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Quantitative Measurement of Octopamines and Synephrines in Urine Using Capillary Column Gas Chromatography Negative Ion Chemical Ionization Mass Spectrometry

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The isomeric octopamines and synephrines were measured in urine by a new assay which combines ion-exchange chromatography, capillary column gas chromatography, and electron capture negative ion chemical ionization mass spectrometry. Deuterium labeled analogues of each compound were added to urine containing 1 mg of creatinine and the acid hydrolysate was subjected twice to cation exchange chromatography. The resultant amine fraction was derivatized with pentafluoropropionic anhydride and, under electron capture negative chemical ionization conditions, the PFP derivatives gave M^- and $(M - HF)^-$ ions which were sufficiently abundant to be suitable for selected ion monitoring. The limit of detection was approximately 100 pg mg^{-1} creatinine. In 10 normal adults, the concentrations of *o*-, *m*-, and *p*-octopamine and *o*-, *m*- and *p*-synephrine were, respectively, 0.6 (±0.2), 2.1 (±0.8), 25 (±19), ND (<0.1), 1.8 (±0.7), and 16 (±10) ng mg^{-1} creatinine. The occurrence of *o*- and *m*-octopamine and *m*-synephrine in human tissue or fluid has not been reported previously.

The development of a radiochemical enzyme assay for the quantitative determination of *p*-octopamine (*1*, *2*) led to its discovery in several invertebrate nerve systems and in sympathetically innervated mammalian organs. However, it was subsequently discovered that the method was not specific for *p*-octopamine because the meta and para isomers were not resolved. The use of a modified method showed that both *m*- and *p*-octopamine were present in rat salivary gland (*3*) and brain (*4*). The radiochemical enzyme assay depends upon norepinephrine *N*-methyltransferase, which can accept all three positional isomers of octopamine as substrates with varying efficacy. The resultant products, the corresponding synephrines, are also substrates for the enzyme (*5*, *6*), and consequently, any one or more of these amines could have been detected and quantified as *p*-octopamine by the unmodified assay.

Gas chromatography/mass spectrometry (GC/MS) techniques permit the unequivocal identification and quantitative determination of the three isomeric octopamines and three isomeric synephrines and there is now conclusive evidence for the natural occurrence in mammalian tissue of *o*-octopamine (*7*), *m*-octopamine (*3*, *4*, *8*), *m*-synephrine (*9*, *10*), and *p*-synephrine (*10*). Our initial attempts to determine naturally occurring isomeric octopamines and synephrines in mammalian urine by electron impact GC (packed column) MS methods were unsuccessful because the concentrations of these amines in most of the samples were below the lower limits of detection (50–100 ng mg^{-1} creatinine) of the method. For this reason, we turned to electron capture negative chemical ionization (NCI) GC/MS because of its reported 10–100-fold increase in sensitivity over electron impact methods (*11*, *12*). The use of an ion-exchange resin (to separate and concentrate very small amounts of amines for analysis by NCI GC/MS) together with an "ultrabond" capillary column has enabled us to demonstrate the natural occurrence of *o*-, *m*-, and *p*-octopamine and *m*- and *p*-synephrine in normal human urine. *m*-Synephrine and *o*- and *m*-octopamine have not been identified previously in any human tissue or fluid.

