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The mammalian metabolism of *R*-(−)-*m*-synephrine

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The metabolism of *R*-(−)-*m*-synephrine (administered orally and by inhalation in man and intraperitoneally in rats) was studied quantitatively by a gas chromatography-mass spectrometry-selected ion monitoring (g.c.–m.s.–s.i.m.) method using deuterated internal standards. When *m*-synephrine hydrochloride was administered orally to humans in normal dosage regimens four main metabolites were excreted in urine: (i) unconjugated *m*-hydroxymandelic acid (MHMA, 30%), (ii) *m*-hydroxyphenylglycol (MHPG) sulphate (6%), (iii) *m*-synephrine sulphate (47%) and (iv) *m*-synephrine glucuronide (12%). The comparable figures after inhalation of the drug were 24, 6, 56 and 5%. Intraperitoneal injection of *m*-synephrine into rats gave: unconjugated MHMA (5%), MHPG sulphate (35%), unconjugated *m*-synephrine (7%) and conjugates of *m*-synephrine (9%:4% as the glucuronide and 5% as the sulphate).

m-Synephrine (phenylephrine, neosynephrine) is an α-adrenoceptor agonist (Trendelenburg et al 1962) which has powerful vasoconstrictor and mydriatic properties. It has been extensively used for many years as a component of nasal decongestant preparations and as a mydriatic agent but, until recently, little was known about its metabolism.

In 1967 Elis et al suggested that 'phenylephrine is a substrate for monoamine oxidase and so, when given orally, is normally largely destroyed in the gut wall and liver'. During the 24 h following oral administration of *m*-synephrine to humans approximately 80% of the dose could be accounted for, almost entirely as conjugates (principally sulphates), but the method employed lacked chemical and spectroscopic specificity (Bruce & Pitts 1968). Subsequently it was discovered (Midgley et al 1979) that *m*-hydroxymandelic acid (MHMA) occurs naturally in human urine and it was suggested that this arises by oxidative deamination of endogenous *m*-octopamine and/or *m*-synephrine. Further investigation (Midgley et al 1980; Durden et al 1980) demonstrated that *m*-synephrine does occur naturally in bovine and mouse adrenal gland. Evidence was then provided that exogenously administered (±)-[^{3}H]*m*-synephrine was preferentially reductively deaminated to *m*-hydroxyphenylglycol (MHPG) by perfused rat heart, although an authentic sample of MHPG was not available for comparison (Rawlow et al 1980). An independent and specific g.c.–m.s. study (Crowley et al 1982) has since shown unequivocally that MHPG is a normal constituent of both human and rat urine, in addition to MHMA.

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These observations prompted us to investigate the metabolism of *m*-synephrine when it is administered orally and by inhalation to humans and by intraperitoneal injection into rats. On completion of this study the pharmacokinetics of (±)-[^{3}H]*m*-synephrine in man were reported by Hengstmann & Goronzy (1982), who concluded that phenylephrine was metabolized largely by conjugation and oxidative deamination to MHMA.

MATERIALS AND METHODS

Materials

These were obtained from the following sources: *R*-(−)-[$^2H_0$]*m*-synephrine, Sterling Chemical Co. (New York); pentafluoropropionic acid anhydride (PFPA), Pierce Chemical Co. (Rockford, Illinois); sulphatase (*Helix pomatia*—type H-1), bacterial β-glucuronidase (Type VII) and MHMA, Sigma Chemical Co. (St Louis, Missouri). [$^2H_3$]*m*-synephrine and [$^2H_0$]MHPG were synthesized as previously described (Midgley et al 1980; Crowley et al 1982); [$^2H_3$]MHMA was prepared by heating a solution of the undeuterated acid in D_2O and DCl at 80 °C for 24 h; [$^2H_5$]MHPG was obtained by the reduction of [$^2H_3$]MHMA with lithium aluminium deuteride. Methanolic HCl was prepared by the slow addition of acetyl chloride (1 ml) to dry methanol (4 ml).

Dristan tablets (Whitehall Labs., Inc., N.Y.) each contained 5 mg of *R*-(−)-*m*-synephrine hydrochloride (equivalent to 4·1 mg of free base).

Neo-Synephrine nasal decongestant (Winthrop) contained *R*-(−)-*m*-synephrine HCl (0·5% w/v).

