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Hyperornithinemia-Hyperammonemia-Homocitrullinuria Syndrome: Low Creatine Excretion and Effect of Citrulline, Arginine, or Ornithine Supplement

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ABSTRACT. Two patients with neonatal onset of hyperornithinemia-hyperammonemia-homocitrullinuria syndrome were studied at 4 and 2½ yr of age, respectively. The aim of the investigation was to assess the effect of supplementing citrulline, arginine, or ornithine (2 mmol/kg per day) while on a protein-restricted diet. The peroral supplementation was carried out during 2 wk for each aminoacid. While ammonia in plasma was not increased the supply of citrulline or arginine led to a reduction of plasma glutamine compared to ornithine supplement or to no supplement (control period). Plasmatic ornithine was raised in all instances. Homocitrulline excretion was lower with all additions compared to the control period. Adding citrulline to the diet (in contrast to supplementing arginine) did not lower tubular lysine reabsorption. A lowered creatine excretion was found which could be normalized by arginine or citrulline. The data are compatible with a product inhibition of arginino-glycine transamidinase suggesting that the enzyme is not located in the mitochondrial matrix in man. Citrulline supplement combined with a protein-restricted diet appears to allow a normal development. The additional finding of a factor VII and X deficiency in one of the patient and reports in the literature of this association in two other patients with hyperornithinemia-hyperammonemia-homocitrullinuria syndrome suggest that the genetic defect leading to the syndrome might be located on chromosome 13. (*Pediatr Res* 22: 364–367, 1987)

Abbreviations

HHH, hyperornithinemia-hyperammonemia-homocitrullinuria
OCT, ornithinecarbamoyl transferase
UV, ultraviolet
WPPSI, Wechsler Preschool and Primary Scale of Intelligence

The HHH syndrome is a rare inborn error of aminoacid metabolism first described by Shih *et al.* in 1969 (1). Up to this point 23 patients have been reported (1–14). Clinical symptoms are vomiting, lethargy, coma, seizures, ataxia, and various degrees of mental retardation, as observed in other hyperammonemic disorders. Only one patient reported so far (patient B,13) showed symptoms as a neonate.

The molecular basis of the HHH syndrome remains unclear. Several investigators have postulated a specific defect in the transport of ornithine across the inner mitochondrial membrane but a definite proof is still lacking due to controversial results (6, 7, 14–19). Previous reports on therapeutic trials using supplements of ornithine, arginine, lysine or citrulline in patients with HHH syndrome for controlling hyperammonemia and the other metabolic disturbancies (hyperornithinemia, homocitrulline, and orotic acid excretion) led to contradictive results (1, 3, 5–10, 12–15, 19). In the present study we assessed the effect of supplementing perorally citrulline, arginine, or ornithine, administered during 2 wk each separately in two unrelated patients with HHH syndrome kept on a protein-restricted diet. The marked variations in the daily excretion of creatine depending on the supplement used is of particular interest.

MATERIALS AND METHODS

Protocol of treatment and sample collection. Both patients were treated for a 2-wk period respectively with citrulline (C), arginine-base (A), and ornithine-base (O) 2 mmol/kg per day given in two doses perorally with the meals at 4 (patient A) and 2½ (patient B) yr of age. The trials were consecutive in the order shown in Tables 1 and 2 without washout periods. For control the investigations were also performed after a separate 2-wk period without amino acid supplement (N). Before control period patient A had been on chronic treatment with citrulline while patient B was without any preceeding supplement. During the study the protein supply was kept constant in quality and quantity (1.2 and 1.5 g/kg per day, respectively, for patient A and B). Blood samples were drawn at the end of each 2-wk period of treatment (or control) at 0900 h after an overnight fast (12 h after the last meal). A 24-h urine was collected, starting 24 h before the blood sampling. No side effects were observed with the different protocols of treatment. The parents were informed and consented to the study.

Analytical methods. Samples for the amino acid and orotic acid determinations were frozen at −20° C. Amino acids were quantified by ion exchange chromatography (Biotronik 7000, Munich, FRG) using norvaline as internal standard. Orotic acid in urine was determined by anion exchange chromatography as described (20). Blood ammonia was assayed with an enzymatic UV method (Boehringer, Mannheim, FRG). Creatinine was determined by a kinetic Jaffé reaction and creatine colorimetrically (21). Urea was determined in urine with urease including

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an analytical blank without urease to account for urinary ammonia.