EXPERIMENTAL SECTION

Reagents. These were obtained from the following sources: pentafluoropropionic anhydride (PFPA), Pierce Chemical Co. (Rockford, IL); *m*-octopamine hydrochloride, Interchim (Montlucon, France); *m*-synephrine, Sterling Chemical Co. (New York, NY); phenylethanolamine hydrochloride, *p*-synephrine, epinine hydrochloride, *p*-octopamine hydrochloride, and epinephrine bitartrate, Regis Chemical Co. (Morton Grove, IL); dopamine hydrochloride and metanephrine hydrochloride, Sigma Chemical Co. (St. Louis, MO); norepinephrine hydrochloride, 3-methoxytyramine hydrochloride, and normetanephrine hydrochloride, Calbiochem (Los Angeles, CA); *m*-tyramine hydrochloride, Vega Fox Biochemicals (Tucson, AZ); *p*-tyramine, Aldrich Chemical Co. (Milwaukee, WI); strong cation-exchange resin (AG 50W-X2, 100/200 mesh, H^+ form), Bio-Rad (Richmond, CA); *o*-octopamine-α,α'-d_2-β-d_1 hydrochloride, Merck, Sharp & Dohme (Montreal, Canada).

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Table I. Composition of Deuterated Amines (%)

compound	d_0	d_1	d_2	d_3	d_4	d_5
o-octopamine-d_3	0.1	1.0	12.2	86.1	0.7	
m-octopamine-d_3	0.1	1.1	15.3	81.5	2.0	
p-octopamine-d_4	0.0	0.0	0.7	6.8	85.8	6.6
o-synephrine-d_3	1.5	6.8	1.4	86.2	4.1	
m-synephrine-d_3	0.3	2.0	13.2	80.7	3.8	
p-synephrine-d_3	0.3	1.0	1.8	93.8	3.2	

Other compounds were synthesized as previously described in the literature: *o*-octopamine (*13*), *o*-synephrine benzoate (-d_0 and methyl-d_3) (*14*), *o*-tyramine (*15*), *m*-octopamine-2,4,6-d_3 hydrochloride (*16*), *m*-synephrine-2,4,6-d_3 hydrochloride (*16*), *p*-synephrine (methyl-d_3) (*16*), *p*-octopamine-α,α'-d_2-3,5-d_2 hydrochloride (*16*), and *N*-methylphenylethanolamine (*17*).

The composition of the deuterated octopamines and synephrines was determined by NCI GC/MS of the corresponding PFP derivatives by using the molecular ions (m/z 591–596 for the octopamines and m/z 605–610 for the synephrines). These results are shown in Table I.

The deuterated standards contained less than 2% of the corresponding nondeuterated species. Each of the six deuterated standards in Table I was examined for possible contamination with the other five amines. Each isomer was found not to be contaminated with its other positional isomers, nor did any of the octopamines contain traces of the synephrines, or vice versa. The conditions of acid hydrolysis employed in the biological experiments were not found to cause a significant change in the deuterium distribution of the deuterated standards.

Instrumentation. This work was performed on a Hewlett-Packard 5985 GCMS system equipped with an EI/CI source with negative chemical ionization capability. The end of the fused silica capillary column ("ultrabond" cross-linked methylsilicone, 25 m × 0.2 mm i.d., 0.33 μm film; from Hewlett-Packard) was inserted into the source of the mass spectrometer where a pressure of 1 torr was maintained by adding methane. The GC injection port and interfacial region between the GC and the MS were maintained at 250 °C. The GC oven was maintained at 80 °C for 0.5 min following injection and then raised to 250 °C at a rate of 30 °C/min. The electron multiplier voltage was 2400 V; source temperature, 100 °C; electron beam current, 300 μA.

Extraction of Urine. A standard solution (100 μL, equivalent to 10 ng each of *o*- and *m*-octopamine-d_3 and 100 ng each of *p*-octopamine-d_4 and *o*-, *m*-, and *p*-synephrine-d_3) was added to a volume of urine containing 1 mg of creatinine in a disposable screw-capped tube. The pH of the mixture was adjusted to a value of 1 with concentrated HCl and the tube was placed in a water bath and maintained at 90 °C for 25 min. The urine was cooled, the pH was adjusted to a value of 6.0 with 2 N NaOH, and the liquid was placed on a column (5 × 0.8 cm) of AG 50W-X2 resin in a disposable pipet. The resin was washed with water (10 mL), sodium acetate (0.1 N, 25 mL), water (25 mL), and ethanol (70%, 1 mL), and the amines were eluted with 1 N ammonia in 65% ethanol (3 mL). The eluate was blown to dryness with a jet of nitrogen and the residue reconsituted in water (1 mL). The pH of the liquid was adjusted to a value of 6.0 with 0.5 N HCl and the resultant solution passed through a second column (1.5 × 0.8 cm) of AG 50W-X2 resin. The resin was washed with water (5 mL), sodium acetate (0.1 N, 10 mL), water (20 mL), and ethanol (70%, 1 mL). The amines were eluted with ammoniacal ethanol (1 mL) as before and the solvent removed under a stream of nitrogen.

