Elimination of Tyramine in Protein Energy-Deficient Wistar Rats

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The in vivo biliary elimination of the dietary amine tyramine was studied in protein energy-deficient rats. Tyramine was administered intraperitoneally at single doses of 1200 μg/kg body weight to Kwashiorkor Wistar rats following cannulation of the bile duct. Excretory products in 24-h samples of feces and urine from both Kwashiorkor and normal cannulated rats, who were similarly dosed with tyramine, were also examined. The feces and urine samples were subjected to spectrophotometric and paper chromatographic analyses that showed the presence of tyramine, *p*-hydroxyphenylethanol, *p*-hydroxyphenylacetic acid, *p*-hydroxyphenaceturic acid, and *N*-acetyltyramine. Thin-layer chromatography of the bile from test rats followed by ultraviolet analysis of the chromatogram confirmed the presence of unchanged tyramine and the metabolite *p*-hydroxyphenylethanol. The results confirm that the bile is an excretory route for the amines and also that nutrition plays an important role in the pharmacological or toxic action of ingested foreign chemicals or compounds.

Keywords: *Tyramine; drug metabolism; protein-energy deficiency; bile duct; liver*

INTRODUCTION

Dietary amines (for examples, trimethylamine, tyramine, histamine, and octopamine) arise from the microbiological decarboxylation of choline, tryptophan, tyrosine, and other amino acids present in foodstuff residues in the intestinal tract. Similarly, the diamines putrescine and cadaverine are formed by microbiological decomposition of basic amino acids of the diet (for examples, ornithine and lysine; Boyland and Manson, 1963).

Tyramine is a sympathomimetic amine that is present in considerable quantities in some Nigerian foods and beverages, in yeast extract (marmite), and in plant materials. Tyramine is normally detoxified by the monoamine oxidase present in the intestine and liver to yield *p*-hydroxyphenylethanol, *p*-hydroxyphenylacetic acid and its glycine conjugate *p*-hydroxyphenaceturic acid, and *N*-acetyltyramine (Nakajima and Sanon, 1964). Most medicinal plants contain large quantities of these dietary amines (Maduagwu and Uhegbu, 1986). Patients treated with monoamine oxidase inhibitor drugs, like tranylcypromine and trifluoperazine, are vulnerable to the toxic action of this dietary amine because its metabolism is blocked. Indeed, cases of hypertensive attacks resulting from eating cheese or marmite have been reported (Blackwell and Marley, 1966).

In Nigeria, where a large percentage of the population dwells in rural areas where people are not exposed to orthodox medicines, medicinal plant extracts (herbal medicines) are consumed in large quantities to treat most ailments. The nutritional status of this rural group of people is always below standard; many are malnourished and some of the elderly group are hypertensive. Hence, the amount of dietary amines in the extracts consumed by these people may constitute a potential hazard to public health.

To examine this potential health hazard, we studied the elimination of tyramine in normal and protein energy-deficient rats. In rats fed a normal diet, tyramine is mainly metabolized to *p*-hydroxyphenylethanol, *p*-hydroxyphenylacetic acid, *p*-hydroxyphenaceturic acid, and *N*-acetyltyramine, and the toxicity of tyramine is due entirely to the inhibition of the activity of the monoamine oxidase enzyme. The elimination pattern of tyramine in the bile of protein energy-deficient Kwashiorkor rats, an elimination route for foreign compounds that has not previously been considered with regard to tyramine and protein energy deficiency, was examined in an attempt to mimic what might be happening in rural Nigerians with poor nutritional status and severe caloric deficiency consume medicinal plant extracts containing tyramine.

MATERIALS AND METHODS

Chemicals. Pure tyramine 4-(2-aminoethyl)phenol, 4-hydroxyphenylacetic acid, 4-hydroxyphenylethanol, and 4-hydroxyphenaceturic acid were purchased from Sigma Chemical (St. Louis, MO), and *N*-acetyltyramine was purchased from Parke-Davies (Munich, Germany). All solvents used were of analytical grade and supplied by May and Baker Ltd. (Degenham, England) or Searle Company, Hopkin and Williams (Chadwell Heath Essex, England).

