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The metabolism and biosynthesis of (±)-*o*-octopamine and (±)-*o*-synephrine in the rat

MICHAEL I. JAMES, JOHN M. MIDGLEY* AND CLYDE M. WILLIAMS†

Department of Pharmaceutical Chemistry, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, UK, and †Veterans Administration Medical Center, and Department of Radiology, University of Florida, College of Medicine, Gainesville, Florida 32610, U.S.A.

The metabolism of (±)-*o*-octopamine and (±)-*o*-synephrine by 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 *o*-octopamine was injected intraperitoneally into rats four metabolites were excreted in the urine: (i) unconjugated *o*-hydroxymandelic acid (OHMA) (16%), (ii) unconjugated *o*-hydroxyphenylglycol (OHPG) (4·5%), (iii) an acid-hydrolysable conjugate of OHPG (28%) and (iv) unconjugated *o*-octopamine (10%). When *o*-synephrine benzoate was similarly administered six metabolites were excreted in urine: (i) unconjugated OHMA (13·5%), (ii) unconjugated OHPG (3·3%), (iii) an acid-hydrolysable conjugate of OHPG (15·6%), (iv) unconjugated *o*-synephrine (10%), (v) an acid-hydrolysable conjugate of *o*-synephrine (8·5%) and (vi) unconjugated *o*-octopamine (0·3%). Adult rats normally excreted OHMA (1·0 μg day^{-1}) but OHPG, *o*-octopamine and *o*-synephrine could not be detected in urine. After the administration of a monoamine oxidase inhibitor, unconjugated *o*-octopamine (0·3 μg day^{-1}) was excreted in urine but OHPG and *o*-synephrine could not be detected. *o*-Tyramine given to rats afforded urinary *o*-octopamine (75 ng day^{-1}) and this was increased 10-fold upon co-administration of a monoamine oxidase inhibitor and *o*-tyramine.

o-Octopamine (*o*-hydroxyphenylethanolamine) is a weakly active α-adrenoceptor agonist but does not exhibit significant β-adrenergic activity in rats (Fregly et al 1979; Ress et al 1980). In 1978 Williams & Couch discovered *o*-octopamine in rat salivary and adrenal glands and also in bovine adrenal gland but the natural occurrence of *o*-synephrine has not been reported to date. The physiological role of *o*-octopamine is not known and there are only two other references to the biological activity of these compounds. Daly et al (1966) showed that *o*-octopamine exhibited low activity in the release of [^{3}H]noradrenaline from mouse heart in-vivo: *o*-synephrine was inactive in this respect. Subsequently it was reported (Rotman et al 1975) that *o*-octopamine possessed little affinity for sites of uptake of noradrenaline in mouse heart in-vivo.

Phenolic amines are metabolized primarily by oxidative deamination to the corresponding phenolic acids, which are excreted in the unconjugated form in urine. Thus either *p*-octopamine (Hengstmann et al 1974; Kakimoto & Armstrong 1962b) or its *N*-methyl derivative, *p*-synephrine (Gjessing & Armstrong 1963; Hengstmann & Aulepp 1978) afford *p*-hydroxymandelic acid (PHMA) in mammalian urine. Similarly, *m*-hydroxymandelic acid (MHMA) may arise from *m*-octopamine (Hengstmann et al 1975; Maruyama et al 1968) or *m*-synephrine (Hengstmann & Goronzy 1982; Ibrahim et al 1983). Recently *o*-hydroxymandelic acid (OHMA) has been identified in normal human urine (Midgley et al 1979) and in much larger amounts (a 30-fold increase) in patients suffering from phenylketonuria (Crowley et al 1980). This disease is a defect in metabolism whereby phenylalanine is not converted to *p*-tyrosine and it is believed (Crowley et al 1980) that the former is hydroxylated in the *o*-position leading ultimately to *o*-octopamine. Indeed the metabolic pathway phenylalanine → *o*-tyrosine → *o*-tyramine has been proposed in man (Udenfriend & Mitoma 1955) and in rats (Mitoma et al 1957). The enzyme, dopamine β-hydroxylase, will accept a number of substrates including *m*-tyramine, *p*-tyramine and phenylethanolamine and *o*-tyramine, which has also been identified in mammalian urine, may be hydroxylated by this enzyme to produce *o*-octopamine and, hence, OHMA (Boulton 1978). However, *o*-hydroxyphenylglycol, the expected neutral metabolite of *o*-octopamine, could not be detected in normal human or rat urine (Crowley et al 1982) and neither *o*-octopamine nor *o*-synephrine could be detected in the urine of

