JCI The Journal of Clinical Investigation

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J Clin Invest. 1980;66(4):684-687. https://doi.org/10.1172/JCI109905.

Research Article

Patients with gyrate atrophy of the choroid and retina have 10- to 20-fold increased ornithine concentrations in body fluids and significantly reduced activity of ornithine aminotransferase in lymphocytes and cultured fibroblasts. We administered intravenous arginine to six patients and six controls to study in vivo inhibition by high ornithine concentrations of arginine-glycine transamidinase, the rate-limiting enzyme in creatine synthesis. Serum arginine concentrations curves after administration were similar for the two groups. The increment in serum ornithine was more than three times as great in patients as in controls. The mean half-times in plasma ornithine were 360 and 97 min, respectively. In the patients, the metabolic clearance of ornithine from the extracellular fluid was significantly delayed. Urinary guanidinoacetate excretion rose markedly in all controls, the excretion rate being higher in females. The patients always excreted less than the controls, the differences within the sexes being highly significant. Differences in creatine excretion after administration were less marked. We conclude that in gyrate atrophy patients, formation of guanidinoacetate, creatine, and possibly phosphocreatine is inhibited at the transaminidation step by the high concentrations of ornithine. Deficiency of the high-energy phosphates may underlie the pathogenesis of the eye and muscle atrophies.

Gyrate Atrophy of the Choroid and Retina with Hyperornithinemia

DEFICIENT FORMATION OF GUANIDINOACETIC ACID FROM ARGININE

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ABSTRACT Patients with gyrate atrophy of the choroid and retina have 10- to 20-fold increased ornithine concentrations in body fluids and significantly reduced activity of ornithine aminotransferase in lymphocytes and cultured fibroblasts. We administered intravenous arginine to six patients and six controls to study in vivo inhibition by high ornithine concentrations of arginine-glycine transamidