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Protein load in argininosuccinic aciduria: thoughts on its biochemical implications

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With 3 figures and 1 table

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Congenital defects of urea synthesis are characterized by protein intolerance. Because of a dislike of protein in older patients and a self selected low protein diet, unequivocal biochemical signs, like hyperammonemia, are not necessarily present in all patients. In these cases a protein loading test may be of diagnostic help, stressing the presumably inhibited pathway of urea formation. In a case of arginino succinic aciduria a protein loading test was performed for to have further insight into mechanisms around disturbances of urea synthesis.

Methods

Plasma ammonia was determined enzymatically as described by *Da Fonseca-Wollheim* within 15 minutes after blood collection (1). Urea and creatinine were determined by routine procedures of the clinical laboratory.

Orotic acid determination in urine followed the method described by *Adachi* et al. (2).

Argininosuccinic acid lyase was determined on erythrocyte hemolysates using the method described by *Bohuon,* which is based on the technique by *Campanini* et al. (3). Plasma from heparinised venous blood was examined by ascending partition chromatography in a butanol/acetone/acetic acid/water mixture (35/35/7/23; V/V/V/V) and by elution chromatography on ion-exchange resin with a *Beckman* amino acid analyser (Multichrom M). Argininosuccinic acid and its anhydrides were identified using the commercially available pure substance (Serva Feinbiochemica Heidelberg, Germany).

The patient was a 22 year old female. Her parents were not consanguineous. The parents and one older sister were healthy with respect to protein tolerance, neurology and mental capacity. The patient was reported to be without symptoms until 6 years of age, when she had hepatitis. From then, as the mother reported, "the girl never recovered totally". The first seizures were reported at an age of 12 years. As described, they were of a psychomotor character. The patient never liked meat, milk or dairy products. Over the years aversion and nausea developed just from looking at food with a heavy protein content. The girl put herself on a vegetarian style diet. At school she had very poor results and worked ultimately as an aide in a hospital laundry service. Her IQ at the time of admission as determined by a psychologist using the Hamburg-Wechsler-Intelligence-Scale for Adults was 56. For the last 6 years the patient was taking varying amounts of antiepileptic drugs. At the time of admission she had a daily intake of 900 mg 2-propylvalerianic acid (Ergenyl®) and 350 mg diphenylhydantoine (Zentropil®). The EEG showed slowed background activity of mainly 6/second theta waves and an occipital delta focus. Bilateral synchronized sharp waves were noted intermittently.

At the time of adminission the patient was on a self-selected low protein diet, revised by us that it finally corresponded to a daily protein intake of about 0.5 g/kg body weight.

Laboratory data at the time of admission:

Blood glucose: 117 mg/dl, urea-N: 5 mg/dl, creatinine: 0.6 mg/dl, uric acid: 6.6 mg/dl, total protein: 5.2 g/dl, albumin: 3.5 g/dl, cholesterol: 145 mg/dl, triglycerides: 67 mg/dl, alkaline phosphatase: 67 U/L, SGPT: 8.5 U/L, SGOT: 12.6 U/L, choline esterase: 7.3 U/L, gamma-GT: 57 U/L.

At the physical examination the patient's liver was found to be enlarged 6 cm.

Urinary amino acid screening with thin layer chromatography revealed a very dark band cochromatographing with pure argininosuccinic acid. By alkalinizing and heating the urine and pure arginino succinic acid at

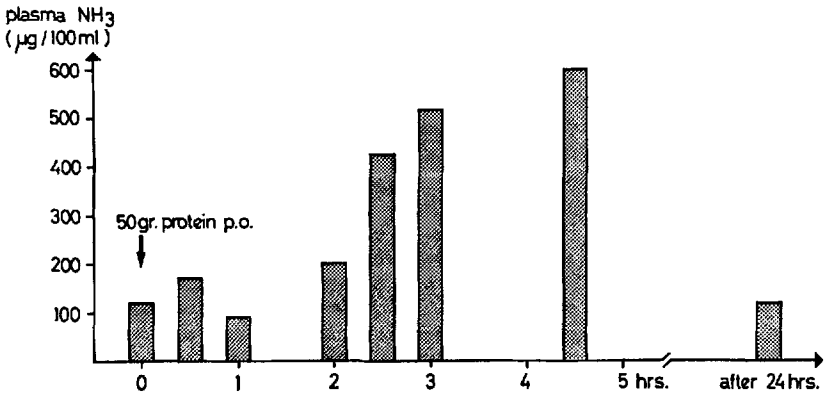

Fig. 1.

Table 1. Plasma amino acid profile in a patient with argininosuccinic aciduria before and 190 minutes after 50 grams of protein (μmoles/l). Values before the load are taken 2 hours after breakfast.

	before 50 g protein	after 50 g protein	normal values according to *Holmgren*
Phosphoserine	31	27	
Taurine	48	48	38 ± 21
Aspartic acid	16	77	10 ± 6
Threonine	109	260	92 ± 40
Serine	101	220	102 ± 43
Asparagine	61	145	44 ± 16
Glutamic acid	114	365	73 ± 73
Glutamine	1070	1451	678 ± 244
Proline	271	804	139 ± 51
Glycine	475	542	216 ± 77
Alanine	400	1032	298 ± 79
Citrulline	271	366	29 ± 12
Valine	95	289	212 ± 83
Cystine	18	–	37 ± 11
Methionine	19	53	21 ± 8
Isoleucine	24	81	65 ± 29
Leucine	45	127	118 ± 51
Tyrosine	52	208	62 ± 26
Phenylalanine	30	54	48 ± 16
Ornithine	54	55	71 ± 22
Lysine	73	241	161 ± 53
Histidine	84	140	–
Tryptophane	24	–	–
Arginine	34	52	77 ± 32
Argininosuccinic acid	153	223	–

100 °C as described by *Westall* (4) anhydride formation could be enhanced. Taking these results together with the patient's history, argininosuccinic aciduria was suspected.

In comparison to five matched controls the argininosuccinic acid lyase activity in the patient's erythrocytes was reduced to 10–12 %. We did not obtain permission for either a liver puncture or for a spinal tap.

The protein tolerance test was performed administering 1 g protein/kg body weight (total of 50 grams) in form of a banana flavoured cottage-cheese-milk drink within 10 minutes. The patient and an accompanying family member was advised about eventual unpleasant side effects that could be provoked by the protein load. Consent was obtained. Urine was collected during 24 hours before (period I) and after (period II) the load.

The ensuring rise in plasma ammonia is demonstrated in figure 1. After an initial lag of about 60–90 minutes plasma ammonia was rising steadily. At values of about 500 μg/dl ammonia the patient felt nauseated and started vomiting. The highest ammonia level was obtained about 5 hours after the load, when blood collection was stopped because it became stressful to the patient. 24 hours after the protein load plasma ammonia had returned to a level similar to that obtained before the test.

Plasma amino acids before (2 hours after breakfast) and 190 minutes after the protein load are demonstrated in table 1. Among the plasma amino acids before protein loading glutamine, glycine and citrulline are elevated compared to normal values (5). Valine leucine and isoleucine are decreased compared to controls. Arginine was found to be very low. Argininosuccinic acid is present in considerable amount. The plasma amino acids 190 minutes after the protein load reflect a general increase. The pattern is mainly characterized by an increment of aspartic acid, glutamine, proline, alanine, citrulline, valine, leucine, isoleucine and argininosuccinic acid.

The urine amino acid pattern before and after the protein load is characterized by heavy argininosuccinic aciduria.

Urea excretion showed basically no change comparing the two urine collection periods (fig. 2).

During period I 3.4 mg orotic acid (5.3 μg/mg creatinine) were excreted. Orotic acid excretion rose steeply up to 86.8 mg (142.6 μg/mg creatinine) during period II (fig. 3).

Discussion

Argininosuccinic aciduria results from a defect in the enzyme argininosuccinate lyase, which mediates the cleavage of arginino succinic acid to arginine and fumaric acid. This defect was first described by *Allan* et al. in 1958 (6). As in our case it is common that the disease does not manifest until childhood (7). *Shih* and *Efron* (8) are dividing the patients into a Late-onset group and an Early-onset group. The same authors report IQ values mainly between 20 and 75, but *Carson* and *Neill* report about a patient with borderline normal intelligence (IQ 92) (9).

The unchanged urea excretion during periods I and II may reflect directly the inability to respond to an increased protein intake with an

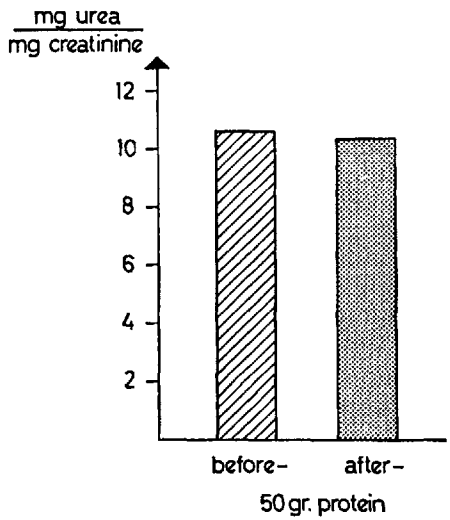

Fig. 2

adequate increase in urea formation. Besides the impairment of urea formation the observed lack of increase of urea after the load could be due to previous long standing protein undernutrition, which is reflected also in the plasma aminogram.

In our patient a residual argininosuccinate lyase activity of about 10 to 12 % of normal controls was found. This is in accordance to *Nicholson* et al. (10) who presented data leading to the conjecture that inborn errors of ammonia detoxification which result in clinical disease are partial defects. Complete defects in ammonia detoxification are lethal. We made the assumption that the patient's enzyme activity in the liver is similarly decreased to that in the erythrocytes.

The plasma amino acid pattern before the test is typical for protein deficiency. The increased glycine levels and the decreased concentrations of the branched chain amino acids: valine, leucine and isoleucine are, according to *Snyderman* (11) early signs of protein deficiency. This may reflect a long term inadequate protein intake of our patient.

The plasma amino acid changes observed 190 minutes after the protein load demonstrate on one hand with its general increase the actual huge amount of protein ingested and reflect on the other with its pattern the inability of metabolism to handle it. The large increment of alanine and aspartic acid reflect the direct involvement of both amino acids in ammonia metabolism through the mediation of alanine aspartate amino transferase and glutamate dehydrogenase (12). The high proline level demonstrates the interconvertibility between glutamine and proline existing in the human organism (13). The block of urea synthesis leads to an increase of citrulline

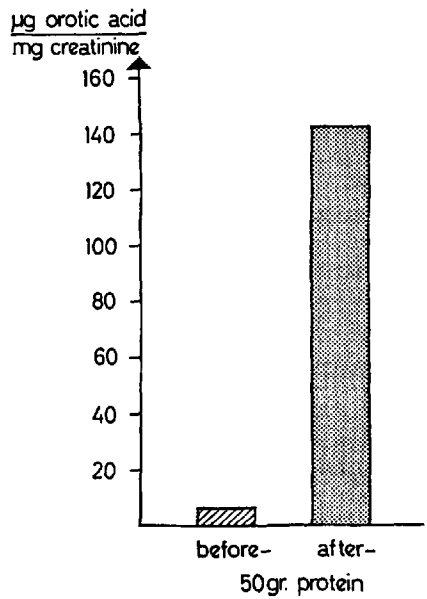

Fig. 3.

which precedes the cleavage of argininosuccinic acid and explains the high glutamine and aspartic acid levels because the ammonia supply for urea formation from these donor amino acids is backed up.

Various authors described orotic aciduria in ornithine transcarbamylase deficiency (14, 15). Orotic acid is an intermediary product of pyrimidine synthesis. The synthesis of pyrimidines, as of urea starts with carbamyl phosphate. The cytosolic carbamylphosphate synthetase reaction for pyrimidine synthesis is glutamine dependent (16). The corresponding mitochondrial reaction for urea synthesis is dependent on N-acetyl-glutamate (16). A block during the course of urea formation, for instance because of an inborn error of the urea cycle, leads primarily to a decreased arginine level and a resulting decreased N-acetyl-glutamate synthetase reaction which has been demonstrated to be arginine dependent (16). A slowing down of urea synthesis is the result. However, the elevation of glutamine and aspartic acid levels, as also in our patient, presents an ideal condition for the synthesis of pyrimidines, if we remember that the cytosolic carbamyl phosphate synthetase is glutamine dependent and that the aspartate transcarbamylase reaction follows after. We are aware that these thoughts are somewhat speculative and that there is still controversy about the existence of two carbamylphosphate synthetase enzymes. It is an intriguing question if orotic aciduria could represent an alternate pathway for nitrogen detoxification. If we compute the actual nitrogen intake of the patient and the nitrogen excretion as urea-N and orotate-N we obtain the following figures:

	Intake mmoles/day	Urea-N excreted mmoles/day	Orotate-N excreted mmoles/day
before load	270	224	0.044
after load	840	208	1.1

This shows that orotic aciduria is important for diagnostic purpose but does not represent a quantitatively important way for nitrogen detoxification.

Argininosuccinic acid has the tendency to spontaneous formation of anhydrides. These are structurally similar to orotic acid and could theoretically interfere with the orotic acid assay (4). The performed orotic acid assay on pure argininosuccinic acid adjusted to urinary pH showed no significant difference from blank values.

Conclusion

A block of urea synthesis favours the enhanced synthesis of orotic acid, a precursor of pyrimidine synthesis. Orotic acid does not present a quantitatively important way for nitrogen detoxification, but it may be of diagnostic value to detect inborn errors of urea synthesis. Our results show that orotic acid excretion is not only a phenomenon observed in ornithine transcarbamylase deficiency as suggested by *Scriver* et al. (15).

In our opinion the enhanced pyrimidine synthesis cannot be explained, as is currently done, by a mere backing up of carbamyl phosphate from the intramitochondrial urea synthesis; but by a de novo synthesis of carbamyl phosphate on the basis of favourable regulatory conditions.

Summary

A patient with argininosuccinic aciduria was charged with 50 grams of protein, which was followed by considerable hyperammonemia. There was no response in further urea formation; but there was a considerable production of orotic acid, a precursor of pyrimidines. This makes orotic acid to an important diagnostic tool for the diagnosis of impaired urea formation. The patient's plasma amino acid pattern led to the suggestion that orotic acid synthesis is initiated by increased de novo formation of carbamyl phosphate in the cytosol and not by deviation of already existing intramitochondrial carbamyl phosphate.

Zusammenfassung

Eine Patientin mit Argininbernsteinsäureerkrankung wurde mit 50 g Eiweiß belastet. Die Belastung wurde von einer beträchtlichen Hyperammoniämie gefolgt. Es erfolgte keine gesteigerte Harnstoffbildung; jedoch trat eine kräftige Orotsäureausscheidung, ein Vorläufer der Pyrimidinsynthese, auf. Hierdurch wird Orotsäure zu einer bei der Diagnose von Störungen der Harnstoffsynthese bedeutsamen Substanz. Die Veränderung der Plasmaaminosäuren der Patientin nach der Proteinbelastung weist möglicherweise auf eine vermehrte zytostolische Carbamylphosphatneubildung und nicht auf die Verwendung von aufgestautem, intramitochondrialem Carbamylphosphat für die Pyrimidinsynthese hin.

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