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Species differences in the *in vitro* metabolic reduction of the amphetamine metabolite, 1-phenyl-2-propanone

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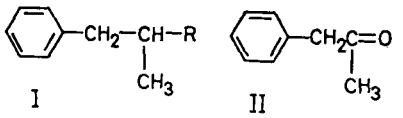
Marked differences were observed, in the ability of fortified 9000 *g* liver homogenate supernatants from three species to reduce 1-phenyl-2-propanone to the corresponding alcohol. This metabolic keto-reduction was negligible in homogenates from the rat and extensive in the rabbit; guinea-pig liver homogenates had intermediate ability. Metabolic oxidation of 1-phenyl-2-propanol was negligible in all three species. The amount of deamination of amphetamine and of *N*-n-propylamphetamine was approximately equal, *in vitro*, in rats and guinea-pigs but two to three times greater in liver homogenates from rabbits. Approximately three times more deaminated products were formed from the *in vitro* metabolism of *N*-n-propylamphetamine than from amphetamine metabolism by all three species.

The *in vitro* metabolism of three closely related analogues of amphetamine (Ia), *N*-n-propylamphetamine (NPA, Id) (Coutts, Dawson & Beckett, 1976), *N*-ethylamphetamine (NEA, Ic) (Beckett & Haya, 1977) and *N*-methylamphetamine (NMA, Ib) (Coutts & Kovach, 1977) differed qualitatively in that 1-phenyl-2-propanol (I) was reported as a metabolite of NEA in rabbits but could not be detected after NMA or NPA metabolism in rats.

Smith, Smithies & Williams (1954) found that 1-phenyl-2-propanone (II) was extensively metabolized to the corresponding alcohol (Ie) and its glucuronide *in vivo* in rabbits. Others (Dring, Smith & Williams, 1970; Beckett & Al-Sarraj, 1972; Gal, Wright & Cho, 1976) have demonstrated the metabolic formation of 1-phenyl-2-propanol from amphetamine *in vivo* and *in vitro*.



I II

Ia R=NH_2; Ib R=$NHCH_3$; Ic R=$NHCH_2CH_3$; Id R=$NHCH_2CH_2CH_3$; Ie R=OH.

In view of these results, we wished to determine whether our failure to detect 1-phenyl-2-propanol in our incubations of NMA and NPA (Coutts & others, 1976; Coutts & Kovach, 1977) was due to a difference in our method of preparation of the liver homogenates, or to a species difference. Thus (±)-amphetamine (Ia), (±)-NPA (Id), 1-phenyl-2-propanone (II) and 1-phenyl-2-propanol (Ie) were incubated with fortified liver homogenates from rats, guinea-pigs, rabbits, and the ketone (II) and/or the alcohol (Ie) formed were measured.

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MATERIALS AND METHODS

Compounds

The following compounds were purchased: glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate ($NADP^+$) (Sigma), 1-phenyl-2-propanone (Aldrich), 1-phenyl-2-propanol (Fluka), cyclohexylacetone (BDH). (±)-Amphetamine hydrochloride (Ingersoll, 1941) and (±)-*N*-n-propylamphetamine hydrochloride (Leonard, Adamcik & others, 1958) were synthesized.

Animals

Male Wistar rats (250–350 g), male Kam Hartley guinea-pigs (300–500 g) and male New Zealand White rabbits (2–5 kg) were used. The animals were starved overnight before cervical dislocation.

Liver homogenates

Livers were removed immediately after death of the animal and cooled in 1·15% KCl at 0°. Homogenates were prepared as 20% w/v suspensions in 1·15% KCl using a Potter homogenizer with a Teflon pestle, and spun at 9000 *g*, 12 000 *g* or 18 000 *g* for 20 min. The volume of the supernatant was adjusted with 1·15% KCl such that 1 ml was equivalent to 0·2 g of original liver. The total 12 000 *g* supernatant protein, microsomal protein (105 000 *g* resuspended pellet) and cytosol protein (105 000 *g* supernatant) was determined by the method of Lowry, Rosebrough & others (1951) as modified by Miller (1959).

