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The effect of *N*-alkyl chain length and stereochemistry on the absorption, metabolism and urinary excretion of *N*-alkylamphetamines in man*

A. H. BECKETT AND E. V. B. SHENOY

Department of Pharmacy, Chelsea College (University of London), Manresa Road, London, S.W.3, U.K.

Urinary excretion in man, of the unchanged drug and metabolite amphetamine, has been investigated after the (+)- and (—)-isomers of methyl-, ethyl-, n-propyl- and n-butyl- amphetamine had been taken by mouth under acidic urinary pH. The total metabolism of (+)-methyl-, ethyl-, and n-propyl-amphetamine was greater than that of the corresponding (—)-isomers but there was no difference in the total metabolism of the (+)- and (—)-n-butylamphetamine. A direct correlation was obtained for the (+)-but not the (—)-isomers between the partition coefficient of the compounds in an n-heptane/aqueous system, their buccal absorption and the total metabolism. The (+)-isomers of methyl- and ethyl-amphetamine were *N*-dealkylated more than their (—)-enantiomorphs but (—)-n-propylamphetamine was *N*-dealkylated more than the (+)-isomer.

Metabolic *N*-dealkylation, an important pathway in drug metabolism, has not been systematically investigated in man. Stereoselective metabolism of amphetamine (Alles & Wisegarver, 1961; Beckett & Rowland, 1965a; Gunne, 1967; Gunne & Galland, 1967), methylamphetamine (Beckett & Rowland, 1965b; Gunne, 1967), and ethylamphetamine (Beckett, Brookes & Shenoy, 1969) has been reported.

We have extended our previous work with the aim of evaluating the importance of alkyl chain length and stereochemistry in the metabolism of a series of *N*-alkyl amphetamines in man.

METHODS

Gas-liquid chromatography (g.l.c.)

The unchanged drug and metabolite amphetamine in the urine samples were determined by g.l.c. and thin-layer chromatography (t.l.c.) as described by Beckett & others (1969); identification of the drug and metabolite from urine extracts were made by comparison with authentic samples and their acetone, acetyl and propionyl derivatives.

Urinary excretion trials

Healthy males (age 24–45) were given oral doses of (+)- or (—)-isomers of methyl-, ethyl-, n-propyl- or n-butyl-amphetamine as their hydrochlorides dissolved in 50–100 ml of water. Acidic urine was maintained by the administration of ammonium

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chloride (Beckett & Brookes, 1967). The subjects refrained from smoking at least for 36 h before, and during the trial period when propylamphetamine was administered, since nicotine interferes with the analysis of this compound.

The urine samples were collected every ½ or 1 h for the first 4 h, hourly up to 12 h and then at longer intervals up to 24 to 48 h. The volume and pH of the urine were recorded immediately and the samples stored at 4° until analysed. A blank urine sample was collected at the time of the drug administration.

Compounds

(+)- and (−)-Methylamphetamine were obtained from Burroughs Wellcome and Smith, Kline and French Laboratories respectively. (+)- and (−)-Ethylamphetamine were prepared from (+)- or (−)- amphetamine by the methods decribed by Leonard, Adamcik & others (1958); (+)- and (−)-n-propyl- and n-butyl-amphetamine were prepared by the same procedure but by substituting propionic and butyric anhydrides respectively instead of acetic anhydride. The purity of the compounds was established by (i) thin-layer chromatography (Table 2); (ii) gas-liquid chromatography (Table 1); (iii) nuclear magnetic resonance. Spectra in $CDCl_3$ or D_2O were recorded using a Perkin-Elmer R-10 nmr spectrometer with tetramethylsilane as the internal standard*; (iv) Optical rotatory dispersion. Spectra of the (+)- and (−)-isomers of the compounds in 0·1 N HCl were recorded using a Bellingham Stanley/Bendix-Ericsson Polarimeter*; (v) CHN analysis: the results of which were in agreement with calculated values.

RESULTS AND DISCUSSION

The retention times on g.l.c. of the compounds and their derivatives are given in Table 1; their R_F values in various solvent systems are given in Table 2.

