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Phenylpyruvic acid may be a direct precursor of mandelic acid without intermediate transamination to phenylalanine

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Abstract—A series of compounds related to phenylalanine was administered to rats. Output of mandelic acid, a major but unexplained metabolite in phenylketonuria, was increased after the administration of phenylethanolamine or phenylpyruvic acid, whereas phenylethylamine or phenylalanine increased its excretion only marginally. Phenylacetic acid, previously suggested as a possible precursor in man, was almost without effect. It seems likely that mandelic acid can be formed from phenylpyruvic acid directly, without intermediate transamination to phenylalanine.

One of the major urinary metabolites in phenylketonuria is mandelic acid [1], but the pathway by which it is formed has not been established. Blau [1] suggested two possibilities. The first, by which the normal small output is generally considered to be generated, is analogous to the pathway by which catecholamines are synthesized and degraded, in which the amino acid is decarboxylated, then undergoes β-oxidation and deamination to a mandelic acid. The second is by α-hydroxylation of phenylacetic acid. Several publications indicate that the latter may occur with substituted phenylacetic acids in some biological systems; for instance, 3,4-dihydroxyphenylacetic acid undergoes α-hydroxylation in *Sarcophaga*, and *p*-hydroxyphenylacetic acid in *Aspergillus niger* and various plant species [2–4], but the only evidence that the reaction occurs in mammalian species is an observation that homogentisic acid yields gentisic acid in rat and rabbit, with 2,5-dihydroxymandelic acid as an intermediate [5–7].

In a previous study [8] it was demonstrated that rat liver, perfused with 30 mM L-phenylalanine, forms relatively large amounts of mandelic acid; at lower concentrations, its excretion is much reduced. Thus, the rat appears to be a suitable animal in which to investigate the metabolism of possible mandelic acid precursors.

Materials and Methods

In each experiment, three or four female white Wistar rats (about 200 g) were injected i.p. with the compound under test. They were kept in metabolism cages for 24 hr with water supplied *ad lib.* but without food. Urine was collected for a period of 24 hr over 0.25 mL 6 M HCl, and stored at −20° until assayed.

A 1 mL aliquot was hydrolysed at 100° with 1 mL 12 M HCl for 2 hr to release mandelic acid from any conjugates; the compound is stable under these conditions. After cooling, the mixture was extracted with ethyl acetate, an ethyl ester-trimethylsilyl derivative was prepared and mandelic acid was measured by gas chromatography, based on previously described methods [9, 10].

Results and Discussion

Mandelic acid excretion after administration of the compounds tested is shown in Table 1. Phenylethanolamine was the only amine precursor to yield a significant amount of mandelic acid, and the yield was surprisingly low, in that it is generally considered to be the immediate precursor of mandelic acid. This may be explained by the finding of Edwards [11] that phenylglycol, the reduction compound deriving from the intermediate aldehyde, is a major metabolite of phenylethanolamine in rats.

The increase in mandelic acid excretion after administration of phenethylamine was quantitatively very low, which suggests that the formation of mandelic acid following decarboxylation of phenylalanine is at best an inefficient pathway, probably contributing little to the observed increase in mandelic acid excretion following administration of phenylalanine, which is itself a poor precursor under these conditions. The findings of Blau *et al.* [8] indicate that its formation does not reach a high level until the liver concentration of phenylalanine is above 30 mM; the concentration is unlikely to have approached this level during these experiments.

There was, at best, a marginal increase in formation of mandelic acid following the administration of phenylacetic

Table 1. Excretion of mandelic acid after i.p. injection of potential precursors

Compound administered	Dose (mmol/kg)	Dose/animal (μmol)	Mandelic acid excreted* (% of dose)
β-Phenylethylamine	0.41	74	0.35 (0.25–0.50)
Phenylethanolamine	0.73	155	5.1 (4.6–6.5)
Phenylacetic acid	0.74	129	0.1 (0–0.2)
Phenylpyruvic acid	0.61	113	1.1 (1.0–1.1)
	3.05	550	0.23 (0.19–0.27)
	10.7	1525	0.16 (0.12–0.19)
Phenylpyruvic acid + carbidopa (60 mg/kg)	0.61	112	0.5 (0.25–0.65)
L-Phenylalanine	0.61	109	0
	3.03	545	0.06 (0.05–0.10)
	10.6	1515	0.005 (0–0.015)
D-Phenylalanine	3.03	555	0.19 (0.11–0.28)

* Values are corrected for endogenous levels: 0.2 (0.02–0.5) μmol/24 hr.

acid, suggesting that α-hydroxylation is a poor pathway for its formation.

The best acidic precursor was found to be phenylpyruvic acid. This is not unexpected, since *p*-hydroxyphenylpyruvic acid spontaneously yields a mixture of *p*-hydroxymandelic and *p*-hydroxyphenylacetic acids *in vitro* [12] (B. L. Goodwin, unpublished results). It seems possible that a similar reaction occurs *in vivo*. The *in vitro* reaction is slow, which suggests that the large amounts observed during the liver perfusion experiments [8] are mainly formed enzymatically. Even so, the formation of mandelic acid from phenylpyruvic acid was quantitatively low, which might have been expected, since reverse transamination of phenylpyruvic acid to phenylalanine is likely to have been the predominant pathway.

In man, the rapid time course for *p*-hydroxymandelic acid formation from L-tyrosine is consistent with *p*-hydroxyphenylpyruvic acid being the immediate precursor [13]; this compound is formed in the catabolism of tyrosine.

Excretion of mandelic acid is lower after phenylalanine administration than after phenylpyruvic acid, again suggesting that phenylalanine is not an obligatory intermediate. Administration of carbidopa, an amino acid decarboxylase inhibitor, in conjunction with phenylpyruvic acid, did not eliminate the formation of mandelic acid, which further suggests that it was formed without prior reverse transamination.

D-Phenylalanine is nearly as good a precursor of mandelic acid as phenylpyruvic acid; this would be expected if phenylpyruvic acid were the immediate precursor of mandelic acid, since the main metabolic pathway of D-amino acids involves oxidation by renal D-amino acid oxidase to the analogous pyruvates [14] (although, additionally, D-phenylalanine may be directly decarboxylated in the rat [15]).

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