Derivatization. Dried urinary extracts or standards were heated with PFPA (100 μL) for 15 min at 60 °C in a screw-capped vial. The PFPA was evaporated under a stream of nitrogen and the residue taken up in hexane.

Identification and Quantitative Determination of Amines. The 1:1 standard containing 1 ng/μL of each of the six octopamines and synephrines and their corresponding deuterated analogues was prepared daily and the amines were derivatized as above. The following ratios of intensities (measured by areas) were determined: $M^-/(M - HF)^-$ for the PFP derivatives of the nondeuterated compounds and $M^-_{nondeuterated}/M^-_{deuterated}$ (or, $M^-_{d_0}/M^-_{d_x}$). The corresponding ratios were determined for each derivatized biological extract (containing the internal standard) and compared to those obtained for the standard. When a 1:1 standard was subjected to the same procedure as the biological sample (acid hydrolysis, ion-exchange chromatography, GC/MS), there was no change in the $M^-/(M - HF)^-$ and $M^-_{d_0}/M^-_{d_x}$ ratios.

RESULTS AND DISCUSSION

Pentafluoropropionyl (PFP) derivatives of biogenic amines are widely used in GC analyses because they are easily formed and are stable for several hours. Although it has been suggested that other derivatives have advantages (*11*), the PFP derivatives have so far proven to be superior for NCI GC/MS analyses of the catecholamines (*18*, *19*). Martin et al. (*18*) have investigated the fragmentation patterns of the PFP derivatives of dopamine, norepinephrine, epinephrine, and their *O*-methyl derivatives under NCI conditions. All of these compounds undergo dissociative resonance electron capture followed by cleavage of the benzylic C–O bond resulting in the formation of the *reagent-specific* ion ($C_2F_5CO_2$, m/z 163) as the base peak; however the molecular M^- and/or $(M - HF)^-$ ions are usually prominent also. We observed that the PFP derivatives of the octopamines and synephrines also exhibit relatively abundant M^- and $(M - HF)^-$ ions when subjected to NCI GC/MS (Table II).

The presence of appreciably intense M^- and $(M - HF)^-$ ions is important not only for sensitivity of the method but also

Table II. Kovats' Indexes and Intensities of Relevant Ions of PFP Derivatives of Some Biogenic Amines

PFP derivative	Kovats' index	mol wt	% total negative ion current		m/z
			M^-	$(M - HF)^-$	
phenylethanolamine	1325	429	0.7	3.8	409
o-octopamine	1365	591	22	18	571
o-tyramine	1370	429	0.0	31	409
N-methylphenylethanolamine	1385	443	5.9	9.9	423
m-octopamine	1430	591	18	12	571
m-tyramine	1435	429	0.4	27	409
o-synephrine	1440	605	3.0	4.0	585
p-tyramine	1463	429	0.1	5.5	409
p-octopamine	1465	591	38	2.7	571
m-synephrine	1470	605	6.0	10	585
norepinephrine	1490	753	33	6.0	733
p-synephrine	1520	605	10	7.0	585
epinephrine	1525	767	23	1.0	747
dopamine	1545	591	2.0	42	571
normetanephrine	1570	621	29	4.0	601
epinine	1590	605	1.0	0.1	585
3-methoxytyramine	1605	459	0.2	5.9	439
metanephrine	1620	635	17	13	615

