Prolinuria: A New Renal Tubular Defect in Transport of Proline and Glycine

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During a screening program for aminoaciduria, a previously not described disorder of renal tubular transport of amino acids was found in two mentally retarded infants. Urinary analyses of amino acids revealed an excessive excretion of both proline and glycine with a normal range of excretion of the other amino acids. Proline and glycine in sera from the two patients were found to be of normal or subnormal levels. Renal clearance rates of proline and glycine were highly elevated in the patients. It was, therefore, suggested that hyperprolinuria associated with hyperglycinuria observed in the patients was of renal type. Genetic aspects for renal transport of proline and glycine were discussed.

During a screening program for aminoaciduria, two mentally retarded infants were found to excrete large amounts of proline and glycine into urine. A detailed examination of aminoaciduria indicated that hyperprolinuria and hyperglycinuria observed in these patients were of renal type. The present disorder was, therefore, thought to be a previously not described entity in which renal transport of proline and glycine was defective.

CLINICAL FINDINGS

Case I, T.Y.: A 9-month-old girl was admitted into our Clinic on May 22, 1964, with a chief complaint of mental retardation. She was born after an uncomplicated, full-term pregnancy and spontaneous delivery with a weight of 3,110 g. Her neonatal period was uneventful. The parents were healthy and not consanguineous. There was a sibling who was living and well.

The mother noticed that the patient might be backward in mental development at the age of 5 months. The patient had no history of convulsions or severe illness. Because of mental retardation, the patient was referred to our Clinic at the age of nine months.

多田啓也、森川利夫、安藤敏幸、吉田稔男、皆川明文

Physical examination on admission revealed a moderately nourished girl with an apathetic countenance and reddish-brown hair. Her weight, height and circumference of the head were within normal limits. She appeared to take no notice of her surroundings. She did not grab or reach after objects, hold up her head, roll over or sit alone. She was able to follow the light and to react to auditory or painful stimuli. The pupils reacted to light and the fundi showed no abnormalities. Moderate strabismus was noticed. The lung and heart were clear to auscultation. Neither the liver nor the spleen was palpable. The extremities were somewhat rigid and all the deep tendon reflexes were intact. No pathological reflexes were elicited.

Laboratory findings: Routine blood studies and determinations of chemical constituents in serum, including protein and its electrophoretic pattern, electrolytes, lipids and non-protein nitrogen, were within normal limits. Liver function tests also were normal.

The urine was clear with an acid reaction and negative for protein, sugar, acetone, bilirubin or phenylpyruvic acid. The sediment was unremarkable.

X-ray films of the skull, chest, abdomen and extremities revealed no abnormalities.

Intravenous pyelography indicated no abnormalities.

A lumbar puncture showed transparent fluid which gave $\pm$ Pandy test and contained 3 white cells per cmm.

Chromosomal analysis showed no abnormality both in number and in configuration.

Electroencephalographic findings: Resting record exhibited an occurrence of HVS (3-4 cycles per second) in all areas. Sleeping record revealed a dysrhythmia but neither paroxysmal discharge nor asymmetry was detected.

Fig. 1. Histological findings of the kidney from Case I.

Pneumoencephalographic findings showed a cerebrocortical atrophy.

Histological findings of the kidney biopsied revealed normal development comparable to the age and no pathologic findings were found (cf. Fig. 1). The thin-layer chromatography of urinary amino acids showed an increase in proline and glycine (cf. Fig. 2).

This specific pattern of aminoaciduria was constantly found on repeated examinations of urine specimens taken during her 6 months' hospitalization and of the specimen taken at the age of 18 months after the discharge.

Case II, K.S.: A 4-month-old boy was admitted to our Clinic because of developmental retardation. He was born, weighing 1,520 g, after nine months' pregnancy and spontaneous delivery. He was nursed in an incubator for a month. His neonatal period was uneventful. The parents were healthy but consanguineous (first cousins). There was no sibling. On admission at the age of 4 months, physical examination showed a small infant whose size corresponded to that of a 2-month-old infant. He could not hold up his head yet. He was able to follow the light and to react to auditory or painful stimuli. Fundoscopic examination revealed no abnormalities. The remainder of the physical examination was unremarkable.

Laboratory findings: Routine examinations for blood or chemical constituents were within normal limits. Liver function tests also were normal. Urinalysis gave no abnormalities. X-ray examinations revealed no pathologic change. Intravenous pyelography revealed no abnormalities. Normal cerebrospinal fluid was obtained by lumbar puncture.

