J. Pharm. Pharmac., 1969, **21**, *Suppl.*, 151 S–156 S *Received May* 1, 1969

Urinary excretion of the drug and its main metabolite in man, after the administration of (±)-, (+)- and (−)-ethylamphetamine

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The urinary excretion of ethylamphetamine and its metabolite amphetamine was studied in man after oral administration of (±)-, (+)-, and (−)-ethylamphetamine hydrochloride. The rates of excretion of these amines are dependant on the pH of the urine. At acid values, the (+)-isomer is metabolized faster and to a greater extent than the (−)-isomer, which is excreted mostly unchanged. The effect of alkyl chain length on stereoselective metabolism of *N*-alkylamphetamines is discussed.

The urinary excretion of amphetamine (Beckett & Rowland, 1964; Asatoor, Galman & others, 1965) and methylamphetamine (Beckett & Rowland, 1965 a,b) is influenced by urinary pH, and differences in the metabolism and excretion of the enantiomorphic forms have been investigated (Beckett & Rowland, 1965; Gunne, 1967). The metabolism of ethylamphetamine and its enantiomorphs in man has not been reported previously.

The purpose of the present work is not only to study the metabolism and excretion of ethylamphetamine in man but also to provide information to help evaluate the importance of alkyl chain length in stereoselective metabolism of *N*-alkylamphetamines.

EXPERIMENTAL

Urinary excretion trials

The five male subjects who participated in these trials were given oral doses of ethylamphetamine hydrochloride ranging from 12 to 36 mg in a total of 23 experiments.

All five were given 20 mg of the (±)-form (equivalent to 16·34 mg of the base) in water (50–100 ml) the urinary pH being maintained at acid values. On another occasion three subjects were given the same dose of the drug but the urinary pH was maintained at alkaline values; in a third experiment, the pH of the urine was not controlled.

Five subjects were given an oral dose of (+)-ethylamphetamine hydrochloride and three of (−)-ethylamphetamine hydrochloride (20 mg), the urinary pH being maintained acidic.

The administration of drug, the collection of urine specimens and measurement of urinary pH were as described by Beckett & Rowland (1965a). The dosage regimen of ammonium chloride and sodium bicarbonate for maintaining acidic and alkaline urine respectively was as described by Beckett & Brookes (1967). The urine was collected over 24 h, extracted by the method of Beckett & Rowland (1965a) and analysed by gas liquid chromatography.

Ethylamphetamine and amphetamine (0·1–10 μg/ml) were added to blank urine from some subjects. The two amines (1 μg/ml) were added to acidic and alkaline urines and stored at 4°. The drug content was determined every third day for two weeks.

In addition, some of the ether extracts of the urines from the experiments were treated as follows:—

(a) About 2 μl acetone was added to 5 μl of the concentrate and the solution heated at about 60° for 1 h and injected on the two columns described below.

(b) Two portions of the concentrate were treated separately with acetic and propionic anhydrides, and injected on the two columns.

Gas-liquid chromatography

Analysis using column 1 (below) was as described for amphetamine and methylamphetamine by Beckett & Rowland, (1965a,b) but aletamine hydrochloride (10 μg/ml in water) was added as an internal standard to the urine before extraction.

A Perkin Elmer F11 gas chromatograph with a flame ionization detector was employed, using the following conditions: both columns were of stainless steel 1 m × $\frac{1}{8}$ in o.d.

Column 1. Acid washed DMCS treated Chromosorb G (80–100 mesh) coated with 10% w/w potassium hydroxide and 10% w/w Apiezon L. Column temperature 160°, injection block temperature ca 250°. Nitrogen flow rate 27 ml/min at room temperature. Hydrogen pressure 14 lb/in², air pressure 26 lb/in². Stream split ratio 1 : 5.

Column 2. Acid washed DMCS treated Chromosorb G (80–100 mesh) coated with 2% w/w Carbowax 20M and 5% w/w potassium hydroxide. Column temperature 165°, injection block temperature *ca* 250°. Nitrogen flow rate 33 ml/min at room temperature. Hydrogen pressure 15 lb/in². Air pressure 20 lb/in². Stream split ratio 1 : 5.

Thin-layer chromatography

Preparative thin-layer chromatography (TLC) was used to obtain samples of ethylamphetamine and amphetamine for infra-red analysis.

Glass plates 20 × 20 cm, coated with adsorbant 0·25 mm thick were developed with the following solvent systems. (a) Methanol–acetone (1 : 1); (b) methanol–chloroform (20 : 80); (c) methanol–chloroform (50 : 50).

Silica gel G (Merck) plates were used for systems (a) and (c) and aluminium oxide G (Merck) for System (b). Dragendorff's reagent and solution of bromothymol blue in ethanol were used to visualize the spots.

RESULTS AND DISCUSSION

Linear calibration curves were obtained for ethylamphetamine and amphetamine. No substance interfering with the determination of the amines was found in urine. Both the amines were stable in urine at 4° for at least two weeks.

Structure of the metabolite

Gas-liquid chromatographic analysis of ethereal extracts of urines from the subjects who had taken the drug, gave two peaks representing unchanged drug and its metabolite, amphetamine. This was shown by the identity of retention times of the compounds themselves and of several derivatives with authentic samples. These

were (min): column 1—amphetamine 2·6, ethylamphetamine 4·4, amphetamine + acetone 4·5, amphetamine-acetyl derivative 11·5, amphetamine-propionyl derivative 16·0, ethylamphetamine-acetyl derivative 21·5, ethylamphetamine-propionyl derivative 28·1; column 2—amphetamine-acetyl derivative 7·2, ethylamphetamine-acetyl derivative 5·0.

Identity of Rf values of the drug and the metabolite with authentic samples was demonstrated in systems (a) 0·25/0·47, (b) 0·87/0·50, and (c) 0·43/0·33. Preparative TLC of the two amines from urine gave two products whose infrared spectra were indistinguishable from ethylamphetamine and amphetamine.

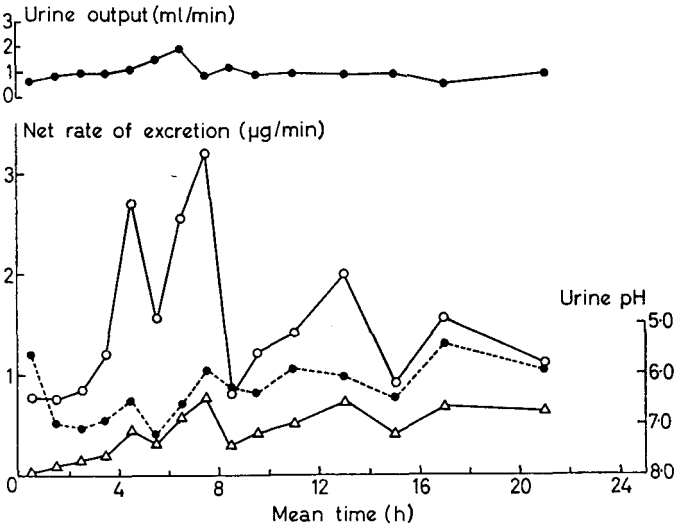

FIG. 1. Urinary excretion of ethylamphetamine and amphetamine over 24 h from subject (4) with no urine pH control, who had taken an oral dose of 20 mg (±)-ethylamphetamine HCl. —○— ethylamphetamine, —△— amphetamine, —●— urine output (ml/min), - -●- - urine pH.