Animals and Diet. Healthy young adult albino rats of the Wistar strain (35−40 g) were obtained from the animal house at the Department of Biological Sciences, Rivers State University of Science and Technology, and kept at room temperature (~28 °C). The rats were divided into two groups of eight. All animals had free access to drinking water, but were starved overnight prior to experimentation. Group one (controls) were fed ad libitum on Pfizer pellets for rats (Pfizer Nigeria Ltd., Ikeja, Nigeria) that contained 21.0% crude protein, 71.5% carbohydrate, 4.0% fiber, and 3.5% oil. The rats in group two (test) were fed ad libitum on a Kwashiorkoregenic diet (Boyd and Carsky, 1969) that contained 3.45% protein (casein), 81.53% carbohydrate (Garri), 8.0% corn oil, 3.0% premixed all vitamin supplement, and 4.0% salt mixture. By the fifth week, the rats in group two showed evidence of Kwashiorkor (i.e., loss of body weight, thinning of hair, reduced liver weight and body weight ratio, and reduced serum albumin).

In all rats, the bile duct was exposed by a midline abdominal incision [rats were anesthetized with Rompum/Ketanest given intraperitoneally (ip) at 0.1 mL/animal]. A polythene cannula was surgically inserted in the bile duct 1 cm from the junction of the duodenum according to the method of Boyland et al. (1961) and Pryor and Slater (1967), and the abdominal slit was sutured.

Treatment. On the 36th day, the test rats (~34 g) were injected ip with tyramine at a dose of 1200 μg/kg. The

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Table 1. Detection of Tyramine and Its Metabolites in Urine, Bile, and Feces of Control and Test Rats[a]

	urine		bile		feces	
compound	control	test	control	test	control	test
tyramine	ND	+	ND	+	ND	+
p-hydroxyphenylethanol	+	ND	ND	+		
p-hydroxyphenylacetic acid	+	ND	ND	+		
p-hydroxyphemaceturic acid	+	ND	ND	+		
N-acetyltyramine	+	ND	ND	+		

[a] + = presence; ND = not detectable.

tyramine dose was administered to animals in ~0.2 mL aliquots of a 200-μg/mL solution of tyramine in physiological saline (0.9% NaCl). The control rats weighed ~40 g and were given the same 1200-μg/kg dose of tyramine as the test rats, each receiving a volume of ~0.25 mL.

The rats in both groups were kept in metabolism cages, and 24-h urine and feces samples were collected into beakers containing a saturated aqueous mercuric chloride to prevent bacterial decomposition of metabolites. The urine and feces samples were frozen until analyzed. Bile exudate from the cannulated rats in both groups was collected initially, at 30, 60, and 120 min, and thereafter at hourly intervals until the animal was moribund.

Analysis of Urine, Feces, and Bile Samples. *Chromatography.* Each sample of urine (0.1 mL) was chromatographed as a band on Whatman no. 1 paper strips (5 cm wide) next to authentic samples with an *n*-butan-1-ol:95% ethanol: water (4:1:2, v/v/v) solvent (Brenmner and Kenten, 1951). The strips were chromatographed for 14 h, removed from the tanks, and dried in a stream of cold air. Tyramine and its metabolites were detected with 0.2 μg of ninhydrin in a mixture of 99 mL of redistilled *n*-butanol and 1 mL of glacial acetic acid according to the method of Mann and Saunders (1975). A yellow-to-blue change in color identified the substances.

Quantitative examination of the bile exudate was carried out on TLC plates (0.5 mm silica gel) developed in the solvent *n*-butan-1-ol:95% ethanol:water (4:2:1) following preliminary investigation of the bile by two-dimensional TLC in the same solvent system and in ethyl acetate:acetone:water (4:5:1) to ensure that each UV fluorescent spot observed migrated as a single band. An aliquot of 0.1 mL of bile diluted twice with 0.1 M orthophosphoric acid (1:2, w/v) was streaked on TLC plates as the authentic samples. Each fluorescent band in the test chromatogram was matched with those in the control chromatogram.