Apparatus

Gas chromatography-mass spectrometry-selected ion monitoring (g.c.–m.s.–s.i.m.) was carried out on a Hewlett-Packard model 5992A using a silanized glass column (1·8 m × 2 mm i.d.) packed with 5% OV-101 on Chromosorb GHP 100/120 mesh (Supelco). The g.c. was operated isothermally at 180 °C for the glycols and acids and at 220 °C for the amines; helium was the carrier gas; column effluent was diverted from the ion source for the first 1·5 min.

Experiments in rats

Urine was collected over a 24 h period from each of three male Wistar rats, 200–250 g, maintained in separate metabolism cages. Each rat was then injected intraperitoneally with 0·9% NaCl (saline) (0·3 ml) and the urine was collected for a further 24 h. This was followed by the intraperitoneal injection of *R*-(−)-*m*-synephrine (250 μg) in saline (0·3 ml) and collection of the urine for two successive periods of 24 h each. The samples of urine were analysed immediately after collection.

Experiments in humans

One male and two female volunteers, 25–35 years, each ingested 6 Dristan tablets over a period of 8 h. Control urine was obtained from each subject during the 24 h immediately before the administration of the drug and a further 24 h collection began at the time of the first ingestion of the drug. These samples also were analysed immediately after collection.

Two weeks later the same three subjects inhaled Neo-Synephrine nasal decongestant in the prescribed manner (three times in each nostril every 3 h during a 9 h period). The total dose of *R*-(−)-*m*-synephrine hydrochloride inhaled by each subject was recorded (equivalent to 10, 24, 34 mg of free base). Urine was collected from each subject for 24 h following the first administration of the drug.

Procedure

Internal Standards. (i) [$^2H_3$]*m*-synephrine, [$^2H_3$]-MHMA and [$^2H_5$]MHPG (10 μg each) were added to each of four aliquots (each equivalent to 1 mg of creatinine) from each specimen of human urine.

(ii) [$^2H_5$]MHPG (150 μg), [$^2H_3$]*m*-synephrine (25 μg) and [$^2H_3$]MHMA (25 μg) were added to each 24 h sample of rat urine which was then divided into four equal parts.

The pH of one of the four aliquots of urine (rat and human) was adjusted to 1 with conc. HCl and the sample was immediately extracted (see below).

Deconjugation. The conjugates in the other three portions of urine (human and rat) were hydrolysed as follows:

(i) acidic hydrolysis was carried out by adjusting the pH of one portion of the urine to 1 with conc. HCl and heating the sample in a boiling water bath for 1 h;

(ii) β-glucuronidase (1000 units) in ammonium acetate buffer (1 ml, 0·2 M, pH 6) was added to the next portion of the urine which was incubated at 37 °C for 18 h;

(iii) sulphatase (30 mg, containing 1200 units of glucuronidase and 750 units of sulphatase) in ammonium acetate buffer (1 ml, 0·2 M, pH 6) was added to the last portion of the urine which was incubated at 37 °C for 18 h.

The pH of portions (ii) and (iii) was then adjusted to 1 with conc. HCl.

Extraction of acids and glycols. The urine was extracted twice with ethyl acetate (2 vol). Carboxylic acids were removed from the combined ethyl acetate extracts by extraction with aqueous $NaHCO_3$ (10%, 2 × 1 ml) leaving glycols in the organic phase. The pH of the aqueous solution was adjusted to 1 with conc. HCl and it was then extracted with ethyl acetate (2 × 5 ml). In each case the solvent was removed from the organic extract by evaporation under reduced pressure at ca 40 °C.

Analysis of acids. The resultant dried extract was reacted with methanolic HCl (100 μl) for 15 min at room temperature (22 °C) (Midgley et al 1979) and, after evaporation of the excess reagent with a stream of nitrogen, the residue was reacted with PFPA (100 μl) for 15 min at 60 °C. Excess PFPA was removed under nitrogen; the residue was dissolved in dry ethyl acetate (500 μl) and an aliquot (1–2 μl) of this solution was then injected directly into the g.c.–m.s. Identification of the pentafluoropropionyl-methyl ester (PFP-Me) derivative of MHMA was carried out using the molecular ion (*m/z* 474) and the $(M–CO_2CH_3)^+$ ion (*m/z* 415). In the derivatized biological extracts the retention times and ratios of the intensities of these two ions were identical, within experimental error, to those of the standard. Quantitative analysis of MHMA was carried out by determination of the ratio of the intensities of the molecular ions, *m/z* 474 and *m/z* 477, produced by [$^2H_0$]MHMA-PFP-Me and [$^2H_3$]MHMA-PFP-Me respectively, in the manner previously described (Crowley et al 1982).

