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Metabolism of cathinone to (−)-norephedrine and (−)-norpseudoephedrine

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S-(−)-Cathinone (*S*-(−)-α-aminopropiophenone) is the major active principle of khat leaves (*Catha edulis*), which are widely used in East Africa and the Arab peninsula as an amphetamine-like stimulant. After oral administration of synthesized cathinone (isomers, racemate), 22–52% was recovered in 24 h urine samples mainly as aminoalcohol metabolites. With GC/MS, HPLC and CD, the main metabolite of *S*-(−)-cathinone was identified as *R*/*S*-(−)-norephedrine and the main metabolite of *R*-(+)-cathinone as *R*/*R*-(−)-norpseudoephedrine. Both aminoalcohols are formed by a stereospecific keto reduction.

Phytochemical, analytical and pharmacological studies (Schorno 1979; Schorno & Steinegger 1979; Zelger et al 1980; Schorno et al 1982; Brenneisen et al 1984; Kalix 1984; Brenneisen & Geisshüsler 1985; Gugelmann et al 1985) have shown that *S*-(−)-cathinone (*S*-(−)-α-aminopropiophenone) is the main active phenylalkylamine of *Catha edulis* Forsk. (khat). The chewing of fresh khat leaves, which produces amphetamine-like CNS-stimulating effects, is very popular in East Africa and South Arabia (Schorno 1982; Kalix 1984).

So far few data on the pharmacokinetics of the 'natural amphetamine' cathinone and other khatamines have been available. Maitai & Mugera (1975) examined the urine of khat consumers and found the phenylpropanolamine *S*/*S*-(+)-norpseudoephedrine to be the principle metabolite. Later experiments with cathinone (Guantai & Maitai 1983) showed the same urinary metabolite, but it is not clear if the racemate or one of the isomers of cathinone was used. Testa & Beckett (1972, 1973, 1974) studied the metabolism of (±)-diethylpropione (Amfepramone D.C.I.), the *N*-diethyl derivative of cathinone, used as an anorectic agent. About 20% of the racemate was metabolized by *N*-dealkylation and reduction to the aminoalcohols *R*/*S*-(−)-norephedrine and *R*/*R*-(−)-norpseudoephedrine.

The purpose of the present work has therefore been to identify the main cathinone metabolites in human urine and to study the possible differences in the metabolic pathway of the optically pure isomers and the racemate of synthetic cathinone.

Materials and methods

General methods

Racemic cathinone hydrochloride was synthesized with a modified version of the Gabriel synthesis using propiophenone as starting material (Schorno 1979; Brenneisen & Geisshüsler 1985). The isomers of cathinone were prepared by separating the racemate with (+)-β-camphorsulphonic acid (Schorno 1979). The optical purity of the cathinone isomers was determined after the method of Berrang et al (1982) by preparing diastereomeric, unsymmetrical ureas and analyzing the products with HPLC. *S*-(−)-Cathinone was 96%, *R*-(+)-cathinone 90% pure.

The drug was administered to three male volunteers orally in the form of capsules, containing *S*-(−)-cathinone oxalate, *R*-(+)-cathinone oxalate or (±)-cathinone hydrochloride, each corresponding to a dose of 24 mg cathinone base. Urine samples were collected over 24 h.

Analytical methods

Extraction and isolation. All chemicals and reagents were of analytical-reagent or HPLC grade. The internal standard solution was prepared by dissolving 136·2 mg of (+)-amphetamine sulphate (Siegfried & Co., Zofingen/Switzerland) in 100·0 ml of water. Of this solution 10·0 ml (corresponding to 10 mg of (+)-amphetamine base) and 10 ml of 2 M hydrochloric acid were added to the 24 h urine samples (1–2 litres). After evaporation under vacuum at 50 °C to about 50 ml, the urine pH was adjusted to >10 with 4 M sodium hydroxide then 400 g of anhydrous sodium sulphate added and the basic compounds immediately extracted several times with a total of 1–2 litres of diethyl ether. The combined organic phases were concentrated under vacuum to about 60 ml and again dried with anhydrous sodium sulphate. After filtration and washing with diethyl ether up to a total filtrate volume of 120 ml, the basic metabolites were precipitated with 10 ml of a 1% w/v solution of oxalic acid in diethyl ether. The precipitation was allowed to stand for 12 h at 5 °C, filtered through a sintered glass funnel and dried under vacuum to give about 30 mg of urine oxalates which were then used for the identification and quantitation of the main metabolites with the aid of GC/MS and HPLC.