Table 1. *Retention times of some* N-*alkylamphetamines and their derivatives.*

Column	Stationary phase	Operating temp. (°C)	Column length	Retention times (min) A	MA	EA	PA	BA	Aletamine
Chromosorb G acid washed DMCS treated 80–100 mesh	10% KOH 10% Apiezon L	130	1 metre S.S. 1/8″ od	4·5	6·4	8·4	13·7	23·6	12·4
				Acetone derivative					
				7·8	—	—	—	—	—
				Acetyl derivative					
				19·9	—	41·0	*48·0	*72·0	—
				Propionyl derivative					
				27·7	—	53·6			
Chromosorb G acid washed DMCS treated 80–100 mesh	5% KOH 2% Carbowax 20 M	165	2 metre S.S. 1/8″ od.	Acetyl derivative					
				7·2	5·2	5·0	—	—	—
				Propionyl derivative					
		140		20·1	14·6	14·0	—	—	—

A = amphetamine; MA = methylamphetamine; EA = ethylamphetamine; PA = n-propylamphetamine; BA = n-butylamphetamine;
N_2 flow rate = 35 ml min^{-1} DMCS = Dimethylchlorsilane.
Other conditions are described by Beckett, Brookes & Shenoy (1969).
* Temp. 150°.

* Data available on request from the Editor, Journal of Pharmacy and Pharmacology, 17 Bloomsbury Square, WC1A 2NN, quoting paper.

Table 2. R_F *values of* N-*alkylamphetamines and their metabolite amphetamine in various solvent systems.*

Solvent System v/v		R_F values				
		A*	MA	EA	PA	BA
(a) CH_3OH - acetone	50:50	0·54	0·23	0·39	0·44	0·39
(b) CH_3OH - $CHCl_3$	20:80	0·35	0·23	0·41	0·55	0·63
(c) CH_3OH - $CHCl_3$ + 5 drops NH_4OH (30%)	90:10	0·30	0·24	0·33	0·39	0·40
(d) CH_3OH - $CHCl_3$	50:50	0·29	0·24	0·32	0·43	0·40

Silica gel G (Merck) 0·25 mm.
* As described in Table 1.
Spray - Dragendorff's (Stahl, 1962) reagent (red spots) or an ethanolic solution of bromothymol blue (blue spots) was used to visualize the compounds.

The plasma concentration of amphetamine is related to urinary excretion only when the urine is maintained acidic (Beckett, Salmon & Mitchard, 1969); thus only under such conditions is it possible to evaluate the effect of chain length and stereochemistry on the metabolism and excretion of these compounds without using a large number of subjects. Increase in chain length is associated with increased metabolism of the (+)-isomers, as indicated by the reduced drug recovery (Table 3; Fig. 1A), and is also correlated with increase in partition coefficient values in n-heptane/aqueous system and buccal absorption (Beckett & Moffat, 1969). No such correlation was obtained for the (−)-isomers (Table 3). Consequently, attempted correlations between metabolic rates and the partition of racemic compounds (i.e. a mixture of two enantiomorphs) are probably of doubtful value for arylalkylamines in general.

No correlation could be shown between the amount of *N*-dealkylation as measured by the determination of amphetamine, and the size of the *N*-alkyl group (Fig. 1B).

Table 3. *Urinary excretion of unchanged drug and metabolite amphetamine over a period of 24 h after oral administration of some* N-*alkylamphetamines to man under -acidic urine control.*

Drug	Dose HCl (mg)	Subject	(+)-isomer % dose excreted		(−)-isomer % dose excreted	
			un-changed	as amphet-amine	un-changed	as amphet-amine
Methylamphetamine	12·45	1	50·7	7·3	59·0	2·3
	12·45	2	45·1	7·4	77·1	4·2
	12·45	3	47·6	8·2	58·5	1·6
Ethylamphetamine	20·00	1	22·6	17·2	66·0	6·5
	20·00	2	29·1	18·4	78·9	7·1
	20·00	3	16·7	12·3	73·3	5·1
n-Propylamphetamine	20·00	1	11·0	12·0	47·2	17·8
	20·00	2	17·9	14·6	46·7	19·5
	20·00	3	20·0	9·7	59·8	10·0
	20·00	6	14·1	9·9	45·8	12·9
n-Butylamphetamine	20·00	1	1·1	8·3	2·4	5·9
	2·000	2	2·0	8·7	2·9	7·2