* Correspondence.

normal humans or phenylketonuric patients (Crowley et al 1980).

These observations prompted us to investigate the metabolism of *o*-octopamine and *o*-synephrine by the rat and to search for these amines in the urine of normal rats and those treated with a monoamine oxidase inhibitor. We report: (i) the metabolism of exogenous *o*-octopamine and *o*-synephrine to OHMA and OHPG, (ii) the identification of endogenous *o*-octopamine in the urine of rats previously treated with a monoamine oxidase inhibitor and (iii) the conversion of *o*-tyramine to *o*-octopamine in both normal rats and those previously treated with a monoamine oxidase inhibitor.

MATERIALS AND METHODS

Materials

These were obtained from the following sources: pentafluoropropionic acid anhydride (PFPA), Tri-Sil'Z', Pierce Chemical Co. (Rockford, Illinois); sulphatase (*Helix pomatia*—type H-1), bacterial β-glucuronidase (type VII), tyrosine decarboxylase and (±)-*o*-tyrosine, Sigma Chemical Company (St. Louis, Missouri); AG 50W-X2 (strong cation-exchange resin; 100–200 mesh; Hydrogen form) and Bio-Rex 70 (weak cation-exchange resin; 200–400 mesh; sodium form, which was converted to the ammonium form by stirring it in ammonium acetate buffer (0·2 M, pH 6), adjusting the pH to 6 with glacial acetic acid and stirring the suspension for 1 h. This procedure was repeated until the pH of the liquid had a constant value of 6 when the resin was removed by filtration under suction and allowed to dry in air), Bio-Rad Laboratories (Richmond, California); iproniazid phosphate, Regis Chemical Company (Morton Grove, Illinois); α,α-[$^2H_2$]-β-[$^2H_1$] *o*-octopamine hydrochloride, Merck, Sharp & Dohme (Montreal, Canada). (±)-[$^2H_0$] *o*-Octopamine (as the free base) was obtained by the method of Kappe & Armstrong (1965); (±)-[$^2H_0$]- and [$^2H_3$] *o*-synephrine benzoate were synthesized as previously described (Crowley et al 1980) using dimethyl sulphate and hexadeuterodimethyl sulphate respectively; [$^2H_0$] OHMA was prepared by the method of Howe et al (1967) and crystallized as the bis-piperazine salt (Midgley et al 1979); [$^2H_3$] OHMA, an extremely viscous pink gum, was synthesized by the reduction of *o*-hydroxyphenylglyoxylic acid (Howe et al 1967) with sodium borodeuteride and the resultant [$^2H_1$] OHMA was treated with [$^2H_2$]O, C[$^2H_3$]COO[$^2H_1$] and [$^2H_1$]Cl as described by Midgley et al (1979). The incorporation of deuterium into the product, estimated by measuring the intensities of the M, M + 1, M + 2, M + 3 and M + 4 ions of the bis-pentafluoropropionyl methyl ester (PFP-Me) derivative, was: [$^2H_0$] 0·0%, [$^2H_1$] 2·4%, [$^2H_2$] 23·7%, [$^2H_3$] 66·7%, [$^2H_4$] 7·3%. An aqueous solution of the crude [$^2H_3$] OHMA was standardized by comparing the intensity of the molecular ion (*m/z* 474) derived from a known quantity of [$^2H_0$]OHMA-PFP-Me with that of the corresponding ion (*m/z* 477) of [$^2H_3$]OHMA-PFP-Me; [$^2H_0$]OHPG was synthesized by the method of Crowley et al (1982); [$^2H_3$]OHPG (m.p. 84–5 °C) was prepared by the reduction of 2,3-dioxo-2,3-dihydrobenzofuran (Huntress & Hearon 1941) with lithium aluminium deuteride. The incorporation of deuterium into [$^2H_3$]OHPG, determined by measuring the intensities of the M, M + 1, M + 2, M + 3 and M + 4 ions of the corresponding PFP derivative was: [$^2H_0$] 0·3%, [$^2H_1$] 1·0%, [$^2H_2$] 21·8%, [$^2H_3$] 69·0%, [$^2H_4$] 8·0%; 3-coumaranol was prepared by the procedure described by Fanta (1977); *o*-tyramine, prepared enzymatically from (±)-*o*-tyrosine and tyrosine decarboxylase (E.C. 4.1.1.25) (added as dried cells of *S. faecalis*) by the method of Udenfriend & Cooper (1953), was obtained as the hydrochloride salt, a colourless deliquescent oil which could not be crystallized; methanolic HCl was prepared by the slow addition of acetyl chloride (1 ml) to dry methanol (4 ml) (Midgley et al 1979).

