Renal Amino Acid Reabsorption in Hyperphenylalaninemic Monkeys Infused with β-2-Thienylalanine[1] (34944)

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β-2-Thienylalanine (α amino-thiopheneproprionic acid), first synthesized by Barger and Easson in 1938 (1), is a potent inhibitor of phenylalanine metabolism in bacteria as shown by the bacterial-inhibition test developed by Guthrie (2) and employed in the screening of human neonates for the detection of phenylketonuria. It has been shown by Godin and Dolan (3) to affect phenylalanine metabolism in rats. When administered to mammals, β-2-thienylalanine is largely excreted by the kidney. The present study examines the effect of β-2-thienylalanine on renal excretion of phenylalanine and other amino acids in the monkey.

Methods. Four healthy, adult, male rhesus monkeys (*Macaca mulatta*) which weighed 6.26–8.75 kg were used. The animals were usually fed Purina Monkey Chow, fruits, and vitamins but were fasted for 8 hr before and throughout the experiment. They were lightly anesthetized with intravenous 5-allyl-5(1-methyl butyl)-2 thiobarbiturate (Surital, Parke-Davis, Co.) 30 mg/kg and an intravenous drip of 2.5% dextrose plus 0.45% sodium chloride was inserted. A collector was attached by tube to the external genitalia so that urine could be collected in a plastic bottle kept in ice. A few thymol crystals were placed in the plastic bottle to prevent bacterial growth. A control blood sample was drawn.

Each monkey was strapped into a special metabolic chair and when full consciousness was regained a control urine specimen was obtained. The animal was infused with 200 mg/kg phenylalanine[2] intravenously as a stat dose. Two monkeys (B56 and E27) were started on an infusion of phenylalanine in physiological saline at concentration of 2.0 g/100 ml and at a rate to deliver 2 g/kg/day. After 24 hr the infusion was changed to 2.0 g/100 ml phenylalanine plus 5 g/100 ml β-2-thienylalanine[3] in saline at the same rate. In the other two monkeys (D43, E65) the reverse order was used. The combination of phenylalanine and β-2-thienylalanine was infused for the first 24 hr followed by phenylalanine alone.

At the end of each 24-hr urine collection suprapubic pressure was applied to empty the bladder completely. During each 24-hr period three blood samples were drawn; at 3, 18, and 24 hr after the infusion was started. Acidified and filtered urine and sera samples (4) were analyzed on a Beckman-Spinco amino acid analyzer by the method of Spackman, Stein, and Moore (5) for neutral and acidic amino acids. β-2-Thienylalanine is clearly separated from phenylalanine by this method, appearing as a peak before tyrosine. Twenty-four-hour excretion and standard clearances were calculated.

Results. The 24-hr excretion and clearance of tyrosine, phenylalanine, and β-2-thienylalanine are shown in Table I. All monkeys increased their excretion of both phenylalanine and tyrosine when receiving β-2-thienylalanine. There was a marked variation in excretion of these amino acids from monkey to monkey but the increase in excretion of phenylalalanine and tyrosine whilst being infused with β-2-thienylalanine as well as

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[2] L-Phenylalanine was purchased from Ajinomoto Corporation, Japan.

[3] β-2-Thienylalanine was purchased from Nutritional Biochemicals Corporation.

TABLE I. The Excretion and Clearance of Phenylalanine and Tyrosine Before and After Thienylalanine Added to Infusion.[a]

	Monkey no.											
	B56			D43			E27			E65		
Amino acid	1	2	3	1	2	3	1	2	3	1	2	3
Excretion in mg/24 hr												
Tyrosine	91.7	227	2.47	102.0	97.4	0.95	59.9	398	6.65	16.8	56.7	3.37
Phenylalanine	216.0	521.0	2.42	69.9	116	1.66	172	375	2.17	19.1	31.9	1.67
Thienylalanine	—	955	—	—	95.6	—	—	508	—	—	1480	—
Clearance in ml/min												
Tyrosine	0.49	1.42	2.90	0.194	0.838	4.32	0.159	0.875	5.5	0.197	0.566	2.82
Phenylalanine	0.29	0.89	3.07	0.0847	0.175	2.07	0.208	0.771	2.71	0.071	0.162	2.28
Thienylalanine	—	4.82	—	—	0.618	—	—	4.65	—	—	20.0	—