Analysis of glycols. The residue containing the glycols (see above) was reacted with PFPA (100 μl) at 60 °C for 15 min. Excess PFPA was evaporated under nitrogen and the resultant residue dissolved in

ethyl acetate (500 μl); 1–2 μl of this solution were injected into the g.c.–m.s. Identification of the PFP derivative of MHPG was carried out by establishing that the retention times and the ratio of the intensities of the molecular ion (*m/z* 592) and the $(M-C_2F_5CO_2H)^+$ ion (*m/z* 428) in the derivatized biological extract were identical, within experimental error, to those of the standard. Quantitative analysis of MHPG was carried out by determination of the ratio of the intensities of the molecular ions, *m/z* 592 and *m/z* 597, produced by $[^2H_0]$MHPG-PFP and $[^2H_5]$MHPG-PFP respectively (Crowley et al 1982).

Extraction of amines. The pH of the residual urine (see above) was adjusted to 6 with 2 M NaOH and this mixture was then passed through a strong cationic exchange resin (AG 50W-X2, 100–120 mesh, Bio-Rad). The resin was washed with water and the amines were eluted with 10–25 ml of NH_4OH (1 M) in ethanol (65%). This eluate was reduced to dryness by rotary evaporation at ca 40 °C.

Analysis of amines. The resultant dried residue was treated with PFPA (100 μl) for 15 min at 60 °C, the excess PFPA was then evaporated with a stream of nitrogen and the derivative dissolved in ethyl acetate (500 μl); 1 μl of this was injected into the g.c.–m.s. Identification of the PFP derivative of *m*-synephrine was carried out by establishing that the retention times and the ratio of the intensities of the molecular ion (*m/z* 605) and the $[CH_2=N(CH_3)(COC_2F_5)]^+$ ion (*m/z* 190) in the derivatized biological extract were identical, within experimental error, to those of the corresponding ions (*m/z* 608 and *m/z* 193 respectively) of the deuterated internal standard. Quantitative analysis of *m*-synephrine was carried out by determination of the ratio of the intensities of the molecular ions (*m/z* 605 and *m/z* 608) in the manner previously described (Midgley et al 1980) for the corresponding TFA derivatives.

RESULTS AND DISCUSSION

The biotransformation pathways of *m*-synephrine in rat and man are shown in Fig. 1.

Metabolism of m-*synephrine after oral administration to humans*

After the ingestion of six tablets (each containing 5 mg of *R*-(−)-*m*-synephrine hydrochloride) over a period of 8 h by each of three humans, the total dose (equivalent to 24·6 mg of *m*-synephrine) could be accounted for by the following four metabolites: (1) unconjugated MHMA, (2) a sulphate conjugate of MHPG, (3) a sulphate conjugate of *m*-synephrine, (4) a smaller amount of a glucuronide conjugate of *m*-synephrine (see Table 1). The quantities of MHMA and MHPG (Crowley et al 1982) excreted in urine normally by humans and rats (before and after injection with saline) were negligible compared with the corresponding amounts of these compounds excreted after the administration of mg quantities of *m*-synephrine. *m*-Synephrine could not be detected in normal human or rat urine.

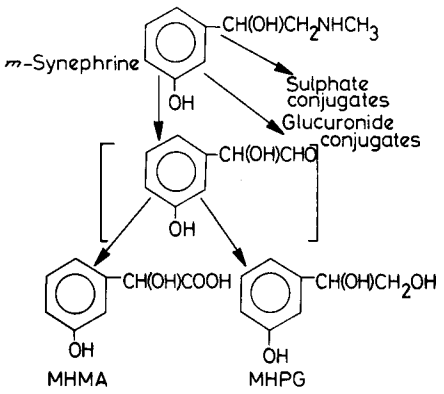

FIG. 1. Metabolites of *m*-synephrine in humans and rats.

In the 24 h following oral administration of 10 mg *m*-synephrine to humans, Bruce & Pitts (1968) obtained a recovery of approximately 80%. However, they did not attempt to identify any of the metabolites and their method would not distinguish between *m*-synephrine and MHPG. The recovery obtained by Bruce & Pitts (1968) comprised sulphate (92%) and glucuronide (8%) conjugates; unconjugated *m*-synephrine/MHPG could not be detected in urine. The results of the present study show that 80% of the total *m*-synephrine/MHPG is excreted as sulphate and 20% as glucuronide, together with a very small fraction in the unconjugated state.