Electroencephalographic findings: Sleeping record showed a slight dysrhythmia and sporadic sharp and wave complexes.

Pneumoencephalography exhibited a finding of cerebrocortical atrophy.

Fig. 2. Thin-layer chromatograms of urinary amino acids in our own patients.

Key: 1) Glycine, 2) Proline, 3) Alanine, 4) Serine, 5) Glutamine, 6) Histidine, 7) Glutamic acid 8) β-Aminoisobutyric acid

The thin-layer chromatography of urinary amino acids showed a remarkable increase in proline and glycine (cf. Fig. 2). These findings were repeatedly confirmed in the different urine specimens taken during the period of 4 to 12 months of his age.

INVESTIGATION OF THE AMINOACIDURIA

In order to clarify whether prolinuria and glycinuria found in these patients are of "overflow" type or of "renal" type, the following investigations were carried out.

Quantitative estimation of proline and glycine were made by Troll and Lindsley's method¹ and Alexander *et al.*'s,² respectively. Results were shown in Table I. Urinary excretion of proline and glycine was remarkably increased in both patients as compared with that in normal infants. Serum levels of proline and glycine were, however, normal or subnormal in both patients. Table II showed serum amino acid pattern determined by the use of Automatic Amino Acid Analyzer (Beckman-Spinco type) according to the standard procedure of

TABLE I. Serum Levels and Urinary Output of Proline and Glycine

	Serum levels (mg/100 ml)		Urinary output (mg/kg/day)		
	Proline	Glycine	Proline	Glycine	Hydroxyproline
Case I (T.Y.)	0.93-4.0	1.23-1.70	4.67-5.22	18.4-18.8	0.42
Case II (K.S.)	2.50-4.35	1.50-3.20	3.22	7.9-17.0	1.69
Normal range (20 cases)	2.0-5.0	1.5-3.5	0.25-0.71	1.31-3.96	0.09-0.86

TABLE II. Serum Amino Acid Levels of the Patients (μ moles/l)

Amino acids	Case I	Case II	Normal range*	
			Average (20 cases)	Range
Aspartic acid	7	10		Trace- 36
Threonine	53	61	58 $\pm$ 16	31- 88
Serine*	286	262	246 $\pm$ 42	187-328
Glutamic acid	54	63	93 $\pm$ 30	45-149
Proline	87	102	155 $\pm$ 44	88-236
Glycine	139	188	235 $\pm$ 57	164-386
Alanine	268	285	313 $\pm$ 112	146-552
Valine	159	172	159 $\pm$ 42	80-245
Isoleucine	45	53	46 $\pm$ 12	21- 72
Leucine	83	98	88 $\pm$ 15	60-117
Tyrosine	37	42	42 $\pm$ 13	20- 69
Phenylalanine	47	49	46 $\pm$ 12	30- 69
Histidine	26	35	36 $\pm$ 14	21- 68
Lysine	139	172	156 $\pm$ 57	75-256
Arginine	20	28		Trace- 41

* The value for serine was expressed as the sum of serine and glutamine, because both fractions were not sharply separated.

Spackman *et al.*⁴ The levels of serum proline and glycine were below normal range in Case I and they were of lower value within normal range in Case II, while the other amino acids were maintained in the middle level of the normal range in both cases.

These results suggest that the excessive output of proline and glycine observed in these patients may be not of overflow type due to a block in intermediary metabolism but of renal type due to a defect in renal tubular reabsorption of the amino acids in question.

Therefore, the renal clearance-rates of proline and glycine were determined in the fasting state by the methods of Cusworth and Dent.⁵ For comparison, the clearance-rate of tryptophan was simultaneously determined. The results obtained were shown in Table III. Both patients showed markedly high rates of renal

TABLE III. Renal Clearance Rates of Proline and Glycine (ml/min/1.73m²)

	Case I	Case II	Normal values (8 cases)	
			Average	Range
Proline	3.73	1.67	0.48	0.28-0.63
Glycine	31.4	12.0	4.96	2.70-5.80
Tryptophan	1.87	3.40	2.80	1.30-3.53

clearance of proline and glycine and normal rate of tryptophan clearance. This indicates that the reabsorption of proline and glycine in the renal tubule is specifically impaired in the patients.

Oral proline loading test: 0.1 g of proline per kg of bodyweight was orally administered. Twenty-four-hours urines were collected before and following the test dose and blood specimens were drawn at intervals of 0, 1, 2 and 4 hours following the loading. Proline and glycine in urine and serum were colorimetrically determined by the methods described above. The results obtained were shown in Table IV. There was no remarkable difference in serum proline levels follow-

TABLE IV. Oral Proline Loading (0.1 g/kg)

Hours following the loading	Serum levels of proline (mg/100ml)				Urinary excretion (mg/kg/day)			
					Proline		Glycine	
	0	1	2	4	Before	After	Before	After
Case I	4.0	14.4	8.5	4.5	4.67	9.47	18.4	23.2
Case II	Undetermined				3.22	7.25	7.9	14.4
Controls	5.3	16.4	14.0	10.0	0.49	0.55	1.9	1.5
	2.9	15.0	9.9	6.6	0.25	0.31	2.3	1.6
	5.7	13.6	8.7	6.0	0.71	0.56	3.4	2.9
	4.0	14.0	9.4	5.7	0.52	0.26	2.1	2.2

ing its loading between the patient (Case I) and controls. Both of the patients, however, revealed great increase of proline excretion by the loading, while controls showed no significant increase of it by the loading. In addition, the patients showed a significant increase in glycine excretion by the loading of proline.

Oral glycine loading test: 0.2 g of glycine per kg of bodyweight was orally administered. Specimens of blood and urine were taken in the same way as the case of proline loading test. As was shown in Table V, serum glycine levels following its loading in the patient (Case I) did not differ from those in controls. Both of the patients, however, exhibited a remarkably higher output of glycine than controls. Proline excretion also showed an increase by the loading of glycine in the patients.

These results again support the above-mentioned presumption that an excessive excretion of proline and glycine in the patients should be ascribed to a disturbance in reabsorption of the amino acids in the renal tubules.

For reference, oral tryptophan loading test was made to the patient of Case I. The results showed no difference in urinary output of tryptophan between the patient and controls (cf. Table VI). Tryptophan loading did not make any significant effect on excretion of either proline or glycine in the patient.

This implies that the defect in renal transport of amino acids in these

TABLE V. Oral Glycine Loading (0.2 g/kg)

Hours following the loading	Serum levels of glycine (mg/100ml)				Urinary excretion (mg/kg/day)			
	0	1	2	4	Glycine		Proline	
					Before	After	Before	After
Case I	1.70	16.50	6.10	1.68	18.4	29.7	4.67	6.64
Case II	Undetermined				7.9	31.7	3.22	5.40
Controls	2.82	11.8	6.90	2.29	2.96	3.48	0.25	0.24
	2.72	15.1	6.32	3.08	4.74	5.33	0.32	0.30
	3.08	16.7	8.92	4.20				
	2.04	17.4	9.80	4.18				

TABLE VI. Oral Tryptophan Loading (0.1 g/kg)

	Urinary excretion (mg/kg/day)					
	Tryptophan		Proline		Glycine	
	Before	After	Before	After	Before	After
Case I	1.10	1.63	4.67	4.50	18.4	19.1
Controls	1.28	1.80				
	1.71	2.18				
	1.65	1.90				
	1.60	2.40				

particular patients may be not common for all the amino acids but be confined to proline and glycine.

In the next place, intravenous loading of proline and glycine, individually or simultaneously, was performed in the patient of Case I and the change in urinary output of these amino acids was estimated.

1) *Intravenous loading of proline:* 10% proline solution (at the rate of 100 mg/kg) was infused into an antecubital vein within 10 minutes in the fasting state. Exactly four-hours-urine was collected by indwelling catheter. For comparison, exactly four-hours-urine was collected in the fasting state without the loading. The amounts of proline and glycine in the urines were determined.

The increment in proline excretion after its loading was greater in the patient than in controls (cf. Table VII). Glycine excretion was enhanced by the

TABLE VII. Intravenous Proline Loading (0.1 g/kg)

	Urinary excretion (mg/kg/4 hrs.)			
	Proline		Glycine	
	Before loading	After loading	Before loading	After loading
Case I	0.87	8.35	3.13	6.70
Controls	0.12	2.86	0.87	0.99
	0.09	3.70	0.54	0.93
	0.10	1.80	0.89	1.32
			0.60	0.90

proline loading both in patient and in controls. The degree of its increment was, however, obviously higher in the patient than in controls.

2) *Intravenous loading of glycine:* 10% glycine solution (at the rate of 100 mg/kg) was infused and exactly four-hours-urine was collected in the same way as the proline loading.