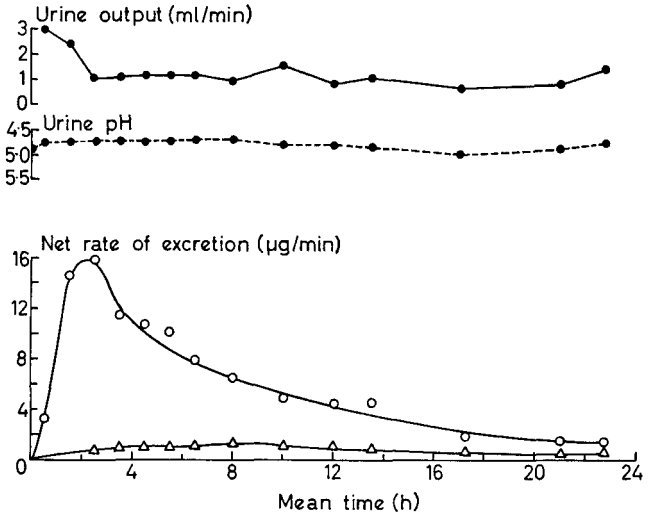

FIG. 2. Urinary excretion of ethylamphetamine and amphetamine over 24 h from a Subject (4) under conditions of acidic urine, who had taken an oral dose of 20 mg (±)-ethylamphetamine HCl. —○— ethylamphetamine, —△— amphetamine, —●— urine output (ml/min), - -●- - urine pH.

Excretion and metabolism

The fluctuations observed in the rate of excretion of (±)-ethylamphetamine and of its main metabolite amphetamine (Fig. 1) were abolished by maintaining the urine at an acidic pH (Fig. 2). When the urinary pH was maintained at alkaline values there was little excretion of the amines (Table 1). The excretion profile of the two

Table 1. *Urinary excretion of ethylamphetamine (EA) and amphetamine (A) over a period of 24 h in subjects receiving an oral dose of 20 mg of (±)-ethylamphetamine hydrochloride under conditions of alkaline and uncontrolled urinary pH*

	Uncontrolled pH				Alkaline pH			
	% dose excreted				% dose excreted			
Subject	EA	A	Total	Ratio EA/A	EA	A	Total	Ratio EA/A
4	12·6	4·4	17·0	2·9:1	0·9	1·2	2·1	0·8:1
2	24·2	10·4	34·6	2·3:1	5·9	3·3	9·2	1·8:1
*3	13·6	6·0	19·6	2·3:1	0·7	0·4	1·1	1·8:1

* Dose 36 mg.

enantiomorphs was similar when the urine was acid but the (−)-isomer was excreted at a faster rate, mostly as unchanged drug. Of the two optical forms the (+)-isomer is metabolized to a greater extent suggesting a stereospecific metabolism (Table 2).

Table 2. *Urinary excretion of ethylamphetamine (EA) and amphetamine (A) over a period of 24 h in subjects receiving an oral dose of (+)-, (−)- or (±)-ethylamphetamine hydrochloride under acidic conditions*

		Dextro				*Racemic*				*Laevo*			
		% Dose excreted				% Dose excreted				% Dose excreted			
Subject	(mg) Dose	EA	A	Total	Ratio EA/A	EA	A	Total	Ratio EA/A	EA	A	Total	Ratio EA/A
1	20	18·6	15·0	33·6	1·2:1	47·3	14·2	61·5	3·3:1				
5	20	22·6	17·2	39·8	1·3:1	42·3	8·4	50·7	5·0:1	66·0	6·5	72·5	10·2:1
4	20	16·7	12·3	29·0	1·4:1	45·5	7·3	52·8	6·2:1	73·3	5·1	78·4	14·4:1
3	20	22·5	17·5	40·0	1·3:1	39·5	6·0	45·5	6·6:1				
2	20					45·9	12·7	58·6	3·6:1	78·9	7·1	86·0	11·1:1
4	12					41·7	8·1	49·8	5·1:1				
3	24·5					45·8	8·3	54·1	5·5:1				
3	12					41·3	10·0	51·3	4·1:1				
3	12·3					42·0	9·5	51·5	4·4:1				
2	12·7	31·7	14·6	46·3	2·2:1								

As with methylamphetamine (Beckett & Rowland, 1965d) the excretion of the unchanged drug is the major route of elimination for ethylamphetamine when the pH of the urine is maintained at acid values. The biological half-life of the (−)-isomer is about 5·2 h and 2·9 h for the (+)-isomer. This last value is significantly different from that of (+)-amphetamine (4·9 h) and (+)-methylamphetamine (4·3 h) (Beckett & Rowland, 1965a,b). The ratio of the metabolite, amphetamine, to the unchanged drug, indicates that (+)-ethylamphetamine is de-ethylated more than its (−)-isomer (Fig. 3) and the sum of drug and amphetamine excreted also shows that there is more metabolism of (+)-ethylamphetamine than of the (−)-isomer by a route or routes other than de-ethylation.