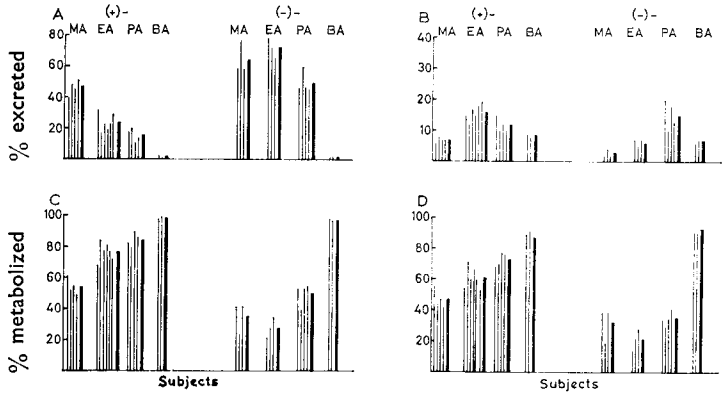

FIG. 1. The importance of stereochemistry and the effect of *N*-alkyl chain length on the metabolism and excretion of *N*-alkylamphetamines in man. (Acidic urine, oral dose, 24 h urine collection). A. Comparison of the urinary excretion of unchanged drug. B. Comparison of the urinary excretion of *N*-dealkylated metabolite amphetamine. C. Comparison of the percentage drug metabolized by all routes. (100 — A). D. Comparison of the percentage drug metabolized by all routes other than N-dealkylation. (100 — (A + B). MA = methylamphetamine. EA = ethylamphetamine. PA = n-propylamphetamine. BA = n-butylamphetamine. Each line represents one subject, the bold line is the mean.

In the case of the (+)-isomers the order of *N*-dealkylation was ethyl > propyl > methyl ≈ butyl and for (—)-isomers propyl > ethyl ≈ butyl > methyl. However, there was a direct trend between metabolism by routes other than dealkylation for the (+)- but not the (—)-isomers (Fig. 1D).

The (+)-isomers of methyl-, ethyl- and propyl-amphetamine are metabolized more than are their corresponding enantiomorphs but no stereoselectivity was observed in the total metabolism of butylamphetamine (Fig. 1C).

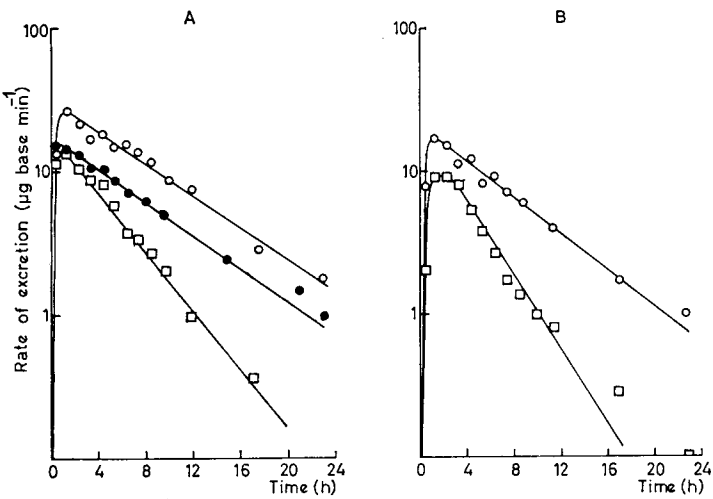

FIG. 2. (A). Comparison of the urinary excretion of ethylamphetamine after oral administration of 20 mg (+)-, (—)- and (±)-ethylamphetamine hydrochloride to a subject under acidic urine control. ○ (—)-isomer. □ (+)-isomer. ● (±)-form.