for satisfying the criteria for identification of an unknown compound by GC/MS SIM. These criteria are (1) that at least two structure-specific ions (preferably one being the molecular ion) should be present, (2) that these two ions be present in the unknown sample at the same retention time as those of the authentic standard, and (3) that the ratio of the intensities of these ions derived from the biological sample should be identical with the corresponding ratio obtained from the authentic standard. Kovats' indexes and intensities of the M^- and $(M - HF)^-$ ions of the PFP derivatives of the octopamines, synephrines, and 12 other biogenic amines are shown in Table II.

The PFP derivatives of the three isomeric octopamines were completely resolved from one another as were the PFP derivatives of the three synephrines. The PFP derivatives of *p*-tyramine, *p*-octopamine, and *m*-synephrine have very similar retention times but they afforded M^- and $(M - HF)^-$ ions at different m/z values.

With our instrumentation, the limit of detection for an on-column injection was 100 fg for the molecular ion of *m*-octopamine–PFP which carries 18% of the negative ion current and 500 fg for the molecular ion of *m*-synephrine–PFP which carries 6% of the total ion current. Our results are very similar to those of Hunt and Crow who have reported that the molecular ion of the (pentafluorobenzylimine)trimethylsilyl derivative of dopamine carries 95% of the negative ion current and that the lower limit of detection for an on-column injection was 25 fg (*11*).

This remarkable sensitivity obtained with pure standards could not be achieved with the biological extracts employed in our experiments. This was because the finite population of electrons which are available in the ion source for ionization of the sample is seriously depleted by relatively small quantities of compounds with high electron affinities, thereby causing a sharp drop in sensitivity (*11*). We have found that the intensity of the negative ion signal becomes nonlinear with regard to concentration when the sample of *m*-synephrine–PFP exceeds 5 ng and that saturation of the negative ion signal occurs with sample size above 20 ng. Unfortunately, biological samples normally contain many compounds which can be derivatized by PFPA to form electron-capturing compounds. If these compounds are present in sufficiently large amounts, and if they elute shortly before (or simultaneously with) the compound of interest, a significant reduction in sensitivity ("quenching") will result. Thus investigations utilizing NCI GC/MS must usually be carried out with relatively small samples and the PFPA used for derivatization must be removed and replaced with hexane before the derivatized sample is injected into the system (*12*). Because of these saturation effects and the presence in samples of other electron-capturing ("quenching") substances, the practical lower limit of detection for the octopamines and synephrines in urine by NCI was found to be about 100 pg mg^{-1} creatinine.

Identification of Octopamines and Synephrines in Urine. Morning specimens of urine were obtained from five male and five female volunteers with ages ranging from 13 to 55. All the subjects were apparently healthy, were not receiving medication, and had abstained from citrus fruit and juice for 48 h prior to collection of the specimens.

In a typical analysis, an aqueous solution (100 μL) of the internal standard containing deuterated *o*-, *m*-, and *p*-octopamine (10 ng, 10 ng, 100 ng, respectively) and the deuterated synephrines (100 ng each) was added to urine (containing 1 mg of creatinine), which was then processed as described above. In each of the NCI GC/MS analyses depicted in Figures 1–3, the tracing at each m/z value was normalized to the highest peak. Figure 1A shows the PFP derivatives of deuterated and nondeuterated *p*-octopamine in the 1:1 standard mixture containing the 12 amines. Figure 1B is the corresponding tracing obtained from a derivatized urine extract. From Table III it is clear that the criteria (vide supra) for the unequivocal identification of *p*-octopamine are satisfied.

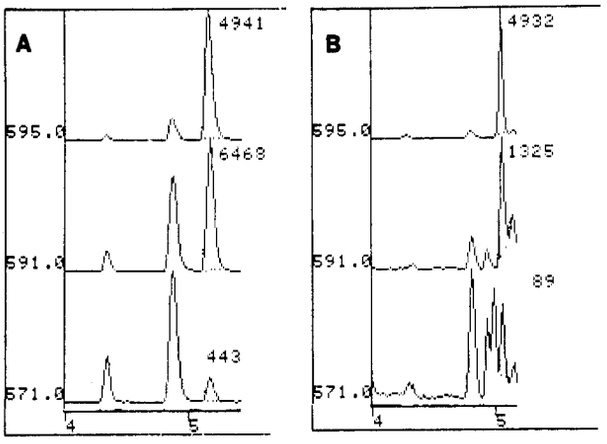

Figure 1. (A) The PFP derivatives of *p*-octopamine-d_0 and -d_4 in the 1:1 standard (corresponding to an on column injection of 250 pg) containing the 12 amines. (B) The corresponding derivatives in an aliquot (3 μL) of a derivatized urinary extract in hexane (25 μL) from urine containing 1 mg of creatinine and 100 ng of the *p*-octopamine-d_4.

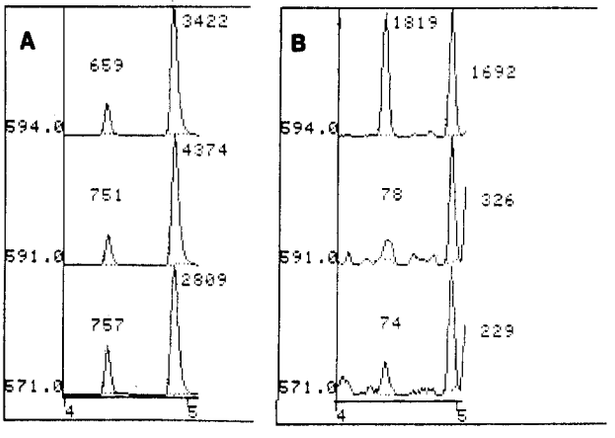

Figure 2. (A) The PFP derivatives of *o*- and *m*-octopamine-d_0 and -d_3 obtained by a repeat injection of the same amount of 1:1 standard shown in Figure 1. (B) The corresponding derivatives in an aliquot (3 μL) of a derivatized urinary extract in hexane (25 μL) from urine containing 1 mg of creatinine and 10 ng each of *o*- and *m*-octopamine-d_3.

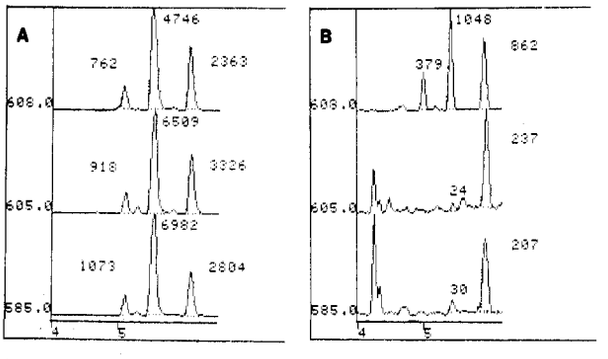

Figure 3. (A) The PFP derivatives of *o*-, *m*-, and *p*-synephrine-d_0 and -d_3 obtained by a repeat injection of the same amount of 1:1 standard shown in Figure 1. (B) The corresponding derivatives in an aliquot (3 μL) of a derivatized urinary extract in hexane (25 μL) from urine containing 1 mg of creatinine and 100 ng each of *o*-, *m*-, and *p*-synephrine-d_3.