All the crystalline compounds described above afforded spectroscopic data and elemental analyses consistent with the assigned structures.

Apparatus

Gas chromatography-electron impact mass spectrometry-selected ion monitoring (g.c.-e.i. m.s.-s.i.m.) was carried out with 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 190 °C for the glycols and acids and at 210 °C for the amines; helium was used as the carrier gas; column effluent was diverted from the ion source for the first 2·0 min.

Gas chromatography-negative ion chemical ionization mass spectrometry-selected ion monitoring (g.c.-n.i.c.i.m.s.-s.i.m.) was performed with a Hewlett-Packard model 5985B using the following conditions: source pressure, 1 torr; emission current, 300 μA; electron energy, 240 eV. The conditions for g.c. were as follows: silanized glass column (1·8 m × 2 mm i.d.) packed with 5% OV-101 on Chromosorb GHP 100/120 mesh (Supelco) and methane was used as the carrier gas. Temperatures employed were:

column, 180 °C; injection port, 250 °C; g.c.-m.s. interface (direct inlet), 250 °C; n.i.c.i. ion source, 100 °C.

Animal experiments

Urine ('control') was collected over a 24 h period from each of three male Sprague-Dawley 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 ('saline injection control') was collected for a further 24 h. Subsequent procedure was as follows:

(i) Metabolism of *o*-octopamine and *o*-synephrine. Each of three rats (a separate group for each drug) was injected intraperitoneally with either *o*-octopamine (free base, 250 μg) or *o*-synephrine benzoate (≡ 250 μg free base) in saline (0·3 ml) and the urine collected for two successive 24 h periods.

(ii) Biosynthesis of *o*-octopamine and *o*-synephrine. Each rat (from a fresh group of three) was injected intraperitoneally with *o*-tyramine (500 μg) in saline (0·3 ml) and urine was collected during the next 24 h. After an interval of two weeks each rat (from a separate group of three) was injected intraperitoneally with a solution of iproniazid phosphate (equivalent to 10 mg kg^{-1} of free base) in saline (0·3 ml) and the 24 h sample of urine was discarded. The injection was repeated 24 h after the first one and urine was collected for a further 24 h. Then *o*-tyramine (500 μg i.p.) in saline (0·3 ml) was injected and the urine was collected over the next 24 h.

Urine samples were analysed immediately.

Procedure

Internal standards

(i) [$^2H_3$] *o*-octopamine (500 ng), [$^2H_3$] *o*-synephrine benzoate (equivalent to 500 ng of free base), [$^2H_3$] OHPG (500 ng) and [$^2H_3$] OHMA (2000 ng) were added to each 24 h sample of control and saline injection control urine.

