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SEPARATION AND QUANTITATION OF SOME URINARY ARYLALKYLAMINES

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SUMMARY

The arylalkylamines *m*- and *p*-tyramine, β-phenylethylamine and tryptamine in their unconjugated forms have been identified and quantitated in urine collected from human volunteers. Their excretion levels (mean ± standard error of the mean in μg/g creatinine) were, respectively, 67 ± 5, 419 ± 37, 4.6 ± 1.2, and 82 ± 11.

INTRODUCTION

Various arylalkylamines have been reported to be constituents of human urine; because of a lack of specificity in the methods used for their detection, however, wide discrepancies exist with respect to their claimed urinary concentration levels. Unconjugated *p*-tyramine has been reported as being excreted in the range of 312–1300 μg/g creatinine[1–9], *m*-tyramine in the range of 5–100 μg/g creatinine[3,10–12], β-phenylethylamine in the range of 3–453 μg/day[13–20], and tryptamine in the range of 62–100 μg/day[1,5,8,9,21–25]. In this study a sensitive and specific procedure based on ion-exchange isolation, derivatization, chromatographic separation, and quantitative high-resolution mass spectrometry (MS) has been utilized to separate, identify and quantitate *m*- and *p*-tyramine, β-phenylethylamine and tryptamine, in their non-conjugated form, in urine samples collected from healthy volunteers.

EXPERIMENTAL

Chemicals and solvents

Reagent-grade solvents as obtained commercially were used without further purification except in the case of the ethyl acetate used as the vehicle for introduction of sample into the mass spectrometer. This solvent was of the Spectranalysed grade. Silica gel thin-layer plates (20 cm × 20 cm) were obtained from Brinkman (Westbury, N.Y., U.S.A.). The deuterated internal standards 1,1-dideutero-2-(4-hydroxyphenyl)-ethylamine (*p*-tyramine-d_2), 1,1-dideutero-2-(3-hydroxyphenyl)ethylamine (*m*-tyramine-d_2), 1,1-dideutero-2-indolylethylamine (tryptamine-d_2), and 1,1-dideutero-

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phenylethylamine (β-phenylethylamine-d_2) were prepared as previously described[26–30]. The extent of deuteration, calculated by measuring the ratio between *m/e* 32 (CD_2NH_2) and *m/e* 30 (CH_2NH_2) in the spectrum of the hydrochloride salts, was shown to be greater than 97% in all cases.

Samples

Urine was collected into polythene bottles over a 24-h period from 21 volunteers in the age range of 19–43. It was frozen at the end of the collection period and then stored at −16° until analysed. In order to assess whether amine levels changed during storage, aliquots of some of the samples were analysed immediately after collection and after various time periods. At the time of analysis, the urine samples were allowed to thaw thoroughly at room temperature, shaken vigorously, and 6-ml aliquots withdrawn, in duplicate, for the analysis of *m*- and *p*-tyramine and tryptamine and 10-ml aliquots for β-phenylethylamine.

Analyses

The overall analytical procedure is outlined in Fig. 1. Briefly, an appropriate quantity of deuterated internal standard (6 μg *m*- and *p*-tyramine-d_2, 1 μg β-phenyl-

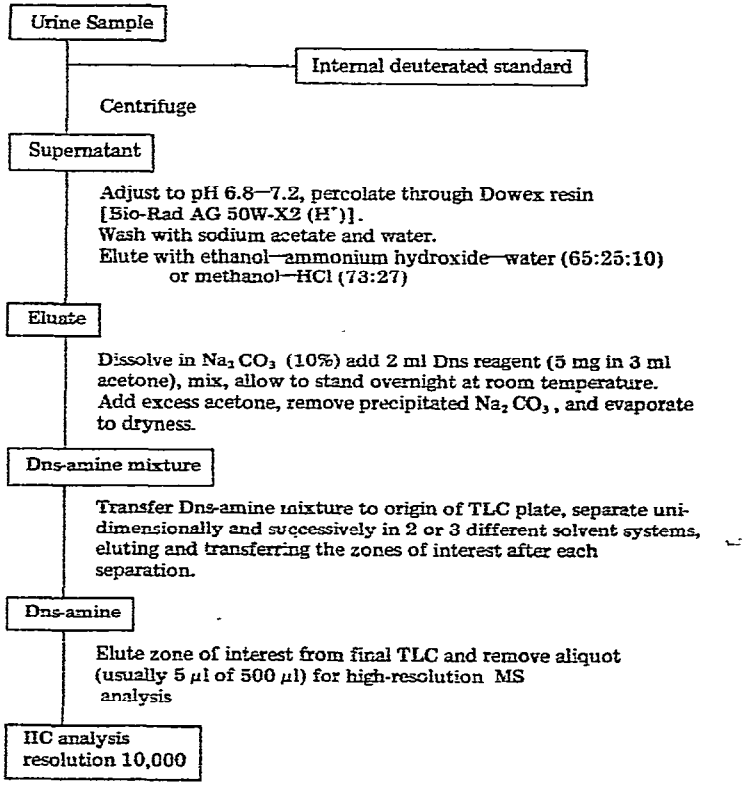

Fig. 1. Procedure for the isolation, derivatization, separation and MS analysis of urinary unconjugated arylalkylamines.

ethylamine-d_2 and tryptamine-d_2) was added to the urine sample (6 ml or 10 ml), which was then centrifuged at low speed for 2–3 min; the clear supernatant, after being adjusted to pH 6.8–7.2, was percolated through a column of Bio-Rad AG 50W-X2 (H^+) (3 cm × 1 cm) as described by Kakimoto and Armstrong[3]. Several blanks (usually three) composed of distilled water to which the internal standard had been added were processed with each batch of urine samples. After the resin columns had been washed with distilled water (10 ml), 0.1 *N* sodium acetate (10 ml) and distilled water (10 ml), *m*- and *p*-tyramine and tryptamine along with other adsorbed materials were eluted with 10 ml 1 *N* ammonium hydroxide in 65% ethanol. *β*-Phenylethylamine analyses were performed separately, since it is necessary to elute this amine with 10 ml methanol–hydrochloric acid solution (73:27) in order that the amine exist as the HCl salt. All eluates were then evaporated to dryness under reduced pressure at 40° and any reactive substances converted to their respective dansyl derivatives by dissolving the eluate in 1 ml 10% sodium carbonate, mixing it with 2 ml dansyl chloride reagent (5 mg dissolved in 3 ml acetone) and allowing the mixture to stand for 16 h at room temperature. Excess Na_2CO_3 was precipitated following the addition of 9 ml acetone and removed by low-speed centrifugation. The supernatant was dried under reduced pressure at 40°, the residue triturated in ethyl acetate (2 × 2 ml), transferred to a clean flask, and dried under a stream of nitrogen. After redissolving in a small volume of ethyl acetate the mixture of Dns derivatives was transferred to the origin of the silica gel thin-layer chromatogram plate. The isomeric Dns-tyramines were separated first in system 1 (where they remain isographic) and then in system 2 (where they separate), Dns-tryptamine in system 1 followed by system 3, and Dns-*β*-phenylethylamine in systems 1, 4, and 5 (*cf.* Table I).

TABLE I

R_F VALUES OF SOME ARYLALKYLAMINES IN SELECTED SOLVENT SYSTEMS

Solvent system	*Composition*	*Proportions (v/v)*	R_F *values*			
			m-Tyramine	*p-Tyramine*	*β-Phenyl-ethylamine*	*Tryptamine*
1	Chloroform–*n*-butyl acetate	4:1	0.63	0.63	0.84	0.47
2	Benzene–triethyl-amine	12:1	0.41	0.38	—	—
3	Benzene–methanol	10:1	—	—	—	0.36
4	Benzene–triethyl-amine	8:1	—	—	0.60	—
5	Carbon tetrachlo-ride–triethylamine	5:1	—	—	0.24	—

Location of the zones containing the Dns-amines of interest was accomplished in UV light (365 nm) and was aided by separating synthetic derivatives in parallel and on the same plates. The intensity and the stability of the fluorescence on the plates was stabilized by spraying with isopropanol–triethanolamine (4:1) in all cases, while the chromatograms were still damp following their removal from the solvent tanks, except after the final separations. The zones of interest were outlined with a metal

stylus and after scraping the gel from the plate the derivatives were eluted and either transferred to other chromatograms or prepared for MS analysis. In the case of the tyramines, elution was into 3 ml benzene–acetone (10:1), tryptamine was eluted into benzene–methanol (10:1), and β-phenylethylamine into ethyl acetate. Following the final separations, each Dns-amine was eluted into 3 ml Spectranalysed ethyl acetate which after drying under a stream of nitrogen was redissolved in 0.5 ml Spectranalysed ethyl acetate.

Aliquots (5 μl) of these solutions including the blanks (all of which contained the appropriate internal deuterated Dns-amine standards) were introduced on a direct insertion probe into the ion source region of an AEI MS 902S mass spectrometer equipped with a Massmaster mass indicator. The ion source temperature was raised to 260° and at a resolution of 10,000 ion current profiles were obtained as previously described[26]. The magnetic field of the mass spectrometer was so adjusted that the molecular ions of the Dns-amines of interest (*m/e* 354.1402 for Dns-β-phenylethylamine, 603.1861 for Dns-tyramine, and 393.1511 for Dns-tryptamine) were focussed in the low mass portion of the peak switching unit and the molecular ions of the Dns deuterated amines (*m/e* 356.1527 for Dns-phenylethylamine-d_2, 605.1987 for Dns-tyramine-d_2, and 395.1637 for Dns-tryptamine-d_2) in the high mass portion. These peaks were located with the aid of the Massmaster mass indicator and confirmed by reference to the ions arising from the heptacosafluorotri-*n*-butylamine standard gas in the mass spectrometer. An IIC recording was obtained during the period of evaporation of the samples from the probe as the switching unit alternately focussed on the appropriate ions of the amine being analysed. The amount of amine in the sample presented for analysis was calculated from a formula of the type

$$\text{Amine} = \frac{\text{Area}_a}{\text{Area}_b - x\%\ \text{Area}_a} \times Y$$

where Area_a represents the area of the IIC profile due to the amine being analysed, Area_b the area of the IIC profile due to the deuterated amine (*i.e.*, appropriate internal standard), and Y the amount of deuterated internal standard added at the start of analytical procedure. It is necessary to correct (*i.e.*, $-x\%\ \text{Area}_a$) for the presence of those ions arising from the isotopes of ^{13}C, ^{18}O, ^{34}S that are associated with the *m/e* value being analysed; it amounts to $x\%$ and is characterized, of course, by the amine being analysed and the resolution.

Creatinine was analysed by the conventional Jaffe procedure using alkaline picrate.

RESULTS AND DISCUSSION

It can be seen from Table II that no systematic variation or reduction in amine concentration occurred during a period of storage, at −16°, for up to at least 128 days.

The spontaneous daily excretions of the four amines are listed in Table III. It was found in the particular volunteer group under study (*i.e.*, laboratory personnel, age range 19–43 years) that males (assessed using the Mann-Whitney U test) excreted less of each amine than females (*p*-tyramine, $p < 0.04$; *m*-tyramine, $p < 0.04$; tryptamine, $p < 0.04$, β-phenylethylamine, $p < 0.03$, respectively) but that there was no

TABLE II

EFFECT OF STORAGE AT −16° ON URINARY ARYLALKYLAMINE LEVELS

Amine	*Storage period (days)*	*Concentration (μg/24 h)*	
		Urine A	*Urine B*
β-Phenylethylamine	0	1.8	1.9
	15	1.4	—
	108	—	3.3
	125	—	3.0
	128	1.8	—
m-Tyramine	0	64	107
	14	69	108
	43	61	107
p-Tyramine	0	770	920
	14	810	890
	43	790	880
Tryptamine	0	220	240
	14	240	270
	43	190	260

change with age. The excretion values obtained for the tyramines[1–12] and tryptamine[1,5,8,9,21–25] agree quite well with those listed in the literature; β-phenylethylamine, however, agrees only with the lowest published value (compare ref. 14 with refs. 8, 13, 15–20). This is almost certainly related to the non-specificity of earlier procedures.

Preliminary studies in which the spontaneous urinary excretion of the above unconjugated amines was assessed in a psychiatric population (129 patients), have revealed that a significant proportion excrete much larger than normal amounts in the case of *p*-tyramine, β-phenylethylamine, and tryptamine. This phenomenon is being studied further in longitudinal studies on selected patients, especially with respect to wether or not urinary amine levels are related to symptoms, diagnosis, and drug therapy.

TABLE III

URINARY ARYLALKYLAMINE LEVELS IN A CONTROL POPULATION

Values are mean ± standard error of the mean; $n = 15$–21, as shown.

Arylalkylamine	*Concentration*	
	μg/g creatinine	*μg/day*
p-Tyramine ($n = 21$)	419 ± 37	489 ± 40
m-Tyramine ($n = 19$)	67 ± 5	83 ± 7
Tryptamine ($n = 18$)	82 ± 11	100 ± 13
β-Phenylethylamine ($n = 15$)	4.6 ± 1.2	4.9 ± 1.0

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