CASE REPORTS

Both in patients A and B the onset of the disease was neonatal. Feeding difficulties, vomiting, hypotonia, and seizures were the predominant symptoms and hyperammonemia was found. ADC (boy), patient A, is the third born of two related parents (first cousins). His older sister presents a mild mental retardation not due to an HHH syndrome. Pregnancy and delivery had been uneventful. At 14 days of life he was admitted to the hospital because of vomiting, hypotonia, and seizures. Biochemical investigations revealed a hyperammonemia (240 μmol/liter) and an increase of serum transaminases (GOT 210 U/liter, GPT 159 U/liter). The patient was then treated with phenobarbital and kept on a protein intake of 1.8 g/kg per day. The diagnosis of the HHH syndrome was established at 2 yr of age during an intermittent attack of ataxia and lethargy. He was treated with a protein-restricted diet (1.2 g/kg per day), supplemented by citrulline (2 mmol/kg per day). At the age of 4 yr weight was 16.5 kg (50th percentile), height was 103 cm (50th percentile) and head circumference was 52 cm (60th percentile). The WPPSI at age of 4 yr and 9 months revealed a full scale IQ of 72 (3rd percentile).

The clinical features of CD (girl), patient B, have already been reported (13). A neonatal hyperammonemia (283 μmol/liter) was assumed to be due to an OCT deficiency. We detected homocitrullinuria and orotic aciduria at 3 months of age. The patients protein intake was restricted to 1.5–1.8 g/kg/day and arginine (later citrulline) supplemented. During a routine hematological investigation, a deficiency of factor VII (21%) and factor X (25%) were additionally found in this patient without manifestation of any clinical symptoms. At 2½ yr of age she weighed 13.5 kg (50th percentile), her height was 88 cm (25th percentile), and head circumference was 49.5 cm (60th percentile). The development quotient by Brunet-Lézine testing was 81 (developmental age 32 months at 40). Since the start of the protein-restricted diet we never observed episodes of hyperammonemia. Hyperornithinemia was absent in several instances when checked during the chronic treatment, while homocitrullinuria persisted. The lowest ornithine in plasma (71 μmol/liter) had been found (13) at 28 days of age when the patient was treated with benzoate (250 mg/kg per day) and low-protein diet (0.75 g/kg per day).

RESULTS

Plasma ammonia concentrations remained normal but plasma ornithine values were elevated with all protocols used (Table 1). Homocitrulline excretion was moderately lowered with all three amino acid supplements compared to the control period (Table 2).

In both patients lysine was never low and its rise in plasma was not followed by an increased homocitrulline excretion. In both patients the variations observed in plasma lysine concentration paralleled those of ornithine ($r = 0.9540$, $p < 0.001$). In both patients glutamine was raised when ornithine was added to the diet or during the period without supplement. Ornithine was the only amino acid whose concentration in plasma increased in both patients when added to the diet. The arginine concentration in plasma always exceeded the upper normal range. No seizures were observed.

The fractional tubular reabsorptions (compared to creatinine clearance) of all the amino acids were normal in the absence of supplement. However, it should be noted that the fractional tubular reabsorption of lysine was lower with arginine supplement (patient A: 96.3%, B: 91.6%) than with citrulline substitution (98.4%; 95.5%). Without treatment the fractional tubular reabsorptions of lysine were 99.3 and 98.4%, respectively. The daily excretion of creatine (Table 2) was below normal in both patients when no amino acids were added to the diet. This value remained below normal with ornithine supplementation in patient B while it reached the normal range both in patient A and B when citrulline and arginine were supplemented.

The pattern of creatine excretion paralleled that of urinary urea (correlation $r = 0.9161$, $p < 0.001$; Spearman $\rho = 0.857$, $p < 0.02$). The excretion of arginine followed a similar pattern. The low creatine excretion was not related to an increased ornithine concentration in plasma.

Table 1. *Plasma amino acids, ammonia, and creatinine (μmol/liter) in patients A and B under different protocols of treatment (C, A, O)* and during control period (N)**

	Patient A				Patient B				Reference values (31)
	C	A	O	N	C	A	O	N	
Taurine	34	67	98	27	59	53	26	141	20–90
Aspartic acid	38	42	38	ND†	42	18	73	23	Tr–10
Threonine	68	69	74	52	48	36	55	61	30–130
Serine	163	162	188	84	133	75	120	127	25–170
Glutamic acid	203	172	152	16	134	39	111	123	25–250
Glutamine	312	375	465	599	276	243	369	609	60–470
Proline	105	106	100	114	117	105	169	134	50–190
Glycine	254	245	387	158	162	127	257	255	60–310
Alanine	287	289	431	185	334	263	426	482	100–310
Citrulline	34	53	57	39	34	9	42	49	10–30
Valine	151	167	173	131	116	107	146	114	60–260
Methionine	26	25	22	33	35	9	31	137	5–30
Isoleucine	55	73	54	40	121	5	46	42	25–95
Leucine	118	126	131	93	66	27	84	139	45–155
Tyrosine	70	86	82	50	51	54	65	146	10–120
Phenylalanine	79	74	73	65	55	99	145	123	20–70
Ornithine	500	638	837	504	405	339	496	419	10–110
Lysine	85	138	180	86	73	45	88	101	45–145
Histidine	67	120	203	71	68	41	94	96	25–110
Arginine	127	120	116	80	110	99	90	125	10–65
Ammonia	29	41	28	35	42	24	38	50	<50
Creatinine	61	53	49	49	43	38	41	49	<65

* C, A, O, N, after 2 wk of supplement with citrulline (C), arginine (A), or ornithine (0) 2 mmol/kg body weight per day or no supplement (N).
† Not detectable.

Table 2. *Urinary excretion of amino acids, orotic acid (μmol/mol creatinine), creatine, creatinine (μmol/kg body wt per day) and urea (mmol/kg body wt per day) in patients A and B*

	Patient A				Patient B				Reference values
	C*	A	O	N	C	A	O	N	(29, 32, 33)
Taurine	20	14	10	26	44	56	20	72	
Aspartic acid	2	ND†	8	ND	13	6	5	9	
Threonine	11	23	17	10	23	11	6	18	<100
Serine	44	75	64	27	73	30	13	63	<340
Glutamic acid	8	9	22	44	6	4	24	18	
Glutamine	102	184	143	36	175	132	109	153	<300
Proline	2	3	2	ND	1	1	2	9	<25
Glycine	100	169	140	85	230	258	225	372	<500
Alanine	57	124	105	53	153	208	154	174	<190
Citrulline	217	38	27	2	1349	67	42	ND	
Valine	9	13	11	7	29	28	16	18	<22
Cystine	9	13	12	7	43	38	34	9	<55
Isoleucine	0.1	0.1	0.1	4	2	12	3	45	<25
Leucine	0.1	9	7	5	10	60	17	45	<20
Tyrosine	8	35	29	21	71	9	13	78	<60
Phenylalanine	8	17	19	10	12	18	9	30	<35
Ornithine	75	310	260	37	205	580	482	42	<13
Lysine	22	87	55	12	76	99	61	33	<120
Histidine	94	191	173	91	179	194	158	219	<320
Arginine	78	60	20	7	214	156	27	6	<7
Homocitrulline	27	75	34	95	34	59	16	78	
Orotic acid	139	132	7.13	172	34	71	254	79	0.08–0.44
Creatine	97	114	57	21	105	65	25	11	41–104
Urea	5.3	5.8	4.8	4.1	6.1	3.9	3.4	2.6	
Creatinine	145	138	152	130	137	112	114	97	88–132

* For abbreviations see Table 1.
† Not detectable.

As shown in Table 2 marked variations in orotic acid excretion were observed in both patients with the different protocols. In patient A orotic acid was lowest with ornithine supplement, while in patient B the lowest orotic acid excretion was achieved with citrulline and the highest occurred with ornithine supplement. The increase in orotic acid was not associated with an increase of homocitrulline excretion or with the elevation of ornithine in plasma.

DISCUSSION

The clinical histories of our patients show that in contrast to previous descriptions (1–12, 14) patients with HHH syndrome can also present with neonatal onset. The syndrome should thus be included in the differential diagnosis of neonatal hyperammonemias. Because of the difficulty to detect small amounts of homocitrulline in urine in the first days of life the HHH syndrome may be confused at the beginning with an OCT deficiency (patient B, 13).

Our study was aimed at testing chronic treatment not acute loads. Because of the good clinical condition we did not consider it to be ethically acceptable to challenge the patients with protein to such an extent that hyperammonemia would occur. Nevertheless the biochemical changes are of interest. A novel finding is the lower creatine excretion in our patients when not supplemented with arginine or citrulline.

The rate limiting enzyme for creatine formation is amidinotransferase (EC 2.1.4.1) catalyzing the formation of guanidino acetate and ornithine from arginine and glycine. The enzyme is mainly found in kidney and pancreas (22). Two isoenzymes have been isolated. Product inhibition of ornithine appears to be relevant *in vivo* (23–25) as exemplified by Sipilä (24). If HHH syndrome is really due to a transport defect of ornithine into the mitochondrion, the low creatine excretion (reflecting its limited formation) could well be due to the inhibition of the amidinotransferase by increased ornithine in the cytosol and in the intermembrane space. The effect appears to be at least partly reversed by increasing the substrate arginine (direct supplementation or through conversion from citrulline). The correlation with urea excretion and low plasmatic glycine values are compatible with such an interpretation. If ornithine leads to product inhibition of transamidinase in HHH patients and if ornithine is depleted in the mitochondria then one would have to conclude that in man the amidinotransferase is not located in the mitochondrial matrix as in chicken (26), and as assumed by Sipilä for HHH patients (24). Our data suggest that in man the amidinotransferase could perhaps be located at the external side of the inner mitochondrial membrane as found in the rat (27), in the intermembrane space, on the external membrane, or in the cytosol. Additional work is needed for definitive proof.

In contrast to ornithine supplement the addition of arginine or citrulline to the diet appears to reduce the ammonia load to the patients as manifest by the lowered plasma glutamine. This is in accordance with the findings of Simell *et al.* (9) showing the ineffectiveness of ornithine in preventing hyperammonemia after alanine load. It is in contrast to the report of Kirsch and McInnes (12) who found the chronic application of ornithine-HCl to be helpful in controlling hyperammonemia. It cannot be ruled out that in the latter treatment not the ornithine but the HCl moiety was effective. Chronic acidosis is well known to stimulate renal ammonia excretion by activating the glutaminase. HCl administration has actually been used in treatment of mild OCT deficiency (28). Citrulline substitution appears to be preferable to arginine supplement in view of the reduction of tubular lysine reabsorption by arginine application. However, the loss of lysine with arginine supplement was not such as to lead to low plasma carnitine. Another reason for substituting citrulline instead of arginine is that it allows the detoxication of one aspartate nitrogen to urea.

A puzzling finding in our and in some patients reported in the literature is that plasma citrulline concentrations are normal or even elevated while orotic acid excretion is increased (3, 6, 9).

This latter is found in hyperammonemia when carbamylphosphate accumulates and when aspartate is not decreased (29). In HHH syndrome this is thought to be due to ornithine depletion in the mitochondria with reduced flux through the OCT. However, this should lead to decreased citrulline production. One could speculate that argininosuccinate synthetase might be inhibited competitively by homocitrulline in the patients since homocitrulline has been shown to be a substrate for argininosuccinate synthetase (30).

In our patients homocitrulline excretion was almost as high with arginine supplement as without treatment, while citrulline and ornithine application led to lower homocitrulline output. We thus wonder if the guanidino group of arginine might be a precursor of the homocitrulline (and even citrulline) in HHH syndrome the acceptor substrate being lysine or a metabolite of this aminoacid.

One of our patients (B) was additionally affected by a factor VII and X deficiency. This has been reported in two other patients with HHH syndrome (3, 9). One thus could suspect that the genetic defect is located on the long arm of chromosome 13. Delimiting the defect of HHH syndrome at the DNA level might help in the future to establish conclusively the cause of HHH syndrome.

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