(ii) [$^2H_3$] *o*-octopamine (25 μg), [$^2H_3$] *o*-synephrine benzoate (equivalent to 25 μg of free base), [$^2H_3$] OHPG (50 μg) and [$^2H_3$] OHMA (50 μg) were added to each 24 h sample of urine obtained from animals injected with *o*-octopamine.

(iii) [$^2H_3$] *o*-octopamine (10 μg), [$^2H_3$] *o*-synephrine benzoate (equivalent to 50 μg of free base), [$^2H_3$] OHPG (50 μg) and [$^2H_3$] OHMA (50 μg) were added to each 24 h sample of urine obtained from animals injected with *o*-synephrine benzoate.

(iv) [$^2H_3$] *o*-octopamine (2 μg) and [$^2H_3$] *o*-synephrine benzoate (equivalent to 2 μg of free base) were added to each 24 h urine sample from animals injected with *o*-tyramine and/or iproniazid phosphate.

Each 24 h sample of urine was divided into four equal parts before deconjugation and extraction of the metabolites. The pH of one such portion was adjusted to 1 with conc. HCl and the sample was extracted immediately (see below).

Deconjugation

Conjugates in all the samples of urine were hydrolysed with acid: those samples of urine obtained after the injection of *o*-octopamine and *o*-synephrine benzoate were also treated enzymatically.

(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 (boiling water bath 1 h);

(ii) β-glucuronidase (1000 units) in ammonium acetate buffer (1 ml, 0·2 M, pH 6) was added to a portion of the urine which was incubated at 37 °C for 24 h;

(iii) sulphatase (45 mg, containing 810 units of β-glucuronidase and 810 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 24 h.

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

Extraction of acids and glycols

The aliquot of urine was extracted twice with ethyl acetate (2 vol.). Carboxylic acids were removed from the combined organic extracts by extraction into aqueous $NaHCO_3$ (10%, 2 × 1 ml), leaving neutral compounds in the organic phase. The aqueous solution was adjusted to pH 1 with conc. HCl, and extracted with ethyl acetate (2 × 5 ml) and 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 5 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 OHMA was carried out using the molecular ion (*m/z* 474) and the $(M\text{-}CO_2CH_3)^+$ ion (*m/z* 415). In the derivatized biological extracts the retention times and ratios of intensities of these two

ions were identical, within experimental error, to those of the standard. Quantitative analysis of OHMA was achieved by determination of the ratio of the intensities of the molecular ions, *m/z* 474 and *m/z* 477, produced by [$^2H_0$]OHMA-PFP-Me and [$^2H_3$]OHMA-PFP-Me respectively, in the manner previously described (Crowley et al 1982).

Analysis of glycols

The residue containing the neutral components (e.g. glycols, see above) was then reacted as follows:
(i) 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 OHPG was carried out by establishing that the retention time and ratio of the intensities of the molecular ion (*m/z* 592) and the $(M\text{-}C_2F_5CO_2)^+$ ion (*m/z* 428) of the unknown peak in the derivatized biological extract were identical, within experimental error, to those of the standard. Quantitative analysis of OHPG was achieved by determination of the ratio of the intensities of the molecular ions, *m/z* 592 and *m/z* 595, produced by [$^2H_0$]OHPG-PFP and [$^2H_3$]OHPG-PFP respectively (Crowley et al 1982).
(ii) with Tri-Sil 'Z' (100 μl) at 60° for 15 min. and 1–2 μl of this solution were injected into the g.c.-m.s. Ions of *m/z* 208 and *m/z* 75 could not be identified at the retention time of the TMS derivative of authentic 3-coumaranol.

Extraction of amines for g.c.-e.i.m.s.-s.i.m.

