Urinary Phenolic Acid Metabolites of Tyrosine

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More than 40 phenolic acids are known to be excreted in the urine of humans ingesting ordinary diets (1). The number can be appreciably reduced by the feeding of a purified diet during the period of urine collection. However, rats continue to excrete several phenolic compounds during starvation; undoubtedly, the endogenous metabolism of aromatic amino acids is involved.

One of the metabolites excreted by rats on a purified diet is p-hydroxyphenylpropionic (phloretic) acid. Recently, a clew as to the origin of phloretic acid was found. Specifically, o-hydroxyphenylpropionic acid was detected in the urine of rats given o-tyrosine by stomach tube. When similar studies were conducted with tyrosine, an increase in the excretion of phloretic acid was observed.

According to Bernhart and Zilliken (2), a 7- to 10-fold increase in the urinary excretion of phenolic acids occurs when rats are fed a diet containing 10% tyrosine with or without the addition of chlortetracycline. p-Hydroxyphenylpyruvic acid, p-hydroxyphenylacetic acid and traces of homogentisic acid were specifically implicated in the phenolic acid fraction.

In this present report it will be shown that p-hydroxyphenylpyruvic, p-hydroxyphenylacetic, p-hydroxyphenyllactic, phloretic, p-coumaric, and p-hydroxybenzoic acids are all urinary metabolites of tyrosine in the rat and rabbit. A suggested scheme of sequential metabolic relationships for these compounds is also presented.

EXPERIMENTAL PROCEDURE

Methods—Collection of the urine and the procedures used for the two-dimensional paper chromatographic detection of the urinary phenolic metabolites have been described previously (3, 4). To facilitate detection of conjugated phenolic compounds, all urine samples were routinely screened by preparing chromatograms of the ether extract of the control, the hydrolyzed ether extract (2 N HCl reflux for 1 hour), ether-extracted urine, and hydrolyzed ether-extracted urine. Frequently, unidentified areas were cut out and eluted from unsprayed chromatograms and various qualitative tests carried out for purposes of identification.

The composition of the diets fed to rats and rabbits has been described previously (5). However, cornstarch was substituted for corn meal in the rabbit diet. All compounds studied were given by stomach tube at a level of 100 mg per rat unless otherwise indicated.

Materials—o-Tyrosine, m-tyrosine, tyrosine, p-hydroxyphenylacetic acid, and p-hydroxybenzoic acid were purchased from commercial sources. Phloretic acid was prepared by hydrogenation of p-coumaric acid in ethanol solution with palladium as a catalyst. The p-coumaric acid was prepared by the method of Vorotko (6). The ethereal sulfate of p-hydroxybenzoic acid was made by treating p-hydroxybenzoic acid with chlorosulfonic acid and chloroform in pyridine (7). p-Hydroxyphenylpyruvic acid was prepared by a method described by Herbst and Shemin (8). A sample of p-hydroxyphenyllactic acid was obtained by hydrogenation of p-hydroxyphenylpyruvic acid with sodium amalgam (9). The chromatographic behavior of these compounds is shown in Table I. All compounds were checked for purity by two-dimensional paper chromatography, and were subjected to the same conditions of acid hydrolysis as the urine fractions in order to distinguish between chemical degradation and metabolic change. The sensitivity of the chromatographic technique used is such that 2 μg of a phenolic acid can be detected in a 5-μg sample of L-tyrosine.

RESULTS

An ether extract of the urine of rats receiving the purified diet described above was subjected to two-dimensional paper chromatography. Three phenolic acids, p-hydroxyphenylacetic, phloretic, and p-hydroxybenzoic, were readily detected. The identity of these compounds was based on their \( R_f \) values in two dimensions, their appearance under ultraviolet (Mineralight), and their colors after spraying with diazotized sulfanilic acid followed by sodium carbonate. The amounts excreted of these three compounds are shown in Table II (control). These data were obtained by visual comparison of the color intensity of spots on the chromatogram containing appropriate aliquots of acid-hydrolyzed urine and known amounts (5 μg or less) of the authentic compounds.

The administration of o-tyrosine to rats by stomach tube (100 mg per rat) led to the detection in the urine of o-hydroxyphenylpropionic and o-hydroxyphenyllactic acids. m-Tyrosine gave rise to m-hydroxyphenylpropionic and m-hydroxyphenyllactic acids. These findings prompted us to investigate the urinary phenolic acid metabolites of tyrosine. The metabolic fate of o-tyrosine and m-tyrosine will be described in more detail in a subsequent report.

When tyrosine itself was given to rats and the 48-hour urine sample was examined chromatographically, distinct increases in p-hydroxyphenylacetic and p-hydroxyphenylpropionic acids were evident when compared with the ether extract of the control urine (Table II). In addition, p-coumaric acid and the ethereal sulfate of p-hydroxybenzoic acid were detected on the chromatogram (Table I).

An understanding was obtained regarding the sequential rela-
The excretion of p-hydroxyphenypropionic acid, p-hydroxybenzoic acid, and p-coumaric acid was increased, whereas the excretion of p-hydroxyphenylacetic acid was decreased and the excretion of p-hydroxyphenyllactic acid was comparable to those obtained for tyrosine, except that in this case the excretion of p-coumaric acid was its ethereal sulfate.

Four of these acids, phloretic, p-coumaric, and p-hydroxybenzoic acid and its ethereal sulfate, were previously encountered and identified as urinary metabolites of naringin (11). Phloretic acid administration resulted in the excretion of p-coumaric acid, the ethereal sulfate of p-hydroxybenzoic acid, and an increase in the excretion of p-hydroxybenzoic acid. In this same report (11) it was also found that the only metabolite of p-hydroxybenzoic acid was its ethereal sulfate.

When p-coumaric acid was administered to rats, large amounts of p-hydroxybenzoic acid and its ethereal sulfate derivative were excreted in the urine. The compound, p-coumaric acid, and its glycone conjugate were also readily detected. Saturation of the double bond in the side chain of the p-coumaric acid would account for the observed increase in the excretion of phloretic acid.

When two antibiotics were added to the basal diet (streptomycin, 23 mg per 100 g and sulfathiazole, 750 mg per 100 g) and fed to rats without added tyrosine, there was no change (decrease) in the normal excretion of phloretic, p-hydroxyphenyllactic, or p-hydroxybenzoic acids. Furthermore, when 10% tyrosine was added to the above antibiotic containing diet, large increases in the excretion of phenolic acids were measured. For example, the ingestion of 1.5 g per rat per day of tyrosine resulted in the minimal excretion of 75 mg of p-hydroxyphenyllactic acid, 75 mg of p-hydroxyphenyllactic acid, 50 mg of p-hydroxybenzoic acid, and 15 mg of p-coumaric acid. Thus, more than 14% (215 mg) of the extra tyrosine fed was excreted in the urine as phenolic acids.

A species difference exists between rats and rabbits, in that the excretion of phloretic acid is not normally detected in the urine of rabbits on a purified diet, whereas it is readily detected in the case of the rat. Both species, however, excrete p-hydroxyphenyllactic acid and p-hydroxybenzoic acid on purified diets containing casein as a protein source. Urine from humans ingesting a high protein diet, is similar to rabbit urine with respect to the absence of phloretic acid and the presence of p-hydroxyphenyllactic and p-hydroxybenzoic acids.

When p-hydroxyphenyllactic acid was administered to rabbits (0.9 g each), an area corresponding to phloretic acid (Table I) was clearly evident. The amount of phloretic acid excreted in the 24 hour urine sample was in excess of 3 mg per rabbit. The amounts of p-hydroxyphenyllactic acid and p-hydroxybenzoic acids were increased in this same urine from control values of 18 and 9 mg to 36 and 45 mg, respectively. An increase in the excretion of p-hydroxyphenyllactic acid was also noted.

When p-hydroxyphenyllactic acid was given to rabbits (0.9 g each), more than 3 mg of phloretic acid were excreted. In the same urine the amount of p-hydroxybenzoic acid including
its ethereal sulfate was increased to 45 mg, but the amount of p-hydroxyphenyllactic acid was not increased (18 mg). Approximately one-fifth of the p-hydroxyphenyllactic acid administered was excreted unchanged.

DISCUSSION

A scheme summarizing the proposed pathways by which tyrosine is converted to p-hydroxyphenyllactic and p-hydroxybenzoic acids is shown in Fig. 1. The interrelationships shown for these compounds are based on the results obtained when the individual compounds were given to rats or rabbits. It should be emphasized that this scheme is simply a working hypothesis which satisfactorily accounts for the observations reported herein (Table II). The directions of the arrows in Fig. 1 indicate only end results, rather than completely balanced reactions.

The conversion of p-hydroxyphenyllactic acid to p-coumaric acid appears to be a new metabolic reaction. Experiments involving radioactive substrates and incubations in vitro are being designed to study in more detail the biological dehydroxylation of a phenolic α-hydroxy acid. Another example of a new metabolic reaction was encountered recently when we discovered the dehydroxylation in vivo of specific phenolic hydroxyl groups (12). Other workers have confirmed this observation (13), but the enzyme system involved has not yet been isolated.

The conversion of tyrosine to phenolic acids does not appear to be dependent on intestinal microorganisms. This was shown by feeding antibiotics (streptomycin and sulfathiazole) to rats with and without extra dietary tyrosine. In neither case did the feeding of antibiotics decrease the excretion of phenolic acids. According to Boscott and Greenberg (14), the administration of aureomycin and phthalysulphathiazole prevented the urinary excretion of phloretic acid but did not significantly alter the levels of the other urinary phenolic acids. The results reported by Bernhart and Zilliken with aureomycin also indicate that the increased phenolic acid excretion by rats fed tyrosine is independent of the influence of intestinal microorganisms (2). The fact that rats starved for more than 72 hours continue to excrete phloretic, p-hydroxyphenyllactic, p-coumaric, and p-hydroxybenzoic acids in the urine is further evidence for the view that these phenolic acids are of endogenous origin. Incidentally xanthurenic acid, a metabolite of tryptophane, is also excreted by starved rats as would be expected when body protein is being metabolized for energy.

The established role of tyrosine involving its conversion to 3,4-dihydroxyphenylalanine, melanin, adrenaline, and homogentisic acid has been intentionally omitted from this scheme, since none of these compounds or their intermediates were detected in the urine by the methods used in this study. From the suggested scheme shown in Fig. 1, it is now possible to account for the occurrence of p-hydroxyphenyllactic, phloretic, p-hydroxyphenyllactic, p-coumaric, and p-hydroxybenzoic acids in the urine of rats maintained on a purified, nutritionally adequate diet. These urinary metabolites of tyrosine, with the exception of phloretic and p-coumaric acids, are also found in rabbit and human urine. However, when rabbits were given either p-hydroxyphenyllactic acid or p-hydroxyphenylpyruvic acid by stomach tube, phloretic acid was readily detected on the chromatogram of the ether extract of the urine together with a 5-fold increase in the excretion of p-hydroxybenzoic acid. Thus, it appears that the rabbit and perhaps man are more efficient than the rat in the conversion of phloretic acid and p-coumaric acid to p-hydroxybenzoic acid by β-oxidation under normal conditions, when the capacities of the enzyme systems involved are not overloaded. Similarly, in a previous report, a species difference was observed, in that m-hydroxyhippuric acid was primarily excreted by humans, whereas the rat preferentially excreted m-hydroxyphenylpropionic acid after the ingestion of chlorogenic acid (3).

Homogentisic acid was not detected in the urine in these experiments even when its immediate precursor, p-hydroxyphenylpyruvic acid, was administered to rats. However, this does not mean that homogentisic acid was not formed; rather that it was rapidly metabolized to other products no longer detectable by the procedures used here. Supporting this view is the finding that only traces of homogentisic acid were detected in the urine when a large single dose of it (100 mg per rat) was given. Bernhart and Zilliken also concluded that only small amounts of homogentisic acid were excreted, as compared to the total phenol and phenolic acid fractions when rats were fed up to 10% tyrosine in the diet (2).

The p-hydroxybenzoic and p-hydroxyphenyllactic acid pathways of tyrosine metabolism described here are of fundamental biochemical interest for at least three reasons: (a) a satisfactory explanation for the occurrence of these phenolic acids in the urine of normal animals is now available; (b) at least one-seventh of the extra tyrosine ingested by normal rats was excreted via these two pathways; and (c) in the presence of defects in the metabolism of phenylalanine and tyrosine via homogentisic acid, the newly proposed pathways may assume an even more significant role. Regarding the latter point (c), a report by Aterman et al. (15) indicates that such is the case. These workers found that, "in contrast to normal animals, scorbatic guinea pigs excreted in the urine p-hydroxyphenyllactic acid as well as smaller amounts of p-hydroxyphenylacetic acid."

Abolishment of the urinary excretion of p-hydroxyphenyllactic, p-hydroxyphenylacetic, and phloretic acids by feeding chlorozone in the diet of rats was reported by Boscott and Greenberg (14). Since our attempts to confirm this effect were negative, we therefore do not concur with the suggestion that the urinary excretion of p-hydroxyphenyllactic acid may be regarded as an early indication of suboptimal intake of ascoboric acid.

SUMMARY

Metabolic pathways involving the conversion of tyrosine to p-hydroxyphenyllactic acid and p-hydroxybenzoic acid in the animal body are presented. At least four urinary phenolic acid intermediates have been identified, including p-hydroxyphenylpyruvic, p-hydroxyphenyllactic, phloretic, and p-coumaric.
Phloretic acid was readily detected in the urine of rats, but not in the urine of rabbits unless p-hydroxyphenylpyruvic acid was given, indicating a more efficient conversion of phloretic acid to p-hydroxybenzoic acid by the latter species. A new metabolic reaction involving the conversion of p-hydroxyphenyllactic acid to p-coumaric acid is suggested by this work.

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