(B). Comparison of the urinary excretion of propylamphetamine after oral administration of 20 mg (+)-and (—)-propylamphetamine hydrochloride to a subject under acidic urine control. ○ (—)-isomer. □ (+)-isomer.

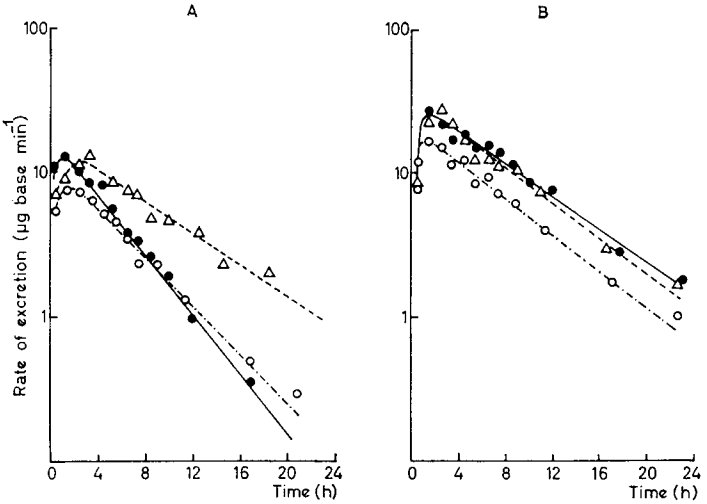

FIG. 3 (A). Comparison of the 24 h urinary excretion of unchanged drug after oral administration of the (+)-isomers of *N*-alkylamphetamines (as HCl) under acidic urine control. Dose: 20 mg HCl.
Subject 2: △ (+)-methylamphetamine*. ● (+)-ethylamphetamine. ○ (+)-propylamphetamine. * an oral dose of 12·45 mg scaled up to 20 mg dose.

B. Comparison of the 24 h urinary excretion of unchanged drug after oral administration of the (—)-isomers of *N*-alkylamphetamines (as HCl) under acidic urine control. Dose: 20 mg HCl.
Subject 2: △ (—)-methylamphetamine*, ● (—)-ethylamphetamine. ○ (—)-propylamphetamine. * an oral dose of 12·45 mg scaled up to 20 mg dose.

The peak rates of excretion of the unchanged drug reached after (—)-ethyl-and (—)-propyl-amphetamine were significantly higher than those for the corresponding (+)-isomers (Fig. 2). The possible reason is a difference in body distribution of the enantiomorphs or alternately the (+)-isomers are metabolized faster than the (—)-isomers on their first pass through the liver. Differences in the absorption of the isomers from the gastrointestinal tract is unlikely since the buccal test indicated comparable absorption for the enantiomorphic pairs of compounds.

The total contribution of *N*-dealkylation to the metabolism of these compounds is small compared to metabolism by other routes (see Fig. 1B and D). The rate of metabolism of both isomers of amphetamine is similar (Beckett & Rowland, 1965a; Gunne, 1967; Dring, Smith & Williams, 1970); consequently the presence of different amounts of (+)- and (—)-amphetamine after giving the enantiomorphs of the different homologues of amphetamine (Fig. 1B) indicates the importance of stereochemistry on dealkylation of the various homologues.

The peak rates of excretion of methyl-, ethyl- and n-propyl-amphetamine occurred between 1–3 h after oral dosage followed by a mono-exponential decline in the rate of excretion; however, n-butyl-amphetamine showed a biexponential elimination. A comparison of a plot of the rate of excretion against time for the (+)-isomers of ethyl- and propyl-amphetamine indicated that their rate of elimination (kd = ke + km) was roughly comparable, but that of (+)-methylamphetamine was slower (Fig. 3A). However, all the (—)-isomers had similar elimination rates (Fig. 3B). The rate of metabolism of (+)-ethyl- and (+)-propyl-amphetamine were similar, and the km values were much higher than that of (+)-methylamphetamine, or of (—)-ethyl and