Incubations

(±)-Amphetamine HCl (10 μmol), (±)-*N*-n-propylamphetamine HCl (10 μmol), 1-phenyl-2-propanone (0·1 μmol) or 1-phenyl-2-propanol (0·1 μmol) were

incubated in open, 25 ml Erlenmeyer flasks containing liver homogenates (equivalent to 0·2 g liver) and cofactors (glucose-6-phosphate 20 μmol, $NADP^+$ 4·4 μmol and $MgCl_2$ 20 μmol) in tris-KCl buffer, pH 7·4 (total volume 6 ml per flask), in a shaking Dubnoff incubator at 37° for 1 h. Control incubations were carried out where (a) substrate (b) liver homogenate and (c) cofactors were separately omitted. The incubations were terminated by placing the flasks on ice.

Extraction and assay

The internal standard (cyclohexylacetone, 0·5 μmol per 0·1 ml 96% ethanol) was added to the incubation flasks and the mixture adjusted to pH 3–4 by the addition of 150 μl M HCl. The contents were extracted with freshly distilled diethyl ether (2 × 5 ml) in tapered centrifuge tubes (15 ml capacity), the ether extracts were concentrated on a water bath to 50 μl and analysed by g.l.c.

Calibrations

Calibration plots were constructed for 1-phenyl-2-propanone and 1-phenyl-2-propanol (correlation coefficients 1·000 and 0·9993 respectively) by adding known amounts of these compounds (0·01 to 1·0 μmol) plus a constant amount (0·5 μmol) of the internal standard to aged 12 000 *g* supernatants (1 ml) in pH 7·4 buffer (4·9 ml) and extracting as described above, at pH 3–4.

Gas liquid chromatography (g.l.c.)

A Hewlett-Packard gas chromatograph model HP 700 was used, equipped with a flame ionization detector, employing a glass column, 2·2 m long, 4 mm i.d., packed with 5% Carbowax 20M and 2·5% KOH on acid-washed Chromosorb W, 80–100 mesh, at an oven temp. of 130°; helium was the carrier gas (60 ml min^{-1}).

RESULTS AND DISCUSSION

A Carbowax-KOH g.l.c. column was selected, which gave adequate separation of 1-phenyl-2-propanone (II, Rt 5·1 min) and 1-phenyl-2-propanol (Ie, Rt 7·5 min) from (±)-amphetamine (Ia, Rt 3·1 min), (±)-*N*-n-propylamphetamine (NPA, Id, Rt 4·4 min) and the internal standard (cyclohexylacetone, Rt 1·8 min). Extractions of the incubates were carried out at pH 3–4 to prevent extraction of the amines (the tail of the NPA peak overlapped slightly with the ketone II). These conditions were not acidic enough to cause hydrolysis of any 1-phenyl-2-propanone oxime which might be formed metabolically from amphetamine or by chemical breakdown of the metabolite *N*-hydroxyamphetamine. Basic metabolites encountered in these experiments, i.e. *N*-hydroxyamphetamine and *N*-hydroxy-NPA were poorly extracted and had long g.l.c. retention times; they did not interfere with the analysis of Ie and II. The identities of the g.l.c. peaks were confirmed by g.c.-ms analysis and by direct comparisons with authentic samples of the alcohol (Ie) and ketone (II).

The quantities of (±)-amphetamine and (±)-NPA incubated (10 μmol per flask) were the same as used earlier (Coutts & others, 1976; Beckett & Haya, 1977) and the quantities of the ketone (II) and alcohol (Ie) incubated (0·1 μmol per flask) were chosen as approximate concentrations that might result from the incubations of amphetamine and NPA with fortified liver homogenates under the same conditions.

Formation of 1-phenyl-2-propanol

Only traces of 1-phenyl-2-propanol (Ie) were detected after the metabolism of (±)-NPA or (±)-amphetamine with fortified rat liver homogenates, confirming earlier work (Coutts & others, 1976) (see Table 1 for a summary of the results). With the same conditions, the metabolism of amphetamine and NPA with liver homogenates from guinea-pigs gave more of the ketone (II) than the alcohol (Ie) though significant quantities of the latter were present; rabbit liver homogenates formed about ten times more alcohol (Ie) than ketone (II) (see Table 1).