Tyramine concentration in bile, urine, and feces samples was determined spectrophotometrically according to the method of Pribyl and Nedbalkova (1967). As a control, ordinary rat bile was spiked with pure tyramine and treated in the same manner as the test bile, and the concentration of tyramine was determined spectrophotometrically. Recoveries of tyramine in bile (0.1 mL) to which standard aqueous solutions of tyramine at 2, 5, 10, and 20 μmol/L were added were ~98%, using 1 mL for the assay.

RESULTS

No traces of tyramine were observed in the feces of the control rats. Tyramine was also not observed in the urine of the control rats, but the metabolites of tyramine were detected (Table 1). In contrast, tyramine was detected in the urine of test rats, but tyramine metabolites were not detected (Table 1). Tyramine and its metabolites were not detected in the bile of control rats but were seen in the bile of the test animals, as shown in Table 1. The concentration of tyramine in the bile of test rats decreased with the time of collection (Table 2), and as little tyramine as 0.75 μg/100 mL was detected in the feces (Table 3).

The observation in the urine of control rats of only metabolites of tyramine suggests that the tyramine was

Table 2. Concentration of Tyramine in Bile of Malnourished Rats[a]

time, min	value of bile excreted, mL	absorbance	tyramine concn, μg/100 mL
30	10.60	0.82	3.15
60	7.50	0.77	2.25
120	6.90	0.73	1.95
180	6.50	0.69	1.56
240	4.60	0.64	1.35
300	3.75	0.58	1.10
360	3.25	0.52	0.96
420	2.75	0.46	0.75

[a] Average of three determinations from eight rats.

Table 3. Concentration of Tyramine in Feces of Malnourished Rats[a]

sample	absorbance	tyramine concn, mg/100 g
feces	0.46	0.75

[a] Average of three determinations from eight rats.

effectively degraded enzymatically. In contrast, the observation in the urine of test rats of tyramine only and no metabolites suggests that tyramine was eliminated into the bile because it was not effectively metabolized in the gut.

Tyramine and its metabolites were not detected in the bile of the control rats, but both were detected in the bile of test rats (Table 1). This result indicates that in the control rats, tyramine was adequately metabolized, but in the test rats, it was eliminated into the bile duct for metabolism. The subsequent decrease in the tyramine concentration with time also suggests that the tyramine was released into the bile duct.

The TLC chromatogram of bile samples (Table 1) shows that the bile from test rats contain mostly tyramine and its metabolites, indicating that the nutritional state of the animal is not adequate for metabolism of the amine.

DISCUSSION

The detection of intact tyramine in the bile of malnourished rats given ip doses of the pure compound and the identical migration of the bile component as a single spot on TLC plates with the pure compound adequately confirm that the bile is an excretory route for the amines. This finding is in agreement with the universally recognized prerequisites for the biliary excretion of xenobiotics, namely high polarity and a fairly large molecular weight (Brawer, 1959; Williams, 1965). The biliary excretion of intact tyramine and its metabolites in the control rats is a detoxication mechanism, judging from the biochemical effect of amine toxicity and poisoning.

The observation that most of the metabolites shown in the urine of the control rats were not detected in the urine of the test rats is evidence that the results of alterations in tyramine metabolism may be due to protein energy deficiency. The inability of the test rats to degrade the tyramine may be due to low monoamine oxidase enzyme activity, which catalyses this reaction. These results tend to agree with the findings of Perry et al. (1966) that a change in the quality or quantity of dietary protein causes an alteration in the rates of metabolism of many xenobiotics by the mammalian liver. Also, alterations in the dietary protein content have been shown to influence mixed function oxidase (MFO) activities as well as enzymes catalyzing conjuga-

tion reactions (Wood and Woodcock, 1974). It has also been reported that protein deficiency causes an increase in the activity of UDP-glucuronyl transferase in rat liver, despite a decrease in the MFO activities (Woodcock and Wood, 1971).

The alterations in tyramine metabolism due to protein deficiency as well as severe caloric restriction confirm that nutrition plays an important role in the pharmacological or toxic action of ingested foreign chemicals (Campbell and Hayes, 1974). Hence, it is of great importance that the nutritional status of patients ingesting medicinal plant extracts for pharmacological purposes be adequate.

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