The pH of the residual urine (see above) was adjusted to 6 with 2 M NaOH and this mixture was passed through a strong cationic exchange resin (AG 50W-X2, 3 g). The resin was washed with water (10 ml) and the amines were eluted with 10 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 by g.c.-e.i.m.s.-s.i.m.

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 solution was injected into the g.c.-m.s. Identification of the PFP derivative of *o*-octopamine was carried out by establishing that the retention time and the ratio of intensities of the molecular ion (*m/z* 591) and the $[CH_2 = NHCOC_2F_5]^+$ ion (*m/z* 176) in the derivatized biological extract were identical, within the limits of experimental error, to those of the standard. Quantitative analysis of *o*-octopamine was achieved by determination of the ratio of the intensities of the molecular ions (*m/z* 591 and *m/z* 594), produced by [$^2H_0$]*o*-octopamine-PFP and [$^2H_3$]*o*-octopamine-PFP respectively, in the manner previously described (Ibrahim et al 1983). Identification of the PFP derivative of *o*-synephrine was carried out by establishing that the retention time and the ratio of 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 the limits of experimental error, to those of the standard. Quantitative analysis of *o*-synephrine was achieved by determination of the ratio of the intensities of the molecular ions (*m/z* 605 and *m/z* 608), produced by [$^2H_0$]*o*-synephrine-PFP and [$^2H_3$]*o*-synephrine-PFP respectively, in the manner previously decribed (Ibrahim et al 1983).

Extraction of amines for g.c.-n.i.c.i.m.s.-s.i.m.

This was carried out essentially by the method of Kakimoto & Armstrong (1962a) with the following modifications. The pH of the sample of rat urine was adjusted to 6 with 2 M HCl and the mixture passed through a strong cationic exchange resin (AG 50W-X2, 3 g). The resin was washed successively with water (10 ml), aqueous sodium acetate (0·1 M, 10 ml) and water (10 ml); the amines were eluted with 10 ml of NH_4OH (1 M) in ethanol (65%). The eluate was concentrated to ca 500 μl by rotary evaporation at ca 40 °C and applied to the top of a column (13 mm × 40 mm) of weak cationic exchange resin (Bio-Rad 70). The amines were eluted from this column with ammonium acetate buffer (0·2M, pH 6) in the fraction 20–120 ml. The eluate was desalted by slow passage through a second column of the strong cationic exchange resin (AG 50W-X2, 3 g). The resin was washed with water (10 ml), the amines were eluted with 10 ml of NH_4OH (1 M) in ethanol (65%) and the eluate was reduced to dryness by rotary evaporation at ca 40 °C.

Analysis of amines by g.c.-n.i.c.i.m.s.-s.i.m.

The resultant dried residue was treated with PFPA (100 μl) for 15 min at 60 °C and the excess reagent removed by a stream of nitrogen. This residue was dissolved in sufficient ethyl acetate to afford a concentration of the internal standards of approximately 1 ng μl^{-1} and 0·5 –1 μl of this solution was injected into the g.c.-m.s. Identification of the PFP derivative of *o*-octopamine was carried out by establishing that the retention time and the ratio of intensities of the M^- and $(M\text{-}HF)^-$ ions (*m/z* 591 and

m/z 571) in the derivatized biological extract were identical, within the limits of experimental error, to those of the standard. Similarly, ions of *m/z* 605 and *m/z* 585 were used to identify *o*-synephrine-PFP. Quantitative analysis of *o*-octopamine was achieved by determination of the ratio of the intensities of the molecular ions (*m/z* 591 and *m/z* 594), produced by [$^2H_0$]*o*-octopamine-PFP and [$^2H_3$]*o*-ocotopamine-PFP respectively. Similarly, ions of *m/z* 605 and *m/z* 608 were used for the estimation of *o*-synephrine.