Metabolism of 1-phenyl-2-propanone produced small quantities of the alcohol (Ie) (3%) using fortified rat liver homogenates; guinea-pig liver homogenates formed ten times more of the alcohol (Ie), and when rabbit liver homogenates were used, metabolism of the ketone (II) was virtually complete.

Most studies on the *in vitro* metabolism of amphetamines have used rabbit liver homogenates and as a result the formation of 1-phenyl-2-propanol from the metabolism of amphetamines has been regarded as a general route (e.g. Caldwell, 1976). Beckett & Haya (1977) found as much of the ketone (II) as alcohol (Ie) was formed from the metabolism of *N*-ethylamphetamine using 9 000 *g* liver homogenate supernatants from rabbits. Using 9 000 *g* rabbit liver supernatants, Wright, Cho & Gal (1977) found that the 1-phenyl-2-propanone produced from amphetamine metabolism was completely reduced to the alcohol (Ie) under their conditions. Hucker, Michniewicz & Rhodes (1971) also found the alcohol (Ie) was a major metabolite of amphetamine, and that the ketone (II) was extensively

Table 1. *Quantities of the alcohol (Ie) and ketone (II) formed from the metabolism of (±)-amphetamine, (±)-N-n-propylamphetamine, 1-phenyl-2-propanone (II) and 1-phenyl-2-propanol (Ie), by fortified liver homogenate supernatants from three species.*

Substrate:	(±)-Amphetamine (Ia)[a]		(±)-*N*-n-Propylamphetamine (Id)[a]		1-Phenyl-2-propanone (II)[b]		1-Phenyl-2-propanol (Ie)[b]		Protein[i]	
Recovered metabolites:	Ketone (II)	Alcohol (Ie)	Ketone (II)	Alcohol (Ie)	Ketone (II)	Alcohol (Ie)	Ketone (II)	Alcohol (Ie)	Supernatant	Microsomal
Rat 9000 *g*[c] (2)[d]	13 ± 0[f]	trace[g]	ND	ND	80 ± 1·8	2 ± 0·25	ND	ND	16·4	5·0
12000 *g* (4)	12 ± 0·68	trace	44 ± 0·48[e]	trace[e]	80 ± 2·3	3 ± 0·41	5 ± 0·75[e]	83 ± 6·5[e]	16·5	4·4
18000 *g* (2)	8 ± 0·75	trace	ND	ND	83 ± 2·2	3 ± 0·25	ND	ND	14·9	2·9
G. Pig 12000 *g* (3)	12 ± 0·76	4 ± 0·17	40 ± 3·8	6 ± 0·60	24 ± 2·2	32 ± 2·5	7 ± 0·56	68 ± 3·7	17·6	5·0
Rabbit 12000 *g* (2)	2 ± 0·25	27 ± 2·2	13 ± 2·3	158 ± 10·9	trace	85 ± 3·3	trace	92 ± 3·9	18·2	2·7
Rabbit[h] 12000 *g* (1)	17 ± 0	160 ± 0·50	83 ± 2·0	583 ± 41·0	trace	89 ± 0	trace	89 ± 0	16·2	7·6

[a]10 μmol per flask; [b]0·1 μmol per flask; [c]x gravity used to prepare liver homogenate supernatant; fortified with an NADPH regenerating system; [d]number of animals used; number of experiments in duplicate; [e]two determinations only; [f]nmol per flask ± s.e.m.; [g] trace = <2 nmol per flask; [h]this animal was not kept under controlled conditions; [i]mg protein per flask.

metabolized to the alcohol (Ie) *in vitro*. However species differences in the *in vivo* formation of 1-phenyl-2-propanol are known. Dring, Smith & Williams (1966) and Dring & others (1970) found that rabbits dosed with [^{14}C]amphetamine excreted 22% 1-phenyl-2-propanone (as an acid labile precursor) and 7·5% of the corresponding alcohol (Ie, as a conjugate), whereas, they could detect neither metabolite in the urine of rats. But failure to detect these metabolites *in vivo* in rats does not preclude their formation since these metabolites were also not detected in guinea-pig urine (Dring & others, 1970) although our results show that both the ketone (II) and the alcohol (Ie) are formed *in vitro*, from amphetamine and NPA, by liver homogenates from guinea-pigs, though in smaller quantities than from rabbit liver homogenates.