Figure 2 shows the results of the corresponding analyses of *o*- and *m*-octopamine–PFP by NCI GC/MS. Examination

Table III. Identification of Octopamines and Synephrines in Human Urine

PFP derivative	retention time, min		ratio of intensities of $M^-/(M - HF)^-$ ions	
	std	unknown	std	unknown
o-octopamine	4.2	4.2	0.99	1.05
m-octopamine	4.95	4.95	1.56	1.42
p-octopamine	5.30	5.30	14.6	14.9
o-synephrine	5.05		0.85	
m-synephrine	5.25	5.25	0.93	0.80
p-synephrine	5.65	5.65	1.19	1.14

Table IV. Recovery and Quenching of Deuterated Standards

deuterated amine	observed recovery, %	quenching, %
o-octopamine	11 (±5)	53 (±14)
m-octopamine	6 (±2)	59 (±16)
p-octopamine	4 (±2)	75 (±15)
o-synephrine	9 (±4)	61 (±11)
m-synephrine	6 (±2)	73 (±10)
p-synephrine	12 (±6)	46 (±14)

of Table III confirms unambiguously that both *o*- and *m*-octopamine are present in the urinary extract. This result was not an artifact arising from the presence of *o*-octopamine-d_0 in the internal standard (ratio of intensities, 0.001: Table I) since the corresponding ratio observed for the biological sample was 0.042.

Similarly Figure 3 was obtained from the analyses of the PFP derivatives of the isomeric synephrines by NCI GC/MS. *o*-Synephrine could not be detected in any urinary extract whereas *m*- and *p*-synephrine were both present (Table III). The ratio of intensities observed for *m*-synephrine ($M^-_{d_0}/M^-_{d_3}$, 0.023) is approximately seven times that expected from the contribution arising from the d_0 component of the internal standard (100 ng). When 10 ng of *m*-synephrine-d_3 was used as an internal standard instead of 100 ng, the anticipated 10-fold increase in this ratio was observed.

Quantitative Determination of Isomeric Octopamines and Synephrines. The amounts of *o*-, *m*- and *p*-octopamine and of *m*- and *p*-synephrine were determined by comparison of the ratio of the intensities of $M^-_{d_0}/M^-_{d_x}$ measured for the derivatized biological sample with that arising from the derivatized 1:1 standard and the results are summarized in Tables IV and V. All the analyses were carried out in duplicate and the percent mean variations were as follows: *o*-octopamine, 20%; *m*-octopamine, 16%; *p*-octopamine, 20%; *m*-synephrine, 24%; *p*-synephrine, 20%.

The recovery of each component was estimated by comparing the intensity of the molecular ion arising from 1 ng deuterated standard with that observed for a theoretically equal quantity in the urinary extract. Recoveries ranged from 4% to 12% in these analyses (Table IV) while the recoveries of similar amounts of internal standard from water were ca. 40%.

The quenching factor (46–75%, Table IV) was estimated in the following way. The derivatized urinary extract was coinjected with the derivatized internal standard, and the intensity of the M^- ion arising from the PFP derivatives of the deuterated amine was measured. This value was compared with the sum of the values arising from the separate injections of corresponding quantities of the derivatized urinary extract and derivatized internal standard. Consequently it may be seen that the apparent recovery of ca. 5–10% was caused in part by quenching due to coeluting components which have a high affinity for electrons: thus the actual recovery of these amines from urine is of the order of 10–20%.

Our choice of acid hydrolysis for deconjugation of the octopamines and synephrines was based on the following considerations: (1) In the human, endogenous *p*-octopamine and *p*-synephrine are excreted predominantly as acid-hydrolyzable conjugates. In the case of *p*-synephrine, this is neither a sulfate nor a glucuronide (*20*). (2) In the human, exogenous *m*-synephrine is excreted predominantly as the sulfate (oral, 80%; nasal 90%) with the remainder as a glucuronide, and both are released completely by acid hydrolysis (*21*). (3) In the human, exogenous (intravenous) *m*-octopamine is excreted free (50%) and as an acid-hydrolyzable conjugate (50%) (*22*). (4) Although nothing is known of the mode of excretion of *o*-octopamine and *o*-synephrine in the human, in the rat exogenous (intraperitoneal) *o*-octopamine is excreted free. *o*-Synephrine is excreted both free (50%) and as an acid-hydrolyzable conjugate (50%) (*7*) which is neither a sulfate nor a glucuronide. Extending the time of acid hydrolysis to 1 h at a higher temperature (boiling water bath) did not increase the observed amounts of the amines.