For the chiroptical discrimination of the diastereomeric phenylpropanolamines norephedrine and norpseudoephedrine with circular dichroism (CD), the same extraction procedure as described before was used, but without addition of internal standard. The bases prepared from 15–18 mg of the urinary oxalates of subject 1 were further separated by preparative TLC on silica gel 60_{F254} (Merck, 200 × 200 × 2 mm) and with chloroform–methanol (9:1) as solvent system. The

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band with a R_F-value of 0·05–0·1 corresponding to the mixture of norephedrine and norpseudoephedrine was extracted with 0·01 M hydrochloric acid. After evaporation to about 5 ml and adjusting the pH to 10 with 4 M sodium hydroxide, the oxalates were precipitated as described before.

Gas chromatography-mass spectrometry (GC/MS). To prepare the trifluoroacetic acid (TFA) derivatives, 4 mg of the urine oxalates were suspended in 1 ml of benzene and treated for 2 min with ammonia gas. After centrifugation, 500 µl of the supernatant was heated for 30 min at 50 °C with 100 µl of 0·05 M triethylamine in benzene and 10 µl of trifluoroacetic acid anhydride (TFAA). To the cold solution 1 ml of water and, after 1 min shaking, 1 ml of 5% ammonium hydroxide were added. The reaction mixture was shaken again for 5 min, centrifuged and 1 µl of the upper benzene phase used for the GC/MS analysis. The mass spectra were recorded on a Hewlett Packard GC/MS system 5990 A + 5992 + 9825 A at an ionizing potential of 70 eV and a scan range from 45 to 400 amu. The GC conditions were: helium carrier gas at a flow rate of 3 ml min^{-1}, a 25 m fused silica OV-1701 capillary column (Carlo Erba, Milano), and a temperature program from 84° (1·5 min hold) to 170° at 3 °C min^{-1}. Cathinone-TFA had a retention time of 18·1 min and showed a mass spectrum identical with that obtained from synthetic cathinone with the ions *m/z* (rel. intensity) 77 (40), 105 (100), 140 (3). Norpseudoephedrine-TFA had a retention time of 25·1 min and showed a mass spectrum identical with that obtained from synthetic norpseudoephedrine (Fluka & Co., Buchs/Switzerland) with the ions *m/z* 77 (45), 79 (80), 105 (15), 107 (100), 126 (5), 141 (80). Norephedrine TFA had a retention time of 25·4 min and the same mass spectrum as norpseudoephedrine-TFA. Amphetamine-TFA (internal standard) had a retention time of 15·6 min and the ions *m/z* 77 (5), 91 (50), 118 (95), 140 (100).

Circular dichroism (CD). The CD measurement of the isolated norephedrine–norpseudoephedrine mixtures and the reference compounds (in water) was performed on a Russel-Jouan Dichrograph 2 instrument. The percentage of the two diastereomeric forms were determined by calculating the ellipticities ψ in relation to those of the reference compounds: *S/S*-(+)-norpseudoephedrine (ψ_{266} = −146), *R/R*-(−)-norpseudoephedrine (ψ_{266} = +146), *S/R*-(+)-norephedrine (ψ_{266} = −245) and *R/S*-(−)-norephedrine (ψ_{266} = +245). From the *R*-(+)-cathinone experiment with subject 1: ψ_{266} (norephedrine + norpseudoephedrine) = +169; 23% *R/S*-(−)-norephedrine and 77% *R/R*-(−)-norpseudoephedrine (calculated). From the *S*-(−)-cathinone experiment with subject 1: ψ_{266} = +233; 88% *R/S*-(−)-norephedrine and 12% *R/R*-(−)-norpseudoephedrine (calculated).

The racemization rate of cathinone was determined with a solution of 4 mg *S*-(−)-cathinone oxalate and *R*-(+)-cathinone oxalate in 100 ml of water.

High-performance liquid chromatography (HPLC). For the quantitative determination of the main cathinone metabolites, 4 mg of the urine oxalates were dissolved in 1 ml of the HPLC mobile phase. A 10 µl-aliquot was injected into the HPLC system. The further analytical procedure was the same as used by Schorno et al (1982) and Brenneisen & Geisshüsler (1985) for the measurement of khatamines in khat samples. The retention times were: cathinone 10·6 min, (+)-amphetamine (internal standard) 12·4 min, *R/S*-(−)-norephedrine 15·0 min, *R/R*-(−)-norpseudoephedrine 15·9 min.

Results and discussion

As shown in Table 1, orally administered cathinone (α-aminopropiophenone) is metabolized to aminoalcohols by reduction of the C-1 keto group and excreted in the urine. With GC/MS and HPLC these main urinary metabolites could be identified as norephedrine and norpseudoephedrine. From the chiroptical discrimination (CD) it was found that the two diastereomers, isolated from 24 h urine samples, are only present in the (−)-form with 1*R* configuration (Fig. 1: III and IV). By

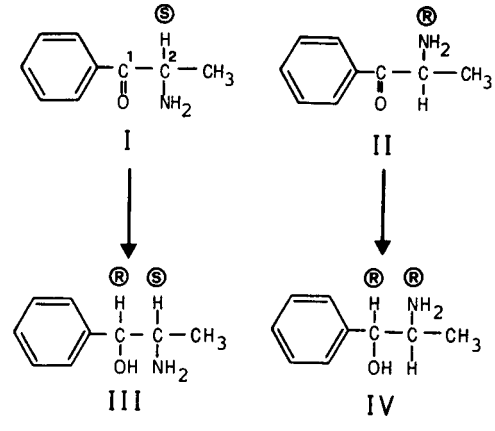

FIG. 1. The structures and the main urine metabolites of *S*-(−)-cathinone and *R*-(+)-cathinone.

(I) (−)-Cathinone

(II) (+)-Cathinone

(III) (−)-Norephedrine

(IV) (−)-Norpseudoephedrine

the *stereospecific* 1*R* keto reduction a second asymmetric centre (C-1) is formed. With the described method 21–50% of the administered cathinone was recovered in the urine as aminoalcohols, 0·6–3·3% as unchanged drug. On comparison of the metabolism of the cathinone-isomers and the racemate, the ratios of the

Table 1. Excretion of cathinone and its aminoalcohol metabolites in 24 h human urine after administration of 24 mg cathinone (isomers, racemate) in capsules.

Compounds administered	Subject	Compounds excreted and recoveries expressed as % of initial dose cathinone (24 mg) Cathione	*R/S*-(−)-Nor-ephedrine	*R/R*-(−)-Norpseudo-ephedrine	Total recoveries
S-(−)-Cathinone	1	2·5	20·1	2·5	25·1
	2	1·8	34·9	5·2	41·9
	3	3·3	30·1	3·7	37·1
R-(+)-Cathinone	1	0·6	5·6	15·8	22·0
	2	1·3	13·8	29·3	44·4
	3	1·8	10·0	24·0	35·8
(±)-Cathinone	1	1·7	12·3	23·0	37·0
	2	1·7	19·3	30·6	51·6
	3	1·6	16·0	29·3	46·9

excreted stereoisomeric metabolites *R/S*-(−)-norephedrine (III) and *R/R*-(−)-norpseudoephedrine (IV) could be clearly differentiated. The 2*S* configurated (−)-cathinone (I) was mainly metabolized at a ratio of 8·8:1·2 (mean value) to the corresponding 1*R*/2*S* configurated erythro-aminoalcohol (−)-norephedrine (III) and to *R/R*-(−)-norpseudoephedrine (IV). The 2*R* configurated (+)-cathinone (II) was mainly metabolized at a ratio of 7·1:2·9 to the corresponding 1*R*/2*R* configurated threo-aminoalcohol (−)-norpseudoephedrine (IV) and to *R/S*-(−)-norephedrine (III). After administration of (±)-cathinone the ratio of the erythro- to the threo-form was 3·6:6·4. The simultaneous occurrence of the corresponding diastereomeric aminoalcohol, e.g. 3·8% (mean value) *R/R*-(−)-norpseudoephedrine after administration of *S*-(−)-cathinone, is due to the fact, that the isomers used are not 100% pure and probably to the partial racemization of the cathinone isomers during absorption and partition. Optically active cathinone racemizes rapidly in hydroxylic solvents (Takamatsu 1956; Berrang et al 1982). We found by CD measurement that the rate of spontaneous racemization of *S*-(−)- and *R*-(+)-cathinone oxalate in water was about 10% over 5 h. The racemization can be explained by the easy enolization of the cathinone's ketoamine structure.

The observed *biotransformation* (reduction and racemization) of the cathinone isomers is therefore *stereoselective*, whereas the *1*R *keto reduction* is *stereospecific*. In this context it is worth mentioning that all naturally occurring neurotransmitters, such as norepinephrine and epinephrine have the same *R* configuration at the corresponding position. Our results further demonstrate that the cathinone-racemate and the racemate of diethylpropione, investigated by Testa & Beckett (1972, 1973, 1974), follow an analogous metabolic pathway and that the amounts of the excreted stereoisomeric aminoalcohols *R/S*-(−)-norephedrine and *R/R*-(−)-norpseudoephedrine are similar.

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