RESULTS AND DISCUSSION

Control and saline injection control urine

24 h samples of urine from each of six rats were hydrolysed with acid, extracted and the derivatized extracts examined by g.c.-e.i.m.s.-s.i.m. for OHMA and OHPG. The former was present in the samples of both control and saline injection control urine [1078(±428) ng day^{-1} and 1054(±127) ng day^{-1} respectively: cf human urine, 4–16 μg day^{-1} OHMA (Midgley et al 1979)]. This result is entirely consistent with the reported presence of *o*-octopamine in rat salivary and adrenal glands (Williams & Couch 1978). On the basis of the metabolic investigations reported here (see below) and our previous observations on the metabolism of endogenous (Crowley et al 1982) and exogenous (Ibrahim et al 1983) *m*-synephrine by rats, we had anticipated that the daily output of OHPG by the animals would be approximately twice that of OHMA. However, OHPG could not be detected (lower limit of detection, 100–200 ng day^{-1}) in any of the samples. Similarly, neither *o*-octopamine nor *o*-synephrine were detectable (lower limit of detection, 200–250 ng day^{-1}) by g.c.-e.i.m.s.-s.i.m.. Consequently g.c.-n.i.c.i.m.s.-s.i.m., which is normally 10–100 times more sensitive than g.c.-e.i.m.s.-s.i.m. (Hunt & Crow 1978; Lewy & Markey 1978), was employed, but *o*-octopamine (lower limit of detection, 10–20 ng day^{-1}) and *o*-synephrine (lower limit of detection, 200–250 ng day^{-1} due to quenching of the internal standard by unknown co-eluting components) could not be detected in either control or saline injection control samples of urine.

Metabolism of o-*octopamine*

After the intraperitoneal administration of (±)-*o*-octopamine (250 μg) to rats five metabolites were detected in the urine excreted during the following 24 h: (1) unconjugated OHMA, (2) unconjugated OHPG, (3) an acid-hydrolysable conjugate of OHPG, (4) unconjugated *o*-octopamine, (5) a small amount of an acid-hydrolysable conjugate of *o*-octopamine (see Table 1). The nature of the latter is unknown but it has been reported previously that urinary *p*-synephrine (and two other aromatic amines) were released from their conjugates by acidic hydrolysis but not by β-glucuronidase or sulphatase (Kakimoto & Armstrong 1962a).

Table 1. Mean excretion (calc. as percentage of total dose) of *o*-octopamine and its metabolites in urine during the 24 h following intraperitoneal injection into rats (n=3).

	Method of hydrolysis			
	Acid	Sulphatase	β-Glucuronidase	None
OHMA	16(±3)	17(±4)	16(±4)	15(±3)
OHPG	33(±9)	5·5(±1·5)	5·5(±0·9)	4·5(±3)
o-Octopamine	14(±2·5)	11(±1·5)	11(±1·5)	10(±0·6)
o-Synephrine	[a]ND	[a]ND	[a]ND	[a]ND
Total	64(±7)	34(±4·5)	33(±4)	30(±5)

[a]ND = not detectable by g.c.-n.i.c.i.m.s.-s.i.m.

Approximately 15% of the administered *o*-octopamine was excreted as unconjugated OHMA which is in excellent agreement with previous observations (Armstrong et al 1956; Midgley et al 1979; Davis & Boulton 1981; Ibrahim et al 1983) that the acidic metabolites of phenolic amines are excreted in the unconjugated state.

Whilst approximately 5% of the administered *o*-octopamine was excreted as unconjugated OHPG, a much larger amount (28%) was excreted as an acid-hydrolysable conjugate which was not a sulphate or glucuronide. This finding is in agreement with that of Ibrahim et al (1983) who administered R-(−)-*m*-synephrine intraperitoneally to rats and observed that 50% of the total dose was excreted as an acid-hydrolysable conjugate of *m*-hydroxyphenylglycol (MHPG). Approximately one-third of the latter was neither sulphate nor glucuronide. These observations are in marked contrast to the secondary metabolic fate of other phenolic glycols: MHPG is excreted naturally in the urine of humans and rats as a sulphate conjugate (Crowley et al 1982) and is similarly excreted by humans after the oral or nasal ingestion of *m*-synephrine (Ibrahim et al 1983). Moreover, 3-methoxy-4-hydroxyphenylglycol, a major metabolite of adrenaline and noradrenaline, is also excreted in the urine as a sulphate conjugate (Axelrod et al 1959). The relative amounts of acid and glycol excreted are also in agreement with the results of previous investigations (Crowley et al 1982; Ibrahim et al 1983) which showed that the reductive pathway of metabolism of hydroxyphenylethanolamines is more important than the oxidative pathway in rats. It was considered that the metabolism of *o*-octopamine (or *o*-synephrine) might afford