The ten samples of urine were also analyzed for unconjugated octopamines and synephrines by omitting the acidic hydrolysis (see Table V).

This is the first report of the presence of endogenous *o*-octopamine in a human biological fluid. The administration of *o*-octopamine to rats (*7*) resulted in the excretion of *o*-hydroxyphenylglycol (OHPG), *o*-hydroxymandelic acid (OHMA), and unchanged amine (ca. 66% as an acid-hydrolyzable conjugate). OHMA, in contrast to OHPG (*7*,

Table V. Urinary Concentration of Octopamines and Synephrines

subject (sex, age)	concentration, ng mg^{-1} creatinine											
	o-octopamine		*m*-octopamine		*p*-octopamine		*o*-synephrine		*m*-synephrine		*p*-synephrine	
	free	total	free	total	free	total	free	total	free	total	free	total
SE (F, 19)	0.6	0.7	1.0	1.7	1.5	33	ND[a]	ND	2.0	2.8	1.0	25
KT (F, 25)	0.3	0.7	1.7	3.4	1.2	32	ND	ND	2.0	2.3	1.9	17
LB (F, 35)	0.2	0.2	0.8	0.8	0.3	6	ND	ND	1.1	0.8	1.2	41
MC (F, 42)	0.5	0.7	1.5	2.0	1.6	61	ND	ND	1.0	0.7	1.9	9
JR (F, 55)	0.1	0.6	1.1	2.2	0.8	7	ND	ND	2.4	2.7	2.5	11
LC (M, 13)	0.4	0.5	1.6	3.1	0.8	9	ND	ND	1.4	1.6	2.5	8
SR (M, 29)	0.4	0.5	1.3	2.6	0.8	12	ND	ND	1.4	1.8	1.2	8
KI (M, 33)	0.4	1.0	1.3	2.1	2.2	49	ND	ND	1.9	2.3	1.4	13
LC (M, 42)	0.3	0.6	0.6	1.2	1.5	28	ND	ND	1.0	1.3	4.0	20
CW (M, 55)	0.3	0.7	0.7	1.6	0.9	10	ND	ND	1.5	2.1	0.4	12
mean	0.3	0.6	1.0	2.1	0.9	25	ND	ND	1.6	1.8	1.8	16
std dev	0.1	0.2	0.5	0.8	1.2	19			0.5	0.7	1.0	10

[a] ND: not detected (<100 pg mg^{-1} creatinine).

23), is a normal constituent of rat (7) and human (24) urine and is found in elevated quantities in the urine of patients with phenylketonuria (14). However, it was not possible to detect endogenous o-octopamine in the urine of normal humans (7) and patients with phenylketonuria (14) by electron-impact GC/MS methods. The present report confirms our earlier proposal (7, 14) that urinary OHMA originates predominantly (if not entirely) from o-octopamine and that the latter is a naturally occurring (i.e., biogenic) amine.

It has been established that m-octopamine is present in rat salivary gland (3, 8), rat and bovine adrenal glands (8), and rat brain (4). However, this constitutes the first account of its occurrence in a biological fluid. Exogenous m-octopamine is metabolized by mammals to m-hydroxyphenylglycol (25) and m-hydroxymandelic acid (22, 25) which both occur naturally in human and rat urine (23). However, part of the administered dose is not deaminated (22, 25) and our finding that m-octopamine is excreted as an acid-hydrolyzable conjugate and as the unconjugated amine (in approximately equal quantities) agrees with that of Hengstmann et al. (22) who administered m-octopamine intravenously to humans.

Unconjugated p-octopamine was identified first in human urine by two-dimensional paper chromatography in amounts of 0.5 ng mg^{-1} creatinine (26) and as an acid-hydrolyzable conjugate in amounts of 0–20 ng mg^{-1} creatine (20). The values reported here are in excellent agreement with these earlier estimates.

o-Octopamine is a poor substrate for norepinephrine N-methyltransferase (6) and James et al. found that o-synephrine could not be detected in urine after the administration of 250 μg of o-octopamine to rats (7). Therefore o-octopamine may not be a precursor of o-synephrine which may account for our failure to detect o-synephrine. However, it should be borne in mind that the lower limit of detection of o-synephrine by our method is 100 pg mg^{-1} creatinine and that o-synephrine might be present in concentrations lower than this limit.

Although m-synephrine has been found to occur naturally in adrenal gland (9, 10) this is the first report of its natural occurrence in mammalian urine and our results indicate that endogenous m-synephrine is present entirely in the unconjugated state. This concurs with the results observed (27) when m-synephrine was administered intravenously to humans: 73% of the excreted amine was unconjugated. In contrast, when administered orally, a substantial proportion (50–60%) of the dose was not deaminated and was excreted almost entirely in the conjugated form (21, 27).

p-Synephrine was identified in human urine by chromatographic means some 20 years ago (20, 28). It is excreted as an acid-hydrolyzable conjugate in amounts which vary markedly from individual to individual (e.g., 0–1600 ng mg^{-1} creatinine) (20). These observations, together with the large variation in the daily excretion of p-synephrine by a given individual, suggested that urinary p-synephrine was primarily of dietary origin. Subsequently, it was discovered that p-synephrine (29) and p-octopamine (30) were present in citrus fruits in appreciable quantities (e.g., p-synephrine, orange juice, 4.8 μg mL^{-1}). Consequently we have analyzed the juice from fresh citrus fruit for the octopamines and synephrines in the manner described above. Unconjugated p-octopamine was present in orange (160 ng mL^{-1}), grapefruit (9 ng mL^{-1}), and lemon (0.8 ng mL^{-1}) while orange and grapefruit juice contained 16 μg mL^{-1} and 8 ng mL^{-1}, respectively, of unconjugated p-synephrine. The other isomeric octopamines and synephrines were absent (lower limit of detection, 100 pg mL^{-1}). When ten human subjects abstained from citrus fruit or juice for 48 h prior to donation of specimens, we observed that p-synephrine was excreted (predominantly as an acid-hydrolyzable conjugate) in amounts varying from 8 to 41 ng mg^{-1} creatinine.

These results demonstrate the utility of the new capillary column electron capture NCI GC/MS assay for isomeric octopamines and synephrines in urine. The high sensitivity of this technique has made possible the detection, for the first time, of o- and m-octopamine and m-synephrine in human specimens. Research, including the development of tandem mass spectrometric methods (MS/MS), is continuing in this area.

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Registry No. o-Octopamine, 2234-25-5; m-octopamine, 536-21-0; p-octopamine, 104-14-3; o-synephrine, 575-81-5; m-synephrine, 532-38-7; p-synephrine, 94-07-5; p-octopamine PFP deriv., 62237-94-9; p-octopamine-d_4 PFP deriv., 90320-70-0; o-octopamine PFP deriv., 90320-71-1; o-octopamine-d_3 PFP deriv., 90320-72-2; m-octopamine PFP deriv., 77745-52-9; m-octopamine-d_3 PFP deriv., 90320-73-3; o-synephrine PFP deriv., 90320-74-4; m-synephrine PFP deriv., 90320-75-5; p-synephrine PFP deriv., 77862-54-5; o-synephrine-d_3 PFP deriv., 90320-76-6; m-synephrine-d_3 PFP deriv., 90320-77-7; p-synephrine-d_3 PFP deriv., 90320-78-8.

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