Species Differences in the Metabolism of Norephedrine in Man, Rabbit and Rat

By JOSEPH E. SINSHEIMER,* L. GRAHAM DRJNG and R. TECWYN WILLIAMS
Department of Biochemistry, St. Mary's Hospital Medical School, London W2 1PG, U.K.

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1. ($\pm$)-2-Amino-1-phenyl[1- 14 C]propan-1-ol ([14 C]norephedrine) was administered orally to man, rat and rabbit and the metabolites excreted in the urine were identified and measured. Pronounced species differences in the metabolism of the drug were found. 2. Three male human subjects, receiving 25 mg each of [14 C]norephedrine hydrochloride, excreted over 90% of the 14 C in the first day. The main metabolite was the unchanged drug (86% of the dose) and minor metabolites were hippuric acid and 4-hydroxynorephedrine. 3. In rats given 12 mg of the drug/kg almost 80% of the 14 C administered was excreted in the first day. The major metabolites in the urine were the unchanged drug (48% of the dose), 4-hydroxynorephedrine (28%) and trace amounts of side-chain degradation products. 4. Rabbits given 12 mg of the drug/kg excreted 85–95% of the dose of 14 C in the urine in the first 24 h after dosing. The major metabolites in the urine were conjugates of 1,2-dihydroxy-1-phenylpropane (31% of the dose) and of 1-hydroxy-1-phenylpropan-2-one (27%) and hippuric acid (20%). The unchanged drug was excreted in relatively small amounts (8%).

Norephedrine (*erythro*-2-amino-1-phenylpropan-1-ol) has been found as a metabolite of amphetamine in the urine of man (Caldwell *et al.*, 1972*b*) and of methamphetamine in man and the guinea pig (Caldwell *et al.*, 1972*a*). It is mainly used mixed with other drugs in preparations made up for the treatment of the common cold. Since marked species differences have been observed in the metabolism of amphetamine and methamphetamine it was decided to examine the related compound norephedrine in various species. Heimlich *et al.* (1961) and Beckett & Wilkinson (1965) have found that in man norephedrine is rapidly excreted predominantly unchanged in the urine. In the present work a pronounced species difference in the nature of the metabolites of ($\pm$)-norephedrine in urine has been found in man, the rat and the rabbit. In man the 14 C-labelled drug is excreted largely unchanged, but in the rat it is metabolized to a considerable extent by aromatic hydroxylation and in the rabbit it is extensively deaminized.

Materials and Methods

Compounds

($\pm$)-Norephedrine hydrochloride, m.p. 190–192°C (Sigma Chemical Co., St. Louis, Mo., U.S.A.), ($\pm$)-4-hydroxynorephedrine [2-amino-1-(4'-hydroxyphenyl)propan-1-ol] hydrochloride, m.p. 198°C

(Aldrich Chemical Co., Milwaukee, Wis., U.S.A.), and 1,2-dioxo-1-phenylpropane, b.p. 123–125°C/23 mmHg (Eastman Organic Chemicals, Rochester, N.Y., U.S.A.), were purchased. 4-Hydroxyhippuric acid, m.p. 234°C (Fischer, 1908), ($\pm$)-1-hydroxy-2-oxo-1-phenylpropane, b.p. 100–110°C/18 mmHg (Hey, 1930), and ($\pm$)-1,2-dihydroxy-1-phenylpropane, m.p. 90–92°C (Zincke & Zahn, 1910), were prepared as described in the literature. The *erythro* configuration of the above dihydroxyphenylpropane has been established by Foltz & Witkop (1957).