3-coumaranol (3-hydroxy-2,3-dihydrobenzofuran) or that the latter might arise via OHPG. The PFP derivative of 3-coumaranol was not suitable for g.c.-m.s. and so the trimethylsilyl derivative of this compound was used. However, 3-coumaranol could not be detected by these means in the neutral fraction of any sample of urine.

N-methylation of *o*-octopamine (to give *o*-synephrine) did not occur to any significant extent. Thus *o*-synephrine could not be detected even by the use of g.c.-n.i.c.i.m.s.-s.i.m. in urine after the intraperitoneal administration of *o*-octopamine (250 μg) to rats. However this analysis was not highly sensitive (lower limit of detection, 500–1000 ng day^{-1}) due to quenching of the peak corresponding to the internal standard by the very large quantity of *o*-octopamine which was eluted immediately before it. In contrast, Maruyama et al (1968) detected *m*-synephrine in the urine of rats and rabbits (but not that of guinea-pigs) by chromatographic means after the oral administration of comparatively large doses of *m*-octopamine (50, 30 and 30 mg kg^{-1} respectively). Although it is known that noradrenaline *N*-methyltransferase (EC 2.1.1.28) will methylate *o*-octopamine in-vitro, the latter is a much poorer substrate than the *m*- or *p*-isomers (Fuller et al 1981). Hence our failure to detect *o*-synephrine after the intraperitoneal administration of *o*-octopamine may mean that either *o*-octopamine is not taken up in-vivo by structures (e.g. adrenal gland, sympathetic nerves, certain tracts in brain) which contain the *N*-methylating enzyme or the enzyme may not methylate *o*-octopamine efficiently enough in-vivo to produce sufficient quantities of *o*-synephrine to be detected by existing techniques.

Analysis of the urine collected 24–48 h after the intraperitoneal injection of *o*-octopamine (or *o*-synephrine) showed that the metabolism of these compounds was essentially complete within 24 h.

Metabolism of o-*synephrine*

The results (see Table 2) of the intraperitoneal administration of (±)-*o*-synephrine benzoate (equivalent to 250 μg of free base) closely resemble those obtained with *o*-octopamine (Table 1). Thus, the amine itself and OHPG were each excreted in the unconjugated form and as an acid-hydrolysable conjugate (neither sulphate nor glucuronide). Moreover, the relative amounts of OHPG and OHMA excreted indicated that the reductive pathway for the metabolism of *o*-synephrine is preferred to the oxidative pathway in the rat. The ratio of glycol to acid was lower in this case than that afforded by

Table 2. Mean excretion (calc. as percentage of total dose) of *o*-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
OHMA	13·5(±4·8)	14·5(±7·3)	14·1(±5·7)	12·7(±6.6)
OHPG	18·9(±4·5)	3·5(±0·5)	3·3(±0·6)	3·3(±0·5)
o-Synephrine	18·5(±1·8)	10·3(±1·4)	9·6(±1·6)	10·1(±0.75)
o-Octopamine	0·33(±0.02)	0·24(±0·04)	0·25(±0·02)	0·28(±0·04)
Total	51·3(±11·2)	28·5(±6·4)	27·3(±4·7)	26·4(±6·6)

metabolism of the *m*-isomer (Ibrahim et al 1983) but, overall, these isomers afforded similar metabolic patterns. However, the excretion of a small amount (0·3%) of unconjugated *o*-octopamine by rats after they had received *o*-synephrine was unexpected. This result was not an artifact since *o*-octopamine was absent from the internal standards and could not be located in blank experiments. There appears to be one previous report (Ramos Aliaga 1974) concerning the *N*-demethylation of such compounds and it was concluded that '*m*-synephrine hydrochloride was not significantly *N*-demethylated by the rat'.

