COMPARATIVE METABOLISM OF SOME AMPHETAMINES IN VARIOUS SPECIES

R. T. WILLIAMS, J. CALDWELL and L. G. DRING
Department of Biochemistry, St. Mary's Hospital Medical School, London, W2 1PG U.K.

FEW DETAILED metabolic studies have been carried out on drugs based upon amphetamine (No. 1 in Table 1). Amphetamine, methamphetamine (No. 2), norephedrine (No. 5) and 4-hydroxyamphetamine have been examined in this laboratory. Other workers have examined, in varying detail, ephedrine (BRALET *et al.*, 1968) (No. 6), ethylamphetamine (BECKETT *et al.*, 1969) (No. 3), Pondinil (LONG, 1970) (No. 4), mephentermine (WALKENSTEIN *et al.*, 1955) (No. 8) and fenfluramine (BRUCE and MAYNARD, 1968) (No. 7).

Metabolism of these drugs in different species may be correlated with a) their physical properties and b) their biological properties such as pharmacological activity and toxicity. At pH 7·4 these drugs occur almost entirely as cations (see Table 1), being over 99 per cent ionised except fenfluramine which is 98 per cent ionised. The unionised drugs, however, differ considerably in lipid solubilities which range over 3000 if one excludes fenfluramine.

The structures of these drugs (see Table 1) suggests several routes of biotransformation,

(a) *aromatic hydroxylation* introducing a phenolic group particularly at the *para*-position of the benzene ring.
(b) N-*dealkylation* in the case of compounds Nos. 2, 3, 4, 6, 7 and 8 of Table 1.
(c) *Oxidative deamination* (following *N*-dealkylation where this occurs) to a ketone which may then be reduced to other compounds or oxidised to benzoic acid.
(d) *Aliphatic hydroxylation* of the carbon atoms in the side chain especially the one in the methylene group next to the benzene ring, except in compounds Nos. 5 and 6 (Table 1.)
(e) N-*Oxidation* to form hydroxylamines or *N*-oxides.

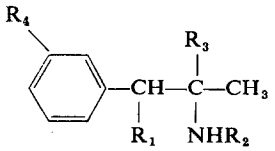
Apart from the above reactions, the drug may be excreted unchanged in the urine to varying extents. There is actually little faecal excretion of these drugs in any of the species examined.

All the above reactions, except possibly (e), have been shown to occur *in vivo*, but the extent to which they occur as measured by the metabolites found in the urine shows a remarkable species variation.

AROMATIC HYDROXYLATION

The *p*-hydroxylation of the benzene ring of the amphetamines is a reaction which occurs extensively and consistently in the rat but not to any great extent in the other species listed in Table 2 except in one or two cases such as Pondinil in man and the *N*-alkylated drugs, methamphetamine, Pondinil and mephentermine, in the dog. There is no evidence of hydroxylation in the *o*- or *m*-positions. In the rat, the extent

TABLE 1. THE STRUCTURE AND SOME PHYSICOCHEMICAL PROPERTIES OF THE AMPHETAMINES
(Data from Vree *et al.*, 1969)



No.	Drug	R_1	R_2	R_3	R_4	pK_a	cation at pH 7·4%	Relative lipid solubility*
1	Amphetamine	H	H	H	H	9·90	99·6	1·00
2	Methamphetamine	H	Me	H	H	10·11	99·8	2·31
3	Ethylamphetamine	H	Et	H	H	10·23	99·8	5·56
4	Pondinil	H	C_3H_6Cl	H	H	—	—	—
5	Norephedrine	OH	H	H	H	9·55	99·3	0·002
6	Ephedrine	OH	Me	H	H	9·60	99·4	0·031
7	Fenfluramine	H	Et	H	CF_3	9·10	98·0	67·40
8	Mephentermine	H	Me	Me	H	10·25	99·8	2·54

* Amphetamine taken as 1·00; calculated from the apparent partition coefficients at pH 7·4 between $CHCl_3$ and H_2O.