Table 4. *Half lives and overall rate constants for elimination, excretion and metabolism of the isomers of some* N-*alkylamphetamines.*

Drug	Subject	$t_{\frac{1}{2}}$ (h)	*Rate constants (h^{-1}) kd	km	ke
**(+)-Methylamphetamine	5	5·0	0·139	0·049	0·090
,,	4	5·0	0·137	0·050	0·087
(—)-Methylamphetamine	2	5·5	0·126	0·029	0·097
,,	3	6·0	0·116	0·049	0·068
(+)-Ethylamphetamine	2	3·1	0·224	0·159	0·065
,,	2	2·9	0·239	0·163	0·076
(—)-Ethylamphetamine	2	5·4	0·128	0·027	0·101
,,	3	5·9	0·117	0·031	0·086
(+)-Propylamphetamine	2	2·4	0·289	0·237	0·052
,,	3	3·4	0·204	0·163	0·041
,,	1	3·5	0·198	0·175	0·023
,,	6	3·5	0·198	0·170	0·028
(—)-Propylamphetamine	2	4·8	0·145	0·077	0·068
,,	3	6·4	0·108	0·043	0·065
,,	6	4·0	0·173	0·094	0·079
,,	1	4·0	0·173	0·091	0·082

* Calculated from $t_{\frac{1}{2}}$ and recovery in urine of the unchanged drug assuming there was no change in elimination half life beyond 24 h. The recovery of unchanged drug after 24 h was less than 2% and did not affect the calculated rate constants.
** Data from Beckett & Tucker (1968).

(—)-propyl-amphetamine (Table 4). The average rate of excretion (ke) in 10 subjects who had been given doses of arylalkylamines under acidic urine control was 0·075 (± 0·03 mean deviation). This variation was not large enough to mask the differences in the km values of these compounds.

Although the km values for the (+)-isomers of ethyl- and propyl-amphetamine were higher than those of their (—)-enantiomorphs (see Table 4), there was more *N*-dealkylation of (+)-ethylamphetamine and (—)-propylamphetamine than of (+)-propylamphetamine (see Fig. 4A and 4B), indicating that there is greater metabolism of the (+)-propylamphetamine by routes other than *N*-dealkylation, than there is

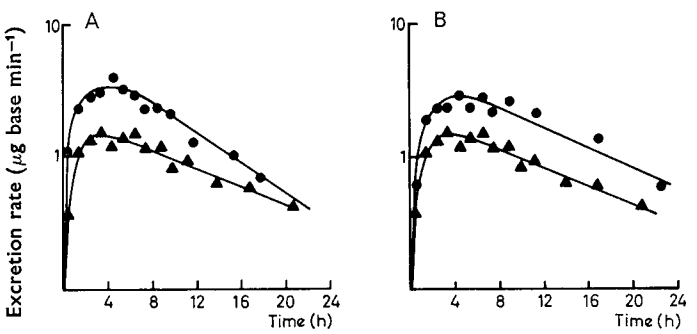

FIG. 4 (A). Comparison of the 24 h urinary excretion of metabolite amphetamine after oral administration of 20 mg each of (+)-ethylamphetamine and (+)-propylamphetamine (as HCl) under acidic urine control. Subject 2: ● amphetamine from (+)-ethylamphetamine. ▲ amphetamine from (+)-propylamphetamine.

(B). Comparison of the 24 h urinary excretion of metabolite amphetamine after the oral administration of 20 mg each of (+)- and (—)-propylamphetamine (as HCl) under acidic urine control. Subject 2: ▲ amphetamine from (+)-isomer. ● amphetamine from (—)-isomer.

for the former two compounds. The excretion of larger amounts of amphetamine after a dose of (−)-propylamphetamine than after (+)-propylamphetamine is therefore indicative of an overall increase in affinity to binding sites and consequent increase in metabolism by all routes, rather than increase in the rate of *N*-dealkylation (see Fig. 4B).

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