[a] Column 1 indicates values while phenylalanine 2.0 g/kg/day infused; column 2, values while phenylalanine 2.0 g/kg/day and thienylalanine 0.5 g/kg/day infused. Column 3 is the ratio of column 2 to column 1. The ratio is significant for phenylalanine excretion ($p < .005$) but not for tyrosine excretion. The clearance ratios are significant for both phenylalanine ($p < .010$) and tyrosine ($p < .005$).

phenylalanine was approximately double that while on phenylalanine alone. The excretion of phenylalanine is expressed as a ratio derived by dividing excretion while on both phenylalanine and β-2-thienylalanine by the excretion while on phenylalanine alone. The ratio was significant for phenylalanine ($p < .005$) but not for tyrosine ($p < .20$). The clearance values for phenylalanine and tyrosine show an even greater ratio which was statistically significant (phenylalanine $p < .010$, tyrosine $p < .005$).

The excretion and clearance of other neutral and acidic amino acids show no consistent alterations when β-2-thienylalanine was added to the infusion. Detailed clearance and excretion values of neutral and acidic amino acids before and after β-2-thienylalanine are shown in Tables III and IV.

Control serum and urine amino acids are shown in Table II. The urinary amino acids were determined from a single untimed sample of urine, rather than stressing the animal by further restriction and fasting. A specific aminoaciduria was not found in the monkeys prior to the experiment, and it is unlikely that a mild generalized renal aminoaciduria would have affected the results. The normal blood levels exclude an overflow aminoaciduria.

Discussion. In the human phenylketonuric patient blood levels of phenylalanine are usually elevated at least 20-fold. Even at levels of this magnitude renal tubular reabsorption of phenylalanine is almost complete (6). Berry and her co-workers (7) were able to show an increase in excretion of phenylalanine in phenylketonurics, using paper chromatography. There was overlap with excretion levels of normal children. No clearance studies were performed, however. It is unlikely that a significant percentage of the filtered load of phenylalanine was not reabsorbed in the phenylketonuric patients.

Competition for renal tubular reabsorption between amino acids is well established (8–10) but the only study on the competition between β-2-thienylalanine and phenylalanine in mammalian kidney by Godin and Dolan (3) showed an increase of ^{14}C derived

TABLE II. Control Urinary and Serum Amino Acid Levels.[a]

	Monkey no.							
	B56		D43		E27		E65	
Amino acid	Urine	Serum	Urine	Serum	Urine	Serum	Urine	Serum
Threonine	1.53	2.24	0.76	1.45	0.42	2.23	0.21	1.28
Serine	0.87	2.21	0.49	3.28	0.27	4.16	0.15	0.81
Glutamine	0.79	7.83	1.61	17.3	2.22	16.4	1.70	17.1
Glutamic acid	0.42	2.59	0.63	1.33	1.41	0.89	0.16	1.79
Glycine	0.11	4.14	1.68	4.15	2.89	4.39	1.84	4.04
Alanine	0.25	4.20	0.39	2.71	0.36	4.00	0.36	2.73
Valine	—	3.06	Trace	1.42	—	2.39	Trace	2.83
Methionine	—	1.40	0.35	0.77	0.10	0.65	0.89	0.59
Isoleucine	—	1.52	0.10	0.73	0.18	1.19	0.45	0.87
Leucine	—	2.94	0.11	1.75	0.12	2.30	—	1.71
Thienylalanine	—	—	—	—	—	—	—	—
Tyrosine	Trace	1.37	Trace	2.02	Trace	1.06	Trace	1.33
Phenylalanine	0.73	2.48	Trace	1.52	0.09	1.51	Trace	1.30

[a] Serum and urine concentration of amino acids in milligrams per 100 ml while on iv saline and dextrose. Trace indicates present but not measurable; (—) indicates not detected.

from phenylalanine in the urine of rats with simultaneous injection of β-2-thienylalanine. Most of this ^{14}C was found as phenylalanine metabolites, only 29% being phenylalanine itself.