($\pm$)-2-Amino-1-phenyl[1- 14 C]propan-1-ol ([14 C]-norephedrine). [*carboxy*- 14 C]Propionic acid was prepared from a mixture of Ba 14 CO $_3$ (94.7 mg and 103 μ Ci/mg) and BaCO $_3$ (3.89 g) as described by Calvin *et al.* (1949) and converted into the corresponding [14 C]propionyl chloride (b.p. 67–69°C; 1.35 g) by the method of Brown (1938). This was then converted into 1-oxo-1-phenyl[1- 14 C]propane ([14 C]propiophenone; b.p. 65–67°C/17–19 mmHg; 1.04 g containing 3 μ Ci/mg) by the method of Read (1922). When the propiophenone (0.98 g) was treated with isopropyl nitrite (2.6 g) as described by Levin & Hartung (1955), ($\pm$)-2-isonitroso-1-oxo-1-phenyl[1- 14 C]propane (m.p. 114–115°C; 0.38 g) was obtained. The isonitroso compound (0.2 g) in ethanol (30 ml) containing 7.5 M-HCl in ethanol (0.6 ml), 5% palladium on charcoal (0.15 g) and 5% platinum on charcoal (0.15 g) was reduced with H $_2$ at a pressure of 210 kPa (30 lbf/in 2) at room temperature for 4 h as described by Wilbert & Sosis (1962). The experiment was repeated with 0.18 g of isonitroso compound. The catalysts were

* Present address: College of Pharmacy, The University of Michigan, Ann Arbor, Mich. 48104, U.S.A.

filtered off and the solvent was removed *in vacuo*. The residue after crystallization from dioxan gave 0.13 g (specific radioactivity 2.45 $\mu\text{Ci}/\text{mg}$) of ($\pm$)-2-amino-1-phenyl[1- ^{14}C]propan-1-ol hydrochloride, m.p. 188–190°C and mixed m.p. 190–192°C with authentic ($\pm$)-norephedrine hydrochloride, m.p. 190–192°C. The radiochemical yield was 3.3% based on the $\text{Ba}^{14}\text{CO}_3$ used and the radiochemical purity was greater than 97% as determined by isotope dilution and recrystallization to constant specific radioactivity from dioxan.

Animals

Female Wistar albino rats ($210 \pm 20\text{g}$) and female Dutch rabbits ($1.7 \pm 0.2\text{kg}$) were used. The drug dissolved in water was administered by stomach tube and the animals were kept singly in suitable metabolism cages. Urine and faeces were collected daily. The animals were kept on a suitable diet, but were not allowed food for the first 24 h after dosing. Human subjects took the drug dissolved in 5 ml of water by mouth, but no attempt was made to control urinary pH.

Radiochemical techniques

The counting of ^{14}C in urine and faeces, the preparation and counting of radiochromatograms

and isotope dilution procedures were carried out as described by Dring *et al.* (1970) and Caldwell *et al.* (1972a,b).

Chromatography

R_F values and colour reactions of norephedrine and its metabolites on paper chromatography and t.l.c. are given in Table 1. The solvent systems B and C were found to require aging for at least 3 days before use to obtain R_F values consistent with those in the literature. T.l.c. was on fluorescent gel so that reference compounds run on the same plate as unknowns could be detected by their quenching of u.v. light. Paper chromatography was used for the separation of the major metabolites, and t.l.c. for their identification and for the separation and identification of minor metabolites that were not readily separated on paper.

Enzyme hydrolysis

Urine samples or fractions separated from the urine were taken up in water and then mixed with an equal volume of β -glucuronidase preparation (Ketodase, William R. Warner and Co. Ltd., Eastleigh, Hants, U.K.) and adjusted to pH 5.0 with 2M-acetic acid. The mixtures were incubated at

Table 1. R_F values and colour reactions of norephedrine and related compounds

For paper chromatography Whatman no. 1 paper was used. For t.l.c., glass plates ($20\text{cm} \times 5\text{cm}$ or $\times 20\text{cm}$) were coated with 0.25 mm of silica gel GF₂₅₄ (E. Merck A.G., Darmstadt, Germany) and then heated for 1 h at 110°C before use. Solvent systems, were: A, butan-1-ol saturated with 1.5M- NH_3 -(NH_4)₂ CO_3 buffer (Fewster & Hall, 1951); B, 3-methylbutan-1-ol–2-methylbutan-1-ol–water–formic acid (5:5:10:2, by vol.; Alleva, 1963); C, 2-methylpropan-1-ol–formic acid–water (50:6:5, by vol.; Matsuda *et al.*, 1971); D, chloroform–diethylamine (199:1, v/v); E, benzene–methanol–acetic acid (10:2:1, by vol.); F, chloroform–diethylamine–methanol (20:1:1, by vol.); G, benzene–methanol–acetic acid (20:2:1, by vol.). With t.l.c. the solvent was allowed to run 14 cm from the origin. Under u.v. light (254 nm; Hanovia Chromatolite lamp) all compounds appeared as dark spots. For colour reactions see Dring *et al.* (1970).