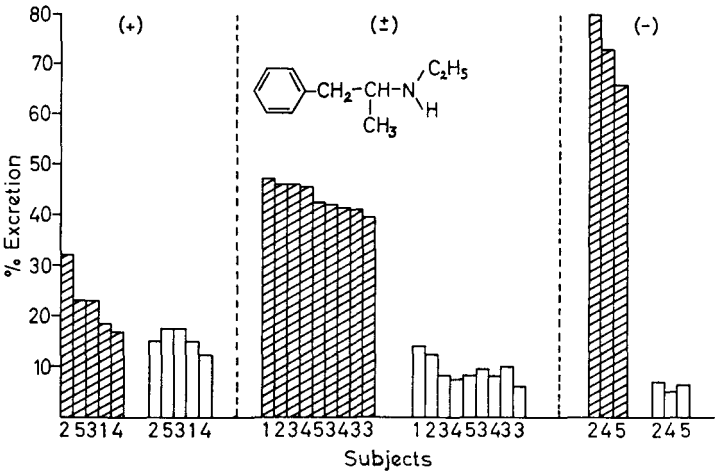

FIG. 3. The importance of stereochemistry in the metabolism and urinary excretion of ethylamphetamine in man (Acidic urine, oral dose, 24 h urine collection). Hatched columns—ethylamphetamine. Open columns—the metabolite-amphetamine.

Although there are only small differences in the excretion and thus metabolism of the isomers of amphetamine, *N*-alkyl substitution increases the total amount of metabolism (see Fig. 4) of the (+)- but not the (−)-isomer, i.e. *N*-alkyl substitution of amphetamine increases the susceptibility to stereoselective metabolism in man. Also, increase in the size of the *N*-alkyl substituent from methyl to ethyl produces relatively more *N*-dealkylation in the metabolically more susceptible isomer (Fig. 4).

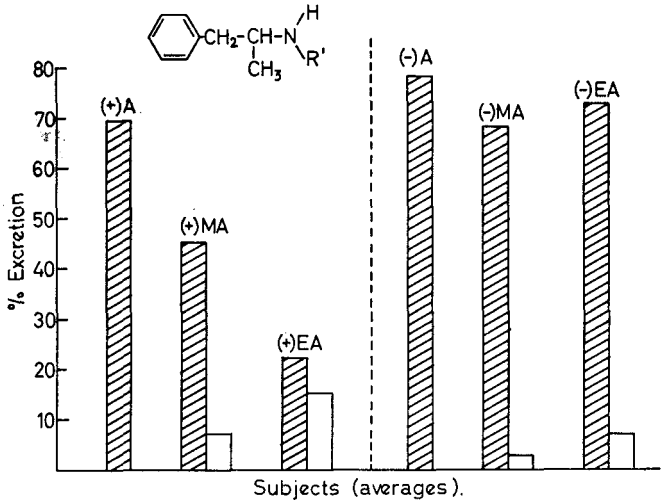

FIG. 4. The importance of stereochemistry and effect of *N*-alkyl substitution of amphetamine in the metabolism and excretion of the drug and the metabolite in man. (Acidic urine, oral dose, 24 h urine collection). Hatched columns—unchanged drug Open columns—the metabolite-amphetamine. A = amphetamine. MA = methylamphetamine. EA = ethylamphetamine.

Acknowledgements

The authors thank all those who participated in the trials, Mr. A. Saunders for technical assistance, and Medical-Riker (Lyon) for the gift of (±)-ethylamphetamine hydrochloride.

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