In the present study, three monkeys showed significant quantities of phenylalanine and tyrosine in their urine while being infused with phenylalanine alone. The excretion and clearance of phenylalanine and tyrosine doubled in all four monkeys when β-2-thienylalanine was infused. This suggests a marked competition between β-2-thienylalanine and phenylalanine for tubular reabsorption. The degree of competition could be more accurately assessed had concurrent

TABLE III. Excretion of Neutral and Acidic Amino Acids Before and After Thienylalanine Added to Infusion in mg/24 hr.[a]

	Monkey no.											
	B56			D43			E27			E65		
Amino acid	1	2	3	1	2	3	1	2	3	1	2	3
Threonine	20.3	30.4	1.50	1.84	13.8	7.50	14.8	36.6	2.47	4.82	0.407	0.19
Serine	10.5	16.8	1.59	4.80	9.66	2.01	7.73	45.04	5.83	4.57	3.46	0.76
Glutamine	96.0	70.3	0.73	11.0	15.8	1.43	28.4	56.8	2.00	31.3	4.44	0.14
Glutamic acid	19.3	8.59	0.44	4.10	14.12	3.44	6.35	7.20	1.13	29.2	22.6	0.77
Glycine	24.2	11.4	0.47	14.9	35.6	2.39	27.5	7.20	0.26	37.2	45.8	1.23
Alanine	6.2	2.5	0.40	1.95	0.36	0.18	3.12	4.63	1.48	15	83.5	5.57
Valine	0.0	0.0	—	3.93	2.34	0.59	—	3.98	—	6.03	1.73	0.29
Methionine	—	4.4	—	1.62	—	—	—	18.3	—	—	1.06	—
Isoleucine	2.54	4.46	1.75	6.72	—	—	0.859	6.94	8.08	—	1.27	—
Leucine	2.54	2.4	0.94	1.90	—	—	0.603	0.66	1.09	—	0.511	—

[a] Column 1 indicates excretion while phenylalanine 2.0 g/kg/day infused; column 2, excretion while phenylalanine 2.0 g/kg/day and β-2-thienylalanine 0.5 g/kg/day infused. Column 3 is the ratio of column 2 to column 1; — indicates amino acid not detected in urine. There is no statistically significant difference in the excretion of these amino acids when β-2-thienylalanine is added to the infusion.

TABLE IV. Clearance in Milliliters per Minute of Neutral and Acidic Amino Acids Before and After Thienylalanine Added to Infusion.[a]

	Monkey no.											
	B56			D43			E27			E65		
Amino acid	1	2	3	1	2	3	1	2	3	1	2	3
Threonine	1.57	1.32	0.84	0.06	0.52	8.67	0.83	1.93	2.32	0.28	0.39	1.39
Serine	0.49	0.59	1.20	0.14	0.24	1.71	0.19	1.73	9.10	0.21	0.16	0.76
Glutamine	0.71	0.46	0.65	0.14	0.07	0.50	0.24	0.65	2.71	0.16	0.03	0.19
Glutamic acid	0.40	0.30	0.75	0.38	1.31	3.45	0.30	0.72	2.4	3.65	2.55	0.70
Glycine	0.69	0.30	0.43	0.37	0.76	2.05	0.59	0.20	0.34	1.08	1.38	1.28
Alanine	0.19	0.07	0.37	0.03	0.01	0.33	0.08	0.12	1.50	0.44	2.97	6.75
Valine	0	0	—	0.06	0.02	0.33	—	.10	—	0.06	0.06	1.00
Methionine	—	0.43	—	0.08	—	—	—	3.28	—	—	0.20	—
Isoleucine	0.23	0.24	1.04	0.18	—	—	0.05	0.35	7.00	—	0.08	—
Leucine	0.13	0.10	0.77	0.06	—	—	0.02	0.02	1.00	—	0.02	—

[a] Column 1 indicates clearance while phenylalanine 2.0 g/kg/day infused; column 2, excretion while phenylalanine 2.0 g/kg/day and β-2-thienylalanine 0.5 g/kg/day infused. Column 3 is the ratio of column 2 to column 1; — indicates amino acid not detected in urine. There is not a statistically significant difference in the excretion of these amino acids when β-2-thienylalanine is added to the infusion.

inulin or creatinine clearance studies been performed. However, the values obtained and the statistical analyses of the results clearly indicate significant competition between β-2-thienylalanine on the one hand and phenylalanine and tyrosine on the other.

Other amino acids were not consistently affected. Thus, there appears to be a specific mechanism for phenylalanine and tyrosine reabsorption in the monkey kidney which is inhibited by β-2-thienylalanine.

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