Compound		R_F value									Colour with diazotized nitroaniline
		Paper			T.l.c.						
		Solvent ...	A	B	C	D	E	F	F then	G	
E											
Norephedrine		0.82	0.39	0.38	—	—	—	—	—	—	—
4-Hydroxynorephedrine		0.64	0.17	0.18	0.00	—	0.15	—	0.21	Purple	
4-Hydroxybenzoic acid		0.00	0.88	0.80	—	—	0.10	—	—	Purple	
4-Hydroxyhippuric acid		—	0.73	0.70	0.06	—	0.00	0.11	—	Purple	
Benzoic acid		0.33	0.93	0.85	0.08	0.53	0.30	0.69	—	—	
Hippuric acid		0.20	0.82	0.80	0.00	0.35	0.13	0.43	—	—	
1,2-Dihydroxy-1-phenylpropane		—	0.81	0.90	0.25	—	0.63	0.82	—	—	
1-Hydroxy-2-oxo-1-phenylpropane		0.73	0.81	0.90	0.89	—	0.99	—	—	—	
1,2-Dioxo-1-phenylpropane		—	0.81	0.90	0.97	—	0.99	—	—	—	

37°C for 48h. Controls containing boiled Ketodase were run at the same time. Similar hydrolyses were carried out with 0.1ml of sulphatase solution (Type H-2; Sigma Chemical Co.) for each 1ml of urine or separated fraction. Although Ketodase contained no sulphatase, the sulphatase preparation contained some β -glucuronidase. Hydrolysis with acid or alkali was not used, since 4-hydroxynorephedrine was decomposed under these conditions.

G.l.c.-mass spectrometry

A Varian Aerograph 1700 gas-liquid chromatograph with a Varian MAT CH5 mass spectrometer was used.

Identification of metabolites

In rat urine. A sample (0.1 ml) of the urine collected during the first 24h after dosing with [^{14}C]norephedrine was chromatographed on Whatman no. 1 paper in systems A, B and C (Table 1). Three main peaks of radioactivity and a minor one were found in each solvent, their R_F values in system A being 0.03, 0.59, 0.79 and 0.25 (minor), and there were corresponding ones in solvents B and C. After hydrolysis of the urine with β -glucuronidase, the peak near the origin was considerably diminished and this was accompanied by an increase in the peak of R_F 0.59 in A and the corresponding one in B and C. Co-chromatography with reference compounds and colour reactions (Dring *et al.*, 1970) indicated that the major peaks found in the original urine were consistent with conjugated 4-hydroxynorephedrine, free 4-hydroxynorephedrine and norephedrine and the minor one with hippuric acid. The presence of 4-hydroxynorephedrine and hippuric acid was confirmed by reverse isotope dilution.

The minor peak had R_F 0.25, 0.82 and 0.83 in solvents A, B and C respectively. The minor band, R_F 0.82, from 1ml of urine chromatographed in solvent B was cut out and eluted with water. The aqueous solution was evaporated to dryness under reduced pressure and the residue dissolved in methanol. This solution was then chromatographed on t.l.c. plates by using solvents D, F or F followed by E (see Table 1). Radiochromatograms were prepared and ^{14}C peaks were found which were consistent with 4-hydroxyhippuric acid, hippuric acid, benzoic acid and 1,2-dihydroxy-1-phenylpropane.