Table 1. Mean excretion (calculated as percentage of the total dose) of *m*-synephrine and its metabolites in urine during the 24 h following oral ingestion by humans (n = 3).

	Method of hydrolysis			
	Acid	Sulphatase	β-Glucuronidase	None
MHMA	35 (±12)	35 (±11)	34 (±10)	30 (±9)
MHPG	9 (±4)	7 (±2)	1·3 (±0·7)	0·7 (±0·4)
m-Synephrine	59 (±6)	59 (±4)	12 (±1·5)	0·3 (±0·1)
Total	103 (±14)	101 (±13)	47 (±12)	31 (±10)

Hengstmann & Goronzy (1982) administered 1 mg (±)-[^{3}H]*m*-synephrine (as the free base) orally to humans and found that radioactivity was virtually cleared from the urine within 12 h. Excretion was

complete 24 h after ingestion of the drug and the total recovery of radioactivity in urine was 77 (±11)% of the administered dose. By extraction and chromatography they obtained the following results (expressed as percentage of the total dose): MHMA (unconjugated, 23·0 ± 5·1), *m*-synephrine (conjugated, 44·5 ± 7·0; unconjugated, 2·5 ± 0·7) (cf Table 1). They did not investigate the possible presence of MHPG and it is very probable that MHPG may account for the discrepancy (7%) between the total cumulative urinary excretion of ^{3}H-activity and the sum of the corresponding figures obtained for the individual excretion products. It is likely that the difference between the recoveries reported in Table 1 and those obtained by Hengstmann & Goronzy (1982) may arise from the different doses administered in the two investigations. If *m*-synephrine is taken up by adrenergic storage vesicles in a manner similar to noradrenaline, this loss would be much more significant with the smaller dose. However in any such comparison of results it must be emphasized that Hengstmann & Goronzy (1982) administered the racemic free base of the drug whilst the investigations reported here are based upon *m*-synephrine (*R*-(−), as the hydrochloride salt) which is normally used therapeutically.

Metabolism of m-*synephrine after inhalation by humans*

The inhalation of mg amounts of *R*-(−)*m*-synephrine hydrochloride by three humans over a period of 9 h afforded results which very closely resemble those obtained when the drug was taken orally (Table 2). The predominance of the sulphate conjugate of *m*-synephrine over the corresponding glucuronide is in accordance with observations concerning the conjugation of phenolic substrates in human lung tissue (Mehta & Cohen 1979).

Table 2. Mean excretion (calculated as percentage of the total dose) of *m*-synephrine and its metabolites in urine during the 24 h following nasal inhalation by humans (n = 3).

	Method of hydrolysis			
	Acid	Sulphatase	β-Glucuronidase	None
MHMA	27 (±9)	29 (±9)	29 (±10)	24 (±8)
MHPG	8 (±3)	7 (±3)	0·9 (±0·2)	0·6 (±0·2)
m-Synephrine	60 (±7)	61 (±6)	5 (±4·5)	1·5 (±0·7)
Total	95 (±19)	97 (±16)	35 (±14)	26 (±8)

Metabolism of m-*synephrine after intraperitoneal injection into rats*

The results of administration of *R*-(−)*m*-synephrine (250 μg) are given in Table 3.

Table 3. Mean excretion (calculated as percentage of the total dose) of *m*-synephrine and its metabolites in urine during the 24 h following intraperitoneal injection into rats (n = 3).

	Method of hydrolysis			
	Acid	Sulphatase	β-Glucuronidase	None
MHMA	6 (±1)	5 (±1)	5 (±1)	5 (±1)
MHPG	50 (±14)	35 (±4)	1·7 (±0·6)	1·5 (±0·5)
m-Synephrine	16 (±2)	16 (±1)	11 (±2)	7 (±1)
Total	72 (±15)	56 (±4)	18 (±1)	13·5 (±1)

The pattern of conjugation resembles that observed when the drug is administered orally or by inhalation to man: MHMA is excreted in the unconjugated state and MHPG mainly as the sulphate conjugate. However, unconjugated *m*-synephrine was detected, together with comparable quantities of sulphate and glucuronide. It may be seen that the proportion of the ingested dose of *m*-synephrine metabolized in the rat is greater than that in the human. Moreover, this occurs by reduction to MHPG rather than by oxidation to MHMA, whereas in man the converse is seen. This confirms our previous conclusion that the reductive pathway of metabolism of endogenous *m*-synephrine is more important in the rat than in man (Crowley et al 1982).

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