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HYDROXYLATION OF β-PHENYLETHYLAMINE IN THE RAT

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SUMMARY

ortho, meta and para Tyramine, as their dansyl derivatives, have been identified and quantitated by mass spectrometry in rat urine. After intraperitoneal injection of phenylethylamine labelled either with deuterium or tritium in the presence of pargyline, the amount of urinary tyramines excreted was 0.87% (para, 0.74%; meta, 0.12%; ortho, 0.008%) of the injected dose. The advantages of involving stable isotopes and mass spectrometry in metabolic studies of pharmacologically active compounds is discussed.

Ortho, meta and para tyramine have been identified in rat and human urine (1-7) and meta and para tyramine in all tissues of the rat so far analysed (8 and Philips, Davis, Durden and Boulton, unpublished observations). The concentration of meta tyramine in all cases is substantially less than that of the para isomer. It has been assumed that para tyramine is formed as a result of decarboxylation of para tyrosine. In the presence of high concentrations of circulating para tyrosine, decarboxylation appears to be very efficient (9,10), at lower levels, however, this seems not to be the case (9-14). It is a fact, however, that ortho and meta tyrosine are much more easily decarboxylated in vivo (11,15,16). meta and para Tyramine are also synthesised in vivo as a result of dehydroxylation of dopa and dopamine when more of the meta isomer is produced (7,17-19). Since decarboxylation and dehydroxylation seemed not to be extensively involved in the biosynthesis of para tyramine however we decided to investigate the alternative biosynthesis, namely hydroxylation of β-phenylethylamine. The hydroxylation of a number of phenolic and phenylalkyl amines and amino acids in the meta and para ring

positions to produce catecholic and phenolic substances have been reported (20-27). Ortho hydroxylation has been reported to arise by non-enzymic mechanisms (28); the urinary excretion of increased amounts of ortho hydroxy metabolites in phenylketonuria has been reported (29).

In this study tyramines labelled either with a radioactive (tritium) or stable (deuterium) isotope have been identified in rat urine following intraperitoneal injection of an appropriately labelled phenylethylamine.

Materials and Methods

Materials

The deuterated amines 1,1,2,2-tetradeutero-2-phenylethylamine (phenylethylamine-d_4), 1,1-dideutero-2-phenylethylamine (phenylethylamine-d_2), 1,1-dideutero-2(4-hydroxyphenyl)ethylamine (para tyramine-d_2), 1,1-dideutero-2(3-hydroxyphenyl)ethylamine (meta tyramine-d_2) and 1,1-dideutero-2(2-hydroxyphenyl)ethylamine (ortho tyramine-d_2) were synthesized as previously described (30,31). The extent of deuteration was calculated by measuring the m/e 32/30, ($CD_2NH_2^+/CH_2NH_2^+$) ratio in the spectra of the HCl salts and of the respective molecular ions of the 5-dimethylaminonapthalene-1-sulphonyl (DNS) derivatives. It was found to be greater than 99%.

β-Phenylethylamine [4-^{3}H(N)], specific activity 4580 mCi/mM was prepared by the Nuclear Chicago Corporation in their New England Laboratories in Boston, Mass. It was purified, before use, in our laboratory by paper chromatographic separation in the solvent system isopropyl ether-methyl ethyl ketone-acetic acid-water, 1:1:5:5 (v/v) followed by elution of the appropriate zone in 0.5 N acetic acid in 65% ethanol. All other chemicals and solvents were of the purest grade commercially available. Pargyline hydrochloride was kindly donated by Abbott Laboratories (North Chicago, Illinois).

Injection Procedure, Urine Collection and Amine Isolation

Male Wistar rats, body weight 150-200 g, were injected intraperitoneally with pargyline-hydrochloride dissolved in physiological saline (75 mg/kg)

one hour prior to the i.p. injection of labelled phenylethylamine. Phenylethylamine-^{3}H (acetate salt) (80, 100 or 120 µCi sometimes supplemented with 10, 100 or 1000 µg of unlabelled amine), phenylethylamine-d_2 (hydrochloride salt, 1 or 10 mg) or phenylethylamine-d_4 (hydrochloride salt, 1 mg) dissolved in 500 µl physiological saline was then injected and the rats maintained individually in polycarbonate metabolic cages without food but with water ad libitum for 24 hours. The urine plus washings from the sides of the collecting vessel were collected into 1 ml 6N HCl. Urines from rats injected only with pargyline or saline were also collected simultaneously in order to permit the estimation of the urinary levels of ortho, meta and para tyramine under these experimental conditions and to act as controls. In this latter case phenylethylamine labelled either with ^{3}H or deuterium was added to the urine in order to demonstrate whether hydroxylation occurred either autocatalytically or in some alternative way (see Table 1).

One tenth or one hundredth of the total urine volume was adjusted to pH 7.0, a phenolic amine fraction isolated and dansylated as previously described (17,31). The dansylated mixture was separated unidimensionally and successively on thin layers of silica gel (Brinkman, Toronto) in the three solvent systems indicated below. The R_f values of ortho, meta and para tyramine are also indicated.

1.	Chloroform-n-butylacetate 5:2 (v/v)	0.75 (ortho)	0.67 (meta)	0.63 (para)
2.	Cyclohexane-ethylacetate 1:1 (v/v)	0.72	0.67	0.65
3.	Benzene-triethylamine 12:1 (v/v)	0.59	0.52	0.47

A
Appropriate zones were located by standards of DNS ortho-, DNS meta- and DNS para-tyramine run alongside, scraped from the plate and the amine derivatives eluted in 2 x 1.5 ml ethylacetate:methanol, 5:1 (v/v). These eluates were then analysed as described below. That the various isomers were adequately separated was proven by showing that labelled p-tyramine (^{3}H and deuterium)

added to the urine did not contaminate either of the recovered ortho or meta chromatographically separated tyramine zones. The overall recovery of para tyramine-^{14}C added to urine and subjected to this isolation procedure was 43.8 ± 2.4% (n=3).

Stable Isotope Analyses

The identification of the ortho, meta and para tyramine isomers both endogenous and those formed by hydroxylation of β-phenylethylamine-d_4 were established from the spectra (see Figure 1 and refs. 7,31) and from the chromatographic properties previously discussed. Quantitation was achieved by adding a known amount (0.2 or 1.25 μg) of the di-deutero tyramines to act as internal standards (31,32) to half of the urine aliquot and the amounts of the deuterated, and non-deuterated isomers in the resultant DNS tyramine eluates evaluated by the mass spectrometric integrated ion current procedure. Briefly this was achieved by placing 5 μl aliquots of the separated DNS-tyramine zones dissolved in 50 μl ethylacetate in the direct insertion probe which after drying was placed in the insertion lock of an AEI MS-902S mass spectrometer. To quantitate the endogenous tyramines the samples containing the internal standards were used. The ions at m/e 603 (603.1861 due to $C_{32}H_{33}N_3O_5S_2$ bis DNS-tyramine) and m/e 605 (605.1987 due to $C_{32}H_{31}N_3O_5S_2D_2$ and 605.1860 from the ^{34}S, ^{18}O and $^{13}C_2$ isotopes of the 603 peak) were recorded as the samples evaporated at 280°C. The ratio between the IIC profile areas corrected for the isotopic contribution at m/e 605 then provides the value for the urinary amine. Similarly the samples not containing internal standard were used to quantitate the tetra-deutero tyramines from the values of the endogenous tyramines from the ratio of the IIC profiles recorded at m/e 607 (607.2113 due to $C_{32}H_{29}N_3O_5S_2D_4$) and m/e 603 (603.1861 due to $C_{32}H_{33}N_3O_5S_2$). Theoretically it is possible to quantitate the tetra-deutero tyramines from the internal standard by comparing the ratio of the areas of m/e 605 and m/e 607. However, in practice the amount of the ion at m/e 607

due to the ^{34}S, ^{18}O and $^{13}C_2$ isotopes associated with m/e 605 obscured the small amount due to the tetra deutero amines.

TABLE 1

Urinary Levels of ortho, meta and para Tyramine in Rats Treated and Untreated with Pargyline

	ortho Tyramine (µg/24h)	meta Tyramine (µg/24h)	para Tyramine (µg/24h)	p/m Ratio
Untreated (n=3)	2.01 ± 0.21	5.89 ± 0.86	14.23 ± 2.54	2.4 ± 0.2
Treated (n=8)	4.23 ± 1.7	6.08 ± 1.06	19.72 ± 4.42	3.3 ± 0.7

Results expressed as mean ± standard deviation.

Radioisotope Analyses

The final DNS meta- and DNS para-tyramine eluates were added to 10 ml scintillation fluid (6 PPO[2,5-diphenyl oxazole] per litre toluene) in vials and counted in a Nuclear Chicago Isocap 300 liquid scintillation counter.

RESULTS

From Table 1 and Figure 1 it can be seen that in the rat the three isomers of tyramine are present. Treatment with the monoamine oxidase inhibitor pargyline produced a modest increase in the case of ortho and para but little change in meta tyramine. It is also apparent from Figure 1 and Table 2 that each isomer of tyramine is formed by hydroxylation of phenylethylamine following its intraperitoneal introduction. Hydroxylation in the para position was the major route accounting for 0.74% of the injected phenylethylamine-d_4 dose in the presence of pargyline, meta hydroxylation (0.12% of the dose) amounted to about 16% of this. Ortho hydroxylation at 0.008% of the injected dose was very small. Data very similar to that listed in Table 1 was obtained after injections of [1 mg (n=3) and 10 mg (n=1)] dideutero phenylethylamine followed by an assessment of meta and para tyramine-d_2.

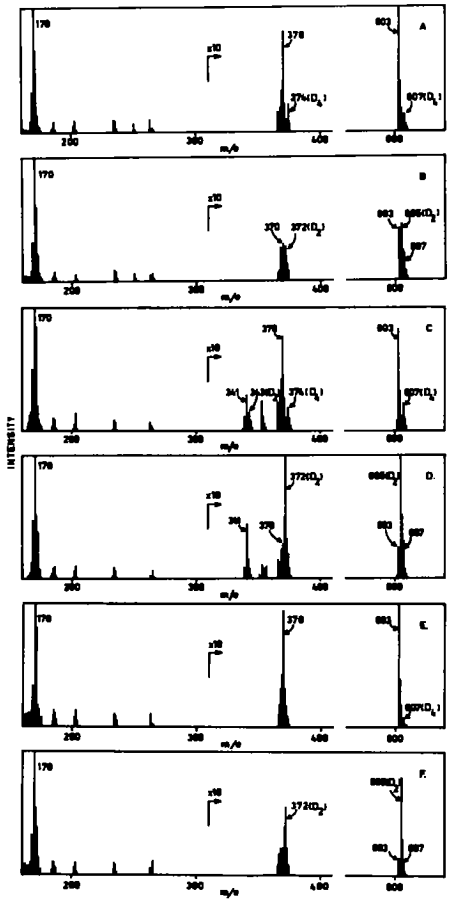

Figure 1. Mass spectra of bis dansyl derivatives of urinary tyramines after injection (i.p.) of 1,1,2,2-tetradeutero-2-phenylethylamine (1 mg) in the presence of pargyline.

A. endogenous p-tyramine and tetradeutero p-tyramine
B. as in A but including dideutero p-tyramine as internal standard
C. endogenous m-tyramine and tetradeutero m-tyramine
D. as in C but including dideutero m-tyramine as internal standard
E. endogenous o-tyramine and tetradeutero o-tyramine
F. as in E but including dideutero o-tyramine as internal standard

TABLE 2

Urinary Excretion of ortho, meta and para Tyramine-d_4 After An Intraperitoneal Injection of Phenylethylamine-d_4

	ortho Tyramine (µg/24h)	% of Dose	meta Tyramine (µg/24h)	% of Dose	para Tyramine (µg/24h)	% of Dose	p/m Ratio
Injected (n=5)	0.08±0.08	0.008	1.19±0.33	0.12	7.40±0.81	0.74	7.0±2.8
Controls (n=2)	0.002±0.000	--	0.15±0.03	--	0.20±0.02	--	--

Results expressed as mean ± standard deviation.

Further confirmation of the formation of meta and para tyramine was obtained following administration of phenylethylamine labelled in the para ring position with tritium (80 to 120 µCi). However, in this latter case the amount of the para tyramine formed was about 50% of that produced when the deuterated phenylethylamine was used as substrate. It is presumed that a kinetic isotope effect is responsible for this reduction even though Daly et al (35) have shown that an apparently complete para to meta ^3H migration occurred during the hydroxylation of amphetamine and phenylalanine. The amount of meta tyramine produced was essentially the same as before. Supplementing the tracer dose with 0.01, 0.1 and 1 mg of phenylethylamine indicated that the various hydroxylations were not dose dependent nor were the para/meta ratios changed.

DISCUSSION

It is apparent that ortho, meta and para tyramine are formed from injected phenylethylamine in the rat in the presence of pargyline. Although the extent of ortho hydroxylation is limited it is unlikely that it arises by non-enzymic means since only a negligible amount of ortho tyramine-d_2 was formed after the addition of phenylethylamine-d_2 to urine (see Table 2).

It is not yet possible to be certain about the major route of para tyramine biosynthesis in mammals. In the presence of high blood levels of

amino acids decarboxylation appears to occur but perhaps predominantly in the kidney and liver. Even in this case, however, the contribution to urinary para tyramine was only about 0.002% of the tyrosine injected (9). meta-Dehydroxylation of catecholic precursors, although apparently a minor route, occurs in the brain and presumably in the peripheral tissues. In Figure 2 an attempt has been made to indicate the metabolic interrelationships that are now known to exist between the catecholamines, phenolic amines and phenylalkylamines. In view of the pharmacological and behavioral

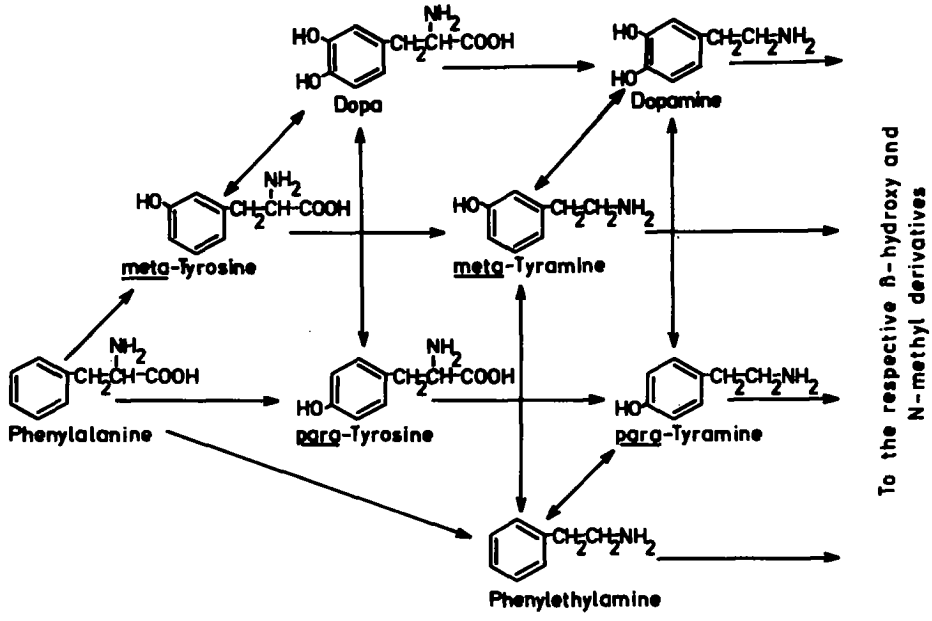

Figure 2. Metabolic interrelationships between some catecholamines, phenolic amines and phenylalkylamines.

properties of some of these amines an investigation on their sites of synthesis both cellular and subcellular and the effects of certain drugs on these syntheses might be profitable.

Since phenylethylamine, which is a normal tissue constituent, is also behaviourally active (31-34) and a potent pharmacological agent (38-42) it might be that hydroxylation is a mechanism of detoxification. Because it is claimed to be a precipitor of migraine (43) and to be excreted in less than normal amounts by patients suffering with depression (44,45), studies on its biosynthesis, metabolism and excretion in humans seems warranted. In this regard, it would be useful to be able to administer to humans relatively large quantities of the amines or their precursors, labelled in a non-toxic and non-hazardous manner so that the formed metabolites and those endogenously present could be identified and quantitated simultaneously by comparing them to a suitably labelled internal standard. The use of compounds labelled with different numbers of deuterium atoms fulfils this criterion.

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