In rabbit urine. The urine (0.1 ml) was chromatographed on paper and treated with β -glucuronidase and sulphatase as for rat urine.

In solvent B, four ^{14}C peaks, nos. 1-4, were found with R_F values 0.12, 0.31, 0.49 and 0.86. These peaks (from 1ml of urine) were cut out of the chromato-

gram, eluted with water and each eluate was chromatographed in solvent C before and after treatment with β -glucuronidase and sulphatase.

Peaks nos. 1 and 4, which had R_F values of 0.17 and 0.83 in solvent C, were unaffected by enzyme treatment. The material eluted from peak no. 1 on t.l.c. in systems F and G had R_F values consistent with those of 4-hydroxynorephedrine. That from peak no. 4 on t.l.c. in systems D, F or F followed by E was resolved into peaks consistent with those of hippuric acid, benzoic acid, 1-hydroxy-2-oxo-1-phenylpropane, 1,2-dihydroxy-1-phenylpropane and 1,2-dioxo-1-phenylpropane.

Peak no. 2 (R_F 0.30 in system C) was completely abolished by sulphatase but not by β -glucuronidase (Ketodase), suggesting that it was a sulphate conjugate. The material produced by hydrolysis with sulphatase was chromatographed in systems B, D and F, and the R_F values obtained were consistent with those of 1,2-dihydroxy-1-phenylpropane.

Peak no. 3 (R_F 0.39 in system C) was partially removed by β -glucuronidase and this suggested that it was, at least in part, a conjugate with glucuronic acid. Chromatography of the material in this peak after treatment with β -glucuronidase in solvents A, B, C and D suggested the presence of norephedrine and the ketol, 1-hydroxy-2-oxo-1-phenylpropane. Chromatography of peak no. 3 before hydrolysis with β -glucuronidase in solvent A was used to establish the presence of norephedrine and to determine its amount in relation to the glucuronide of the ketol.

Identification of phenylpropanes by g.l.c. and mass spectrometry. A hydrolysate of rabbit urine (10ml), which had been treated with the above sulphatase preparation (which contains both arylsulphatase and β -glucuronidase), was adjusted to pH 2.0 with 2M-HCl and then extracted with ethyl acetate (3 $\times$ 75ml). The extract was washed with 1M-acetate buffer, pH 5.9, dried (anhydrous Na_2SO_4) and the solvent removed under reduced pressure. The residue was taken up in diethyl ether (0.4ml) and the solution divided into two halves. One-half was subjected to g.l.c. on a Hewlett-Packard gas chromatograph model 702. The other half was evaporated and the residue treated with acetic anhydride (0.1ml) and pyridine (1ml) and kept at room temperature for 18h. The solvents were removed under reduced pressure and the residue was taken up in methanol (0.1ml). The solution was then subjected to g.l.c. in the Hewlett-Packard instrument and to g.l.c.-mass spectrometry in a Varian MAT CH5 mass spectrometer connected with an Aerograph model 1700 gas chromatograph. Extracts of normal rabbit urine were subjected to exactly the same procedures. Authentic samples of 1,2-dioxo-1-phenylpropane, 1-hydroxy-2-oxo-1-phenylpropane and 1,2-dihydroxy-1-phenylpropane were also subjected to g.l.c.

before and after acetylation as above, and to g.l.c.-mass spectrometry.

For g.l.c. a 1.82m (6ft) glass column (internal diam. 3mm) packed with SE-30 (3%, w/v) on AW-DMCS-treated Chromosorb G (100–120 mesh) was used, the conditions being injection port temperature 240°C, column 135°C, detector 270°C, N₂ flow 30ml/min at 275 kPa (40lb/in²), air pressure

165kPa (24lb/in²) and H₂ pressure 140kPa (20lb/in²).

For g.l.c.-mass spectrometry a 1.52m (5ft) column (internal diam. 2mm) packed with 3% OV-101 on Celite AW-DMCS (100–200 mesh) was used with temperature programming from 110°C at 6°C/min. The injection port temperature was 190°C, detector 165°C, interface 155°C, Biemann-Watson-

Table 2. *G.l.c. of some derivatives of phenylpropane*

Extracts of the urine of rabbits receiving norephedrine were prepared as described in the text. These extracts and solutions of the oxidized phenylpropanes were subjected to g.l.c. as described in the text.

