>
<td>1509.7</td>
</tr>
<tr>
<td></td>
<td>fat</td>
<td>42.2</td>
<td>67.5</td>
<td>59.8</td>
<td>209.1</td>
</tr>
<tr>
<td></td>
<td>muscle</td>
<td>111.0</td>
<td>108.0</td>
<td>215.7</td>
<td>611.2</td>
</tr>
<tr>
<td></td>
<td>liver</td>
<td>128.3</td>
<td>560.6</td>
<td>541.2</td>
<td>1983.5</td>
</tr>
<tr>
<td></td>
<td>kidney</td>
<td>911.0</td>
<td>1327.8</td>
<td>1578.8</td>
<td>4053.1</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;30-150&lt;/sub&gt; tissue per AUC&lt;sub&gt;30-150&lt;/sub&gt; blood</td>
<td>brain</td>
<td>7.9</td>
<td>7.2</td>
<td>6.2</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>fat</td>
<td>1.0</td>
<td>1.45</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>muscle</td>
<td>2.7</td>
<td>2.3</td>
<td>2.8</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>liver</td>
<td>3.1</td>
<td>12.0</td>
<td>7.1</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>kidney</td>
<td>21.8</td>
<td>28.4</td>
<td>20.6</td>
<td>12.4</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (min)</td>
<td>blood</td>
<td>29.4</td>
<td>35.4</td>
<td>38.5</td>
<td>169.1</td>
</tr>
<tr>
<td></td>
<td>brain</td>
<td>36.7</td>
<td>49.9</td>
<td>43.3</td>
<td>216.6</td>
</tr>
<tr>
<td></td>
<td>fat</td>
<td>45.3</td>
<td>62.4</td>
<td>53.3</td>
<td>216.6</td>
</tr>
<tr>
<td></td>
<td>muscle</td>
<td>29.6</td>
<td>43.3</td>
<td>35.5</td>
<td>203.9</td>
</tr>
<tr>
<td></td>
<td>liver</td>
<td>26.6</td>
<td>41.0</td>
<td>51.7</td>
<td>161.2</td>
</tr>
<tr>
<td></td>
<td>kidney</td>
<td>39.8</td>
<td>59.2</td>
<td>51.7</td>
<td>364.8</td>
</tr>
</tbody>
</table>

The data are presented as means; no SEM is shown because the data are calculated from the average concentrations. AUC<sub>30-150</sub> area under the curve from 30 min to 150 min; T<sub>1/2</sub> elimination half-life.
Figure 1.

Intravenous administration

- Trans-4R,5R
- Trans-4S,5S
- Cis-4R,5S
- Cis-4S,5R

Intraperitoneal administration

4-Methylinorex concentration in blood (µg/ml)

Oral administration

Time (min)
Figure 2.

The figure shows the concentration of 4-methylaminoxyx in different tissues over time. The tissues include Blood, Brain, Fat, Muscle, Liver, and Kidney. The concentration is measured in µg/ml or µg/g and is plotted on a logarithmic scale. The time is measured in minutes. The plots show the concentration decline over time for different conformations: Trans-4R,5R, Trans-4S,5S, Cis-4R,5S, and Cis-4S,5R.