Effect of monoamine oxidase inhibition on the metabolism of o-*octopamine (Table 3)*

Iproniazid phosphate (equivalent to 10 mg kg^{-1} of free base) was injected intraperitoneally into rats on two successive days. The urine samples collected during the 24 h following the second injection were processed for amines in the manner described above and the derivatized extracts were examined by g.c.-n.i.c.i.m.s.-s.i.m. *o*-Synephrine could not be identified in any of the samples (lower limit of detection ca 200–250 ng day^{-1}) but *o*-octopamine was present (mean excretion 306(±40) ng day^{-1}). Fig. 1 shows the result of a typical analysis: in (A) it

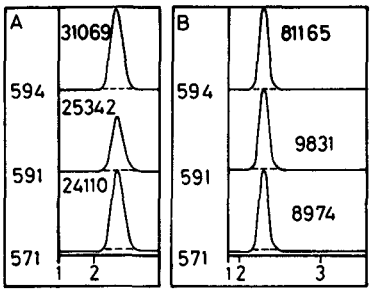

FIG. 1. Analysis by g.c.-n.i.c.i.m.s.-s.i.m. of (A) 0·5 ng (on column) of $[^2H_0]$*o*-octopamine-PFP and $[^2H_3]$*o*-octopamine-PFP (1:1) (B) 1/1600th part (corresponding to 1·25 ng of $[^2H_3]$*o*-octopamine-PFP) of derivatized extract of 24 h urine sample from rat treated with monoamine oxidase inhibitor alone.

may be seen that the ratio of the intensities of the M^- (m/z 591) and $(M\text{-}HF)^-$ (m/z 571) ions of authentic [$^2H_0$]*o*-octopamine-PFP is 1·05; in (B) the corresponding ratio is 1·09. Similarly it may be calculated that this particular 24 h sample of urine contained 295 ng of *o*-octopamine. This first report of the presence of *o*-octopamine in a biological fluid clarifies our earlier hypothesis (Midgley et al 1979; Crowley et al 1980) concerning the origins of urinary OHMA and provides compelling evidence that it originates predominantly (if not entirely) from *o*-octopamine.

Table 3. Mean excretion (ng day^{-1}) of *o*-octopamine and *o*-synephrine in urine during the 24 h following intraperitoneal injection of *o*-tyramine/iproniazid into rats.

	o-Octopamine	*o*-Synephrine
Control (n=6)	[a]ND	[a]ND
Saline injection control (n=6)	[a]ND	[a]ND
o-Tyramine (n=3)	75 (±10)	[a]ND
Iproniazid (n=5)	306 (±40)	[a]ND
Iproniazid + *o*-tyramine (n=4)	854 (±317)	[a]ND

[a]ND = not detectable by g.c.-n.i.c.i.m.s.-s.i.m.

The investigations reported here show unequivocally that exogenous *o*-octopamine and *o*-synephrine are metabolized to OHMA. However, in order to test our hypothesis (Crowley et al 1980) that endogenous *o*-octopamine may arise from *o*-tyramine, the latter (500 μg) was administered to normal rats and those treated with a monoamine oxidase inhibitor. The appropriate 24 h samples of urine (see above) were processed and analysed for *o*-octopamine and *o*-synephrine as before. The latter compound could not be detected in any of the samples but the increased output of *o*-octopamine (Table 3) provides conclusive evidence for the proposed biosynthetic pathway of this compound in the rat.

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