The reverse reaction, i.e. dehydrogenation of the alcohol (Ie) to 1-phenyl-2-propanone, occurred in liver homogenates from guinea-pig and rat, but only to a minor extent; under our conditions this oxidation was not evident in fortified rabbit liver homogenates. In contrast, Hucker & others (1971) found that under their conditions about 20% of 1-phenyl-2-propanol was oxidized to the ketone (II) by rabbit liver 9 000 *g* supernatant; reduction of the ketone (II) to the alcohol (Ie) however, was more extensive (70%).

Deamination of (±)-amphetamine and (±)-NPA

1-Phenyl-2-propanone was formed from both (±)-amphetamine and (±)-NPA by fortified liver homogenates from all species examined by us and three to four times more ketone (II) was formed from NPA than from amphetamine. These relative yields of deamination products are consistent with observations by others that the rate of *N*-dealkylation of amphetamines increases with chain length (Beckett, Brookes & Shenoy, 1969; Vree, Gorgels & others, 1971). Factors affecting dealkylation should similarly affect deamination since both processes occur primarily by oxidation of a carbon atom α to the nitrogen. As the alkyl chain length increases the lipophilicity of the compound will increase and its rate of partition into the microsomes, where dealkylation and deamination occurs, will increase. Steric factors, however, may be equally important.

Deamination activities of the guinea-pig and rat liver homogenates were roughly equal. In contrast, rabbit preparations caused two to four times more deamination, as measured by the total ketone (II) plus alcohol (Ie) formed. (The liver from one of the rabbits had even higher activity [see Table 1] but the exact history of that rabbit is unknown and it may have had elevated cytochrome P_{450} concentrations).

Variation of the centrifugal force used to prepare the rat liver homogenate supernatants had virtually no effect on the metabolic reduction of 1-phenyl-2-propanone. However the amount of 1-phenyl-2-propanone formed from amphetamine decreased with increasing spin speed. This was reflected in the lower yields of microsomal protein, particularly in the 18 000 *g* supernatants, as the spin speed was increased. Some co-precipitation of the microsomal protein with cell debris and sub-cellular structures may explain this observation.

In summary, a marked species difference exists in the ability of rats, guinea-pigs and rabbits to reduce 1-phenyl-2-propanone. *In vitro* the rat liver homogenates had negligible 1-phenyl-2-propanone reductase activity whilst the rabbit homogenates almost totally reduced the ketone (II); guinea-pig homogenates had intermediate activity. These observations are compatible with available *in vivo* data on amphetamine metabolism indicating only the rabbit excretes significant quantities of 1-phenyl-2-propanol (as a conjugate; Dring & others, 1970). The centrifugal force used to prepare the rat liver homogenate supernatants had virtually no effect on their reductase activity.

In studies on the *in vivo* metabolism of [^{14}C]-amphetamine, Dring & others (1970) found that the rat excreted only 3% of the radioactivity as benzoic acid (or its conjugates) whereas the guinea-pig excreted 62%; neither 1-phenyl-2-propanone nor 1-phenyl-2-propanol was detected in the urine of either species. In the present work, the guinea-pig was unique in that 30–40% of the 1-phenyl-2-propanone incubated with fortified liver homogenates was metabolized to products other than the alcohol (Ie); similarly, 20–30% of the 1-phenyl-2-propanol was metabolized to products other than the ketone (II).

Although the rabbit deaminates amphetamine more than the other species, the recovery of benzoic acid *in vivo* is less than half of that recovered from guinea-pigs under the same conditions (Dring & others, 1970). Our findings that 1-phenyl-2-propanone is most extensively reduced by rabbit liver homogenates, but most extensively metabolized by additional routes by guinea-pigs, *in vitro*, are consistent with the *in vivo* data collected by Dring & others (1970).

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