of aromatic hydroxylation seems correlated with lipid solubility since the more lipid soluble drugs, amphetamine and methamphetamine are more extensively hydroxylated than the less lipid soluble drugs, ephedrine and norephedrine. Much of the latter drugs are excreted unchanged by the rat (see Table 6). In the rabbit and guinea pig, aromatic hydroxylation is a minor reaction, and may not occur in the guinea pig with amphetamine and methamphetamine. In the dog, some 20–30 per cent of those drugs containing an *N*-alkyl group, except ephedrine, are hydroxylated. In man, aromatic hydroxylation is a minor reaction except with Pondinil and methamphetamine, and the hydroxylation of amphetamine is approximately the same (2–3 per cent of the dose) in normal and tolerant subjects.

TABLE 2. THE EXTENT OF AROMATIC HYDROXYLATION OF VARIOUS AMPHETAMINES IN DIFFERENT SPECIES
(Values are taken from various sources in the literature)

	Rat	Rabbit	Guinea pig	Dog	Man	Tolerant Man
Amphetamine	60	6	0	6	2	3
Methamphetamine	53	—	0	30	18	—
Norephedrine	28	3	—	—	0–1	—
Ephedrine	14	11	1	1	0	—
Pondinil	58	6	15	22	37	—
Mephentermine	32	5	—	28	—	—
Fenfluramine	—	—	—	0	0	—

Since in the rat the extent of aromatic hydroxylation of these drugs seems to be correlated with their lipid solubility and as the aromatic hydroxylation of many compounds occurs in the lipid containing liver microsomes, then this reaction would be expected to occur in rat liver microsomes. Although amphetamine is hydroxylated in the liver, it is not hydroxylated by liver microsomes (DINGELL and BASS, 1969).

N-DEALKYLATION

Three drugs have been examined for this reaction in various species, namely methamphetamine, ephedrine and mephentermine, which contain an *N*-methyl

group. From the data available it occurs significantly (20–90 per cent) in the rat, rabbit, guinea pig and dog (see Table 3) but not extensively in man (about 10 per cent). On the limited data the rabbit and guinea pig appear to be the species likely to carry out this reaction to the greatest extent. Unlike aromatic hydroxylation, this reaction occurs in the liver microsomes (AXELROD, 1955).

TABLE 3. EXTENT OF *N*-DEALKYLATION OF CERTAIN AMPHETAMINES IN DIFFERENT SPECIES
(Values are taken from various sources in the literature)

	Rat	Rabbit	Guinea pig	Dog	Man
Methamphetamine	28	—	79	45	14
Ephedrine	20	93	39	58	10
Mephentermine	45	27	—	44	—

DEAMINATION

Data for the extent of deamination of four of these drugs are summarised in Table 4. Deamination is a very minor reaction in the rat, an extensive one in the rabbit and guinea pig and a relatively minor one in man except for amphetamine. The possible mechanisms of this reaction are discussed later.

Only in the metabolism of amphetamine can the rabbit and guinea pig be compared. After deamination, considerable differences occur between these two species in the subsequent fate of the ketone produced. In the guinea pig, the major urinary metabolites of both amphetamine and methamphetamine are benzoic acid and its conjugates (CALDWELL *et al.*, 1972a; DRING *et al.*, 1970). In the guinea pig, the ketone produced is mainly oxidised to benzoic acid whereas in the rabbit it also undergoes reduction and/or some form of conjugation. A major metabolite of amphetamine in the rabbit is a conjugate of benzyl methyl ketone together with appreciable amounts of conjugated phenylpropan-2-ol (DRING *et al.*, 1970), and after norephedrine considerable amounts of conjugated 1,2-dihydroxy-1-phenylpropane and 1-hydroxy-2-oxo-1-phenylpropane are excreted (SINSHEIMER *et al.*, 1973).