The increment in glycine excretion after its loading was greater in the patient (Case I) than in controls (cf. Table VIII). Proline excretion was enhanced

TABLE VIII. Intravenous Glycine Loading (0.1 g/kg)

	Urinary excretion (mg/kg/4 hrs.)			
	Glycine		Proline	
	Before loading	After loading	Before loading	After loading
Case I	3.13	17.4	0.87	1.40
Controls	0.87	4.85	0.12	0.13
	0.54	4.95	0.09	0.10
	0.89	5.00	0.08	0.09

by the glycine loading in the patient but not clear in controls.

3) *Intravenous loading of proline and glycine:* A mixture of 10% proline solution and 10% glycine solution was infused at the rate of 100 mg/kg, respectively, and exactly four-hours-urine was collected by the catheter.

The patient (Case I) showed highly excessive excretions of proline and glycine after the loading as compared with controls (cf. Table IX). The increment of each amino acid was found to be more exaggerated than that found in the case of single loading of the respective amino acids, especially in the patient and to some extent in controls, too.

4) *Intravenous loading of lysine:* 10% lysine solution (at the rate of 100 mg/kg) was infused to the patient of Case I in the same way and the amounts of proline and glycine were determined in four-hours-urine following the loading. There was no significant increase in excretion of proline or glycine (cf. Table X).

Table XI showed the levels of proline in the cerebrospinal fluid. The level of the patient (Case I) was of the lower limit of the normal.

TABLE IX. Intravenous Loading of Proline Combined with Glycine

	Urinary excretion (mg/kg/4 hrs.)			
	Proline		Glycine	
	Before loading	After loading	Before loading	After loading
Case I	0.87	14.1	3.13	27.6
Controls	0.12	5.41	0.87	7.63
	0.09	4.52	0.54	5.68

TABLE X. Intravenous Loading of Lysine (100 mg/kg)

	Urinary excretion (mg/kg/4 hrs.)			
	Proline		Glycine	
	Before loading	After loading	Before loading	After loading
Case I	0.87	1.20	3.13	2.39

TABLE XI. Proline Level in Cerebrospinal Fluid

	Proline (γ /100 ml)
Patient (Case I)	100
Normal	
Average (15 cases)	161 $\pm$ 36
Range	110–240

FAMILY STUDIES

The parents of both patients (Cases I and II) revealed no excessive excretion of proline or glycine on thin-layer chromatograms.

The mother and an elder sister of the patient (Case I) were investigated by the intravenous proline loading test (cf. Table XII). Both the mother and sister showed a slightly higher excretion of proline after the loading than control individuals.

TABLE XII. Intravenous Proline Loading to the Sister and the Mother of the Patient (Case I)

	Urinary excretion of proline after the loading (mg/kg/4 hrs.)
Sister	4.50
Control infants	1.87
	2.86
	3.70
Mother	2.10
Control adults	1.53
	1.85
	1.92

Note: The amounts of proline infused were 100 mg per kg to the sister and control infants, and 50 mg per kg to the mother and control adults.

DISCUSSION

In 1962, Schafer *et al.*⁶ described a new disorder in amino acid metabolism, "familial hyperprolinemia", which was associated with a characteristic aminoaciduria consisting of proline, hydroxyproline and glycine. The patient with this particular disorder was reported to show mental retardation, defective renal development, microscopic hematuria and deafness. Four siblings of the patient were found to have elevated plasma levels of proline and increased urinary excretion of proline, hydroxyproline and glycine. The authors postulated that the block responsible for hyperprolinemia would seem to be in the conversion of proline to glutamic acid, possibly due to deficiency of the enzyme proline dehydrogenase, and that increased excretion of glycine and hydroxyproline would be secondary to the excessive urinary excretion of proline.

The present cases of our own are distinctly different from the familial hyperprolinemia of Schafer *et al.*, since our own patients showed normal or subnormal levels of proline in serum. The results of oral loading of proline or glycine in our patients do not imply disturbance in intermediary metabolism of proline or glycine. On the contrary, high rates of renal clearance of both amino acids were found in our own patients. It is, therefore, reasonable to presume that the illness of our own patients belongs to a disorder of defective renal

transport of proline and glycine.

In the kidney, major part of amino acids in the glomerular filtrate is reabsorbed from the proximal tubules against a chemical gradient.^{7,8} Such a reabsorption of the amino acids in the renal tubules is thought to take place by active transport which consists of several mechanisms determined genetically depending on the kind of amino acids. Genetic defect of renal transport of the amino acid is well exemplified in cystinuria, in which renal transport of only cystine and the di-amino acid (lysine, ornithine and arginine) is defective.