Compound	Retention times (min)			
	Before acetylation		After acetylation	
	Authentic compound	Urine extract	Authentic compound	Urine extract
1,2-Dioxo-1-phenylpropane	0.6	0.6	—	—
1-Hydroxy-2-oxo-1-phenylpropane	1.4	1.4	3.9	3.9
1,2-Dihydroxy-1-phenylpropane	2.3	2.3	9.5	9.5

Table 3. *G.l.c.-mass spectrometry of acetyl derivatives of norephedrine metabolites in rabbit urine*

Extracts of rabbit urine and the authentic phenylpropane derivatives were acetylated and subjected to g.l.c.-mass spectrometry as described in the text. Retention times and relative abundances of the more important ions (>3%) are given. Metastable ions at *m/e* 58.3, 76.8 and 115.6 were present in all four spectra, whereas a metastable ion at *m/e* 102 was only seen in the spectrum of the diol and metabolite 2.

<i>m/e</i>	Retention time (min)	...	1-Hydroxy-2-oxo-1-phenylpropane	1,2-Dihydroxy-1-phenylpropane	Metabolite 1	Metabolite 2
			4.75	6.5	4.75	6.5
193			2.9*	2.9*	2.3*	2.7*
192			12.9*†	15.7*	13.1*	18.5*
177				15.0*		15.7*
176				11.4		12.9
150			4.3	4.3	3.9	5.7
149			34.3	35.7	30.7	40.0
134				11.4		10.0
108			7.1	8.6	7.7	8.6
107			71.4	71.4	60.0	70.0
105			5.4	7.1	6.2	5.7
91			4.3	7.1	3.1	8.6
87				7.1		5.7
79			30.0	11.4	18.5	10.0
77			14.3	11.4	11.5	8.6
52			8.6			
51			10.0	5.7	6.2	4.3
45				4.3		
44			5.7	7.1	10.8	7.1
43			100.0	100.0	100.00	100.0

* These values are ×10.

† Molecular ion.

type molecular separator 145°C, inlet line 135°C and the source 140°C. The helium flow was 40ml/min at 195kPa (28lbf/in²) and the electron beam energy 70eV.

The retention times of authentic 1,2-dioxo-, 1-hydroxy-2-oxo- and 1,2-dihydroxy-1-phenylpropane before and after acetylation and those of extracts from rabbit urine described above are shown in Table 2.

The retention times and the relevant peaks in the mass spectrum of the acetylated compounds and urine extracts are shown in Table 3.

In human urine. Samples (1ml) of the urine passed in the first 24h after dosing were chromatographed on Whatman 3MM paper by using solvents A, B and C (see Table 1). On radiochromatogram scanning only one clear peak was found in all three solvents and this corresponded to unchanged norephedrine, which was confirmed by reverse isotope dilution. But on cutting up the radiochromatogram and counting the pieces for radioactivity in the liquid-scintillation spectrometer, small amounts of other metabolites were detected. These included hippuric acid and conjugated 4-hydroxynorephedrine. Hippuric acid and 4-hydroxynorephedrine (after hydrolysis with β -glucuronidase) were determined by reverse isotope dilution.

Results

Table 4 shows that in rat, rabbit and man some 80–90% of the ¹⁴C of small oral doses of [¹⁴C]norephedrine are rapidly excreted in the urine in 24h. The excretion of ¹⁴C in the faeces of rats and rabbits is small (1–2% of dose). The results of the quantitative examination of radiochromatograms of the urine of rats, rabbits and humans excreted in 24h after the

oral administration of [¹⁴C]norephedrine hydrochloride are shown in Table 5.

Rat

In 24h after dosing nearly one-half (48%) of the dose of norephedrine is excreted unchanged in the urine, and the only metabolite that is excreted in substantial amounts (about 28% of the dose) is 4-hydroxynorephedrine, which occurs in both the free and conjugated state. Thus of the ¹⁴C excreted in 24h 60% occurs as unchanged drug and 35% as total 4-hydroxynorephedrine. There is slight deamination of the drug to give side-chain degradation products (1% of dose in 24h), the main one being benzoic acid excreted largely as hippuric acid. Small amounts of 4-hydroxyhippuric acid and 1,2-dihydroxy-1-phenylpropane were detected. The major metabolic reaction of norephedrine in the rat is thus aromatic hydroxylation.

Rabbit

In the rabbit, the metabolism of norephedrine is completely different from that in the rat. About 8% of the dose is excreted unchanged and about 3% is hydroxylated to 4-hydroxynorephedrine, but some 76% is excreted as deamination products. Three of these were found in the urine as major metabolites, namely 1,2-dihydroxy-1-phenylpropane (about 31%), 1-hydroxy-2-oxo-1-phenylpropane (about 27%) and benzoic acid (about 24%). The use of Ketodase (β -glucuronidase) and sulphatase from Sigma Chemical Co. (which contains both arylsulphatase and β -glucuronidase) suggests that 1,2-dihydroxy-1-phenylpropane may be present largely as a sulphate

Table 4. Excretion of ¹⁴C after administration of ($\pm$)-[¹⁴C]norephedrine hydrochloride

($\pm$)-[¹⁴C]Norephedrine hydrochloride dissolved in water was administered orally. Mean values are given with ranges in parentheses.

Animal (no., sex)	Dose		Time after dosing (days)	¹⁴ C excreted (% of dose)		
	(mg/kg)	(μ Ci/kg)		Urine	Faeces	Total
Rat (3, F)	12	28	1	80 (78–81)	—	—
			3	—	1.7 (1.3–1.9)	—
			4	82 (81–83)	—	84 (82–88)
			4	89 (83–96)	—	—
Rabbit (3, F)	12	3.75	1	—	2.3 (1.4–3.3)	—
			3	—	—	—
			4	91 (86–97)	—	93 (88–100)
			4	94 (92–96)	—	—
Man (3, M)	25*	3.9*	1	97 (95–99)	—	—
			2	—	—	—

* Dose of drug and ¹⁴C are per person; the drug dose was approx. 0.36mg/kg.

Table 5. *Quantitative aspects of the metabolites of ($\pm$)-[^{14}C]norephedrine in urine*

The doses of ($\pm$)-[^{14}C]norephedrine hydrochloride are given in Table 4. The urine analysed was that collected for the first 24h after dosing. Metabolites in the upper half of the table were determined by scintillation counting of radiochromatograms developed on paper in solvent B for human and rabbit urine and in solvent C for rat urine. The values given in the upper half of the table are the means for three animals with ranges in parentheses. The values in the lower half of the table were obtained by scintillation counting of thin-layer chromatograms developed in system D or system F followed by system E (see Table 1) and are for one animal.

Metabolites	% of dose found in urine excreted in 24h		
	Rat	Rabbit	Man
Norephedrine	48.1 (44.0–54.7)	8.4 (2.6–15.2)	89.6 (84.9–94.5) 86.3 (84.4–88.8)*
4-Hydroxynorephedrine			
Free	10.4 (7.2–14.1)	2.9 (1.8–3.6)	0.0 (0.0–0.0)
Total	27.8 (20.4–32.8) (30.7)*	2.9 (1.8–3.6) (1.0)*	0.6 (0.0–1.8) (0.8)*
Sulphate† of 1,2-dihydroxy-1-phenylpropane	—	30.7 (24.2–40.0)	—
Glucuronide† of 1-hydroxy-2-oxo-1-phenylpropane	—	26.8 (23.2–32.6)	—
Other side-chain degradation products	1.1 (0.6–1.9)	18.5 (4.7–26.0)	3.4 (1.0–7.1)
Sum of above metabolites	77.0 (76.6–77.4)	87.3 (82.6–95.8)	93.6 (92.0–95.5)
^{14}C in the urine	79.9 (77.6–81.1)	88.5 (83.0–95.8)	93.6 (92.0–95.5)
Side-chain degradation products‡			
Benzoic acid	0.1	3.8	—
Hippuric acid	0.7 (0.2)*	20.1 (21.1)*	2.5*
4-Hydroxyhippuric acid	0.03	—	—
1,2-Dioxo-1-phenylpropane	—	0.1	—
1-Hydroxy-2-oxo-1-phenylpropane	—	0.2	—
1,2-Dihydroxy-1-phenylpropane	0.1	1.8	—
Sum of above products	0.93	26.0	—
^{14}C in sample examined	1.9	26.0	7.1

* Values obtained by reverse isotope dilution.

† Tentative identification of conjugate (see the text).

‡ Urine from one animal only examined, this being the one with the largest amount of this fraction shown in the upper half of the table.

ester and 1-hydroxy-2-oxo-1-phenylpropane largely as a glucuronide (see Table 5).

When the extracts of rabbit urine hydrolysed with sulphatase were subjected to g.l.c. and to g.l.c.–mass spectrometry, two peaks were observed. The retention times of these peaks before and after acetylation corresponded to those of free and acetylated 1-hydroxy-2-oxo-1-phenylpropane and 1,2-dihydroxy-1-phenylpropane (see Tables 2 and 3). This was confirmed by mass spectrometry (see Table 3). The probable cracking pattern for the acetylated derivatives of these two compounds is given in Scheme 1 (m/e values are given in parentheses).

The peaks in the mass spectra (see Table 3) at m/e 108, 105, 91, 77 and 43 are probably $\text{C}_7\text{H}_8\text{O}^+$, $\text{C}_7\text{H}_5\text{O}^+$, C_7H_7^+ , C_6H_6^+ and $\text{C}_2\text{H}_3\text{O}$ respectively. The transitions m/e 192 $\rightarrow$ 149, 176 $\rightarrow$ 134, 149 $\rightarrow$ 107 and 107 $\rightarrow$ 79 are supported by the presence of

metastable ions at m/e 116, 102, 76.8 and 58.3 respectively. The molecular ion m/e 236, which would correspond to 1,2-diacetoxy-1-phenylpropane, was not observed when the authentic compound was injected into the mass spectrometer.

The peak R_f 0.49 in solvent B was only partially removed by β -glucuronidase, and the component of this peak that was not affected by β -glucuronidase was unchanged norephedrine. Separation, elution and rechromatography of this peak in solvent A suggested that it was composed roughly of 1 part of norephedrine to 3 parts of conjugated ketol. The benzoic acid present was largely conjugated in the form of hippuric acid, which occurred in one urine to the extent of 20–21% of the dose. Small amounts of 1,2-dihydroxy- and 1-hydroxy-2-oxo-1-phenylpropane and benzoic acid were found in the free state together with small amounts of 1,2-dioxo-1-phenyl-

Scheme 1. Probable cracking pattern for the acetylated derivatives of 1-hydroxy-2-oxo-1-phenylpropane and 1,2-dihydroxy-1-phenylpropane
m/e values are given in parentheses.

propane, which might well be an artifact since the ketol is readily oxidized to the dione (cf. Warren *et al.*, 1971).

Man

The metabolism of norephedrine in man was different from that in the rabbit and from that in the rat. In fact, the drug was largely excreted unchanged, nearly 90% of the dose of ^{14}C being excreted in 24h as the unchanged drug and only about 4% as transformation products. Hydroxylation to 4-hydroxy-norephedrine was only detected in appreciable amounts in one of the three subjects examined (see Table 5). Deamination of the side chain was also low, ranging from 1 to 7% in the three subjects, the main product being hippuric acid.

Discussion

The metabolic fate of norephedrine with the (–)-isomer has been examined previously by Axelrod (1953) in the dog and rabbit and by Beckett & Wilkinson (1965) in man. In the dog, Axelrod (1953) found 72% of intravenously injected (–)-norephedrine to be excreted in the urine, whereas in the rabbit only 3% of the dose (intraperitoneal) was excreted unchanged. In man, Beckett & Wilkinson (1965) found 91% of the orally administered drug to be excreted unchanged. The present work with the radioactive (±)-isomer confirms that norephedrine is extensively metabolized in the rabbit but not in man

(see Table 6), and shows that there are marked differences in the metabolism of the drug in man, rat and rabbit.

In the rat, more than one-half of (±)-norephedrine is excreted unchanged (see Table 6) and it is possible that only the (–)-isomer is metabolized, since Nagase *et al.* (1967) have suggested that in the rat the *N*-methyl derivative, (+)-ephedrine, is not metabolized whereas its (–)-isomer is extensively metabolized. The major reaction of norephedrine in the rat is aromatic hydroxylation and therefore its metabolism in this species is similar to that of the other phenylisopropylamines that have been examined, namely amphetamine (Dring *et al.*, 1970), methamphetamine (Caldwell *et al.*, 1972a) and ephedrine (Nagase *et al.*, 1967; Bralet *et al.*, 1968). With these four compounds side-chain deamination and degradation occurs to only a minor extent. The main route of transformation of norephedrine in the rat is therefore as follows:

As far as the rabbit is concerned, (±)-norephedrine is extensively metabolized, not by aromatic hydroxylation as in the rat, but by deamination and degradation of the side chain (see Table 6). Norephedrine thus behaves in the rabbit as does amphetamine (Dring *et al.*, 1970), methamphetamine (Smith & Dring, 1970) and ephedrine (Matsuda *et al.*, 1971). The metabolites of norephedrine in the rabbit are mainly those containing the three-carbon side chain intact after loss of the NH_2 group, and the main route of metabolism is probably as outlined in Scheme 2.

Average values for three animals are given to the nearest whole number.

Table 7. Some physical properties of phenylisopropylamines and metabolism

Compound and formula	True partition		Approximate % of dose excreted unchanged in		
	pK _a *	coefficient*	Man	Rat	Rabbit
Amphetamine PhCH ₂ CH(NH ₂)CH ₃	9.90	146	30†	13†	4†
Methamphetamine PhCH ₂ CH(NHMe)CH ₃	10.11	565	30‡	11‡	2‡‡
Norephedrine PhCH(OH)CH(NH ₂)CH ₃	9.55	0.03	90§, 91	48§	8§
Ephedrine PhCH(OH)CH(NHMe)CH ₃	9.60	2.42	79	42¶, 32‡‡	0.7**, 0.1††

References: * values from Vree *et al.* (1969); † Dring *et al.* (1970); ‡ Caldwell *et al.* (1972a); § the present paper; || Beckett & Wilkinson (1965); ¶ Nagase *et al.* (1967); ** Matsuda *et al.* (1971); †† Axelrod (1953); ‡‡ Smith & Dring (1970).

ephedrine and ephedrine) are almost completely metabolized irrespective of their lipid solubilities (see Table 7), but the main reaction is deamination preceded where necessary by *N*-demethylation. With amphetamine, deamination has been shown to occur in liver microsomal fractions (Axelrod, 1955) but no aromatic hydroxylation occurs in these preparations (Daly *et al.*, 1967). It will be recalled that at pH 7.4, these bases occur to the extent of 99% as cations that are water-soluble, and it would appear that rabbit liver microsomal fractions can metabolize these water-soluble cations. This recalls (see Renwick & Williams, 1972) the ability of the rabbit to metabolize to a significant extent (30% of dose) the strong base cyclohexylamine, which is only slightly metabolized in rat, guinea pig and man (1–7%).

This investigation was approved by the Ethical Committee of St. Mary's Hospital Medical School and the informed consent was obtained from the three male volunteers. We are grateful to Miss Helen Turbert for technical assistance in certain experiments. J. E. S. thanks the American Foundation for Pharmaceutical Education for a Pfeiffer Memorial Research Fellowship.

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