TABLE 4. EXTENT OF DEAMINATION OF VARIOUS AMPHETAMINES IN DIFFERENT SPECIES
(Values are taken from various sources in the literature)

	Rat	Rabbit	Guinea pig	Dog	Man	Tolerant Man
Amphetamine	3	54	62	30	24	23
Methamphetamine	4	—	74	—	6	—
Norephedrine	1	76	—	—	3	—
Ephedrine	3	91	—	—	10	—

ALIPHATIC C-HYDROXYLATION

Amphetamines contain a straight side-chain of three carbon atoms, each of which could be hydroxylated. There is no evidence for an attack on the terminal C atom (C-3) which might yield phenylalanine (DRING *et al.*, 1970).

Hydroxylation of the C-2 atom giving an unstable carbinolamine (BRODIE *et al.*, 1958) has indirect support from recent studies (HUCKER *et al.*, 1971; PARLI *et al.*, 1971) and has been invoked in one mechanism for the deamination of amphetamines.

The hydroxylation of the 1-carbon atom, a reaction of importance in explaining tolerance to amphetamines has been definitely proved. Table 5 shows the extent

of this reaction in four species as measured by the urinary output of norephedrine and 4-hydroxynorephedrine. This table suggests that β-hydroxylation is a relatively minor metabolic reaction of the amphetamines. In the guinea pig and rat, β-hydroxylation occurs more extensively with methamphetamine than with amphetamine and paredrine. The values of 16 per cent of the dose in the rat and 19 per cent in the guinea pig are for doses of methamphetamine of 45 mg/kg, whereas the 1 per cent quoted for the guinea pig was obtained with 10 mg/kg. This might suggest that in the guinea pig the production of norephedrine is dose dependent. In normal humans β-hydroxylation is about 3–5 per cent but in the amphetamine tolerant human, β-hydroxylation is higher (5 per cent) than in normals (3 per cent) (CALDWELL *et al.*, 1972*b*; SEVER *et al.*, 1973). If these values are acecpted as being different, the probable explanation is that the normal humans had been given (±)-amphetamine and the tolerant subjects (+)-amphetamine, whilst only (+)-amphetamine is a substrate for dopamine β-hydroxylase (GOLDSTEIN and ANAGNOSTE, 1965).

TABLE 5. THE URINARY EXCRETION OF NOREPHEDRINE AND 4-HYDROXYNOREPHEDRINE AFTER CERTAIN AMPHETAMINES

(Values are taken from various sources in the literature)

	Rat		Guinea pig		Rabbit		Normal Human		Tolerant Human	
Drug	N	HN	N	HN	N	HN	N	HN	N	HN
Amphetamine	0·3	0·3	—	—	—	—	2·4	0·4	4·1	1·3
Methamphetamine	0	16	19	0	—	—	2	1·7	—	—
4-Hydroxyamphetamine	—	4	—	3	—	—	—	4·7	—	—
Norephedrine	48	28	—	—	8	3	86	0–1	—	—

Norephedrine and especially 4-hydroxynorephedrine are regarded as false neurotransmitters. Several authors have suggested that some aspects of the development of tolerance to amphetamines may be related to the metabolic production of 4-hydroxynorephedrine (BRODIE *et al.*, 1970; LEWANDER, 1971). In man, it would appear that 4-hydroxynorephedrine is produced from 4-hydroxyamphetamine but hardly at all from norephedrine. The formation of 4-hydroxynorephedrine from amphetamine or methamphetamine requires both an aliphatic and an aromatic hydroxylation. It is possible in man that the intermediate in the production of 4-hydroxynorephedrine from these drugs is 4-hydroxyamphetamine since more 4-hydroxynorephedrine is excreted after methamphetamine than after amphetamine (Table 5) and there is considerably more aromatic hydroxylation of methamphetamine than amphetamine by man (see Table 2).

It is also of interest to compare production of norephedrines from methamphetamine in the rat and guinea pig. Only 4-hydroxynorephedrine is excreted by the rat whilst only norephedrine by the guinea pig. If current theories are correct, this should mean that the guinea pig would not become tolerant to those effects of the amphetamines in which 4-hydroxynorephedrine apparently plays a role. However, no work which compares the two species with respect to tolerance development is recorded.

N-OXIDATION

The biological oxidation of nitrogen occurring in aliphatic or aromatic amines, in amides or as an atom in a heterocyclic system is well-known (BRIDGES *et al.*

1972). Several amphetamines are reported to yield hydroxylamines in liver microsomal preparations of rat, rabbit and guinea pig (BECKETT, 1971) but their formation *in vivo* has not yet been satisfactorily shown. Such metabolites, however, could be excreted as the more stable *N*-*O*-glucuronides or even sulphates (IRVING, 1971). These hydroxylamines have been suggested as intermediates in deamination.

EXCRETION OF AMPHETAMINES UNCHANGED

In Table 6, the literature data on the extent to which different amphetamines are excreted unchanged in the urine are shown. The amphetamines are extensively metabolised by rabbits and guinea pigs and least metabolised by man.

TABLE 6. EXTENT TO WHICH SOME AMPHETAMINES ARE EXCRETED UNCHANGED IN VARIOUS SPECIES

(The values given are from various sources in the literature and are intended to indicate the approximate extent to which the drugs are excreted unchanged)

Drug	Rat	Rabbit	Guinea pig	Dog	Man
Amphetamine	12	4	19	30	34 [tolerant 44]
Methamphetamine	11 (14)	2 (2)	1–3 (4–16)	20 (35)	23 (26)
Ethylamphetamine	—	—	—	—	17 (24)
Pondinil	1	18	3	17	1
Norephedrine	48	8	—	—	86
Ephedrine	42 (45)	0·5 (2·5)	2 (41)	6 (64)	61 (73)
Fenfluramine	—	—	—	—	7 (10)
Mephentermine	0 (13)	0 (22)	—	0 (16)	—

Values in brackets are the sum of *N*-alkylamine and free amine.

In man, the least lipid soluble drugs (see Table 1), ephedrine and norephedrine, are the most extensively excreted unchanged (70–80 per cent of the dose). The more lipid soluble drugs, amphetamine, methamphetamine and ethylamphetamine (in order of increasing lipid solubility) the excretion unchanged (Table 6) diminishes as lipid solubility (Table 1) increases, but the excretion of primary amine plus alkyl amine for each of these compounds is about 30–40 per cent. The excretion of total amine could be greater than this since this depends upon urinary pH (BECKETT and ROWLAND, 1965). The only drugs almost completely metabolised in man are the highly lipid-soluble Pondinil (only 1 per cent excreted unchanged) and Fenfluramine (7 per cent, or 10 per cent as total amine). Metabolism of these drugs in man is thus correlated with the lipid solubility of the un-ionised forms.

The rat is similar to man except that all the drugs are metabolised to a greater extent than in man. Thus, some 40–50 per cent of the least soluble drugs, ephedrine and norephedrine, are excreted unchanged by the rat compared with over 70 per cent in man, and about 12 per cent of the more soluble drugs, amphetamine and methamphetamine, in the rat compared with some 30 per cent in man. The dog appears to be between man and rat in this respect.

In the rabbit and guinea pig, there is no correlation between the extent of metabolism and lipid solubility since they metabolise some 90 per cent of the dose of all these drugs. In the rabbit these drugs, despite the fact that at pH 7·4 they occur almost entirely as cations, are metabolised by the liver microsomes which, according to accepted views on drug metabolism, are mainly concerned with the biotransformation of non-polar lipid-soluble compounds. Several explanations are possible,

one being that rabbit liver microsomes are different from those of the rat, in allowing cations to penetrate them or in being able to metabolise the small amount of un-ionised drug present (<1 per cent) very rapidly so that all the ionised form is quickly converted to un-ionised drug as required by the laws of ionisation equilibria.

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