The present disorder could be regarded as another example of defect in renal transport confined to proline and glycine. There is, however, a conflicting observation in this respects. In familial glycinuria described by De Vries *et al.*⁹ is known to be due to a defective renal transport of glycine alone and no prolinuria is observed in this particular disorder. Therefore, glycine transport in the renal tubule seems to be controlled by a single gene.

Then, how shall we explain the etiology of the present disorder in genetic basis?

A concomitant mutation of two genes does not seem to occur.

There are several findings suggestive for that renal transport of proline and glycine shares in a common transport mechanism. Evered and Randall¹⁰ have found the competition of proline in glycine uptake in mucosa of small intestine of rat and suggested a common pathway for transport of glycine and proline. Our own results of the infusion experiments of amino acids (proline, glycine and lysine) to the patient and controls also suggest an intimate relation of renal transport between proline and glycine (cf. Tables VII to X).

In Hartnup disease, impairment in renal transport of most of the neutral amino acids is thought to be the result of a specific genetic mutation, but there is little impairment in renal transport of proline and glycine.¹¹⁻¹³

During the early infancy, the infant has relatively elevated urinary excretion of proline and glycine due to insufficient reabsorption in the renal tubule, while the excretion of the other amino acids is comparable to that observed in later life.¹⁴

These evidences favor that a gene may be involved in renal transport of proline and glycine. It might be possible that transport mechanism of amino acid is controlled by more than one gene. For instance, some part of the mechanism may be specific for proline or glycine, respectively, that is, controlled by different genes; and other part of the mechanism may be common for both proline and glycine under a single genetic control. If this were the case, familial hyperglycinuria would be caused by a mutation of the former mechanism and the present disorder would be caused by a mutation of the latter.

In 1958, Joseph *et al.*¹⁵ described a familial disorder characterized by severe convulsions, increase in albumin content in the cerebrospinal fluid and hyperamino-

aciduria. According to their report, the increase of proline was prominent on urinary paperchromatograms of their patients and they supposed that this prolinuria might be of renal type. The present cases of our own are different from Joseph *et al.*'s cases in a view-point of that our patients showed neither convulsion nor increase in albumin in the cerebrospinal fluid. In addition, laboratory data in Joseph *et al.*'s paper seem to be insufficient to conclude that their patients have a specific defect in renal transport of proline.

References

- 1) Troll, W. & Lindsley, J. *J. biol. Chem.*, 1959, **215**, 655.
 - 2) Alexander, B., Landwehr, G. & Seligman, A.M. *ibid.*, 1945, **160**, 51.
 - 3) Tada, K. *Rinsho Shoniigaku* (Jap.), 1964, **12**, 129.
 - 4) Spackman, D.H., Stein, W.H. & Moore, S. *Anal. Chem.*, 1958, **30**, 1190.
 - 5) Cusworth, D.C. & Dent, C.E. *Biochem. J.*, 1960, **74**, 550.
 - 6) Schafer, I.A., Scriver, C.R. & Efron, M.L. *New Eng. J. Med.*, 1962, **267**, 51.
 - 7) Brown, J.L., Smailly, L.M. & Pitts, R.F. *Amer. J. Physiol.*, 1961, **200**, 370.
 - 8) Christensen, H.N. & Jones, J.C. *J. biol. Chem.*, 1962, **237**, 1203.
 - 9) De Vries, A., Kochwa, S., Lazebnik, J., Frank, M. & Djaldetti, M. *Amer. J. Med.*, 1957, **23**, 408.
 - 10) Evered, D.F. & Randall, H. *Nature*, 1963, **197**, 386.
 - 11) Baron, D.N., Dent, C.E., Harris, H., Hart, E.W. & Jepson, J.B. *Lancet*, 1956, **2**, 421.
 - 12) Milne, M.D., Crawford, M. A., Gross, C.R. & Loughridge, L.W. *Quart. J. Med.*, 1960, **29**, 407.
 - 13) Scriver, C.R. & Shaw, K.N.F. *Canad. med. Ass. J.*, 1962, **86**, 232.
 - 14) O'Brien, D. & Butterfield, L.J. *Arch. Dis. Childh.*, 1963, **38**, 437.
 - 15) Joseph, R., Ribierre, M., Job, J.C. & Girault, M. *Arch. franç. pédiat.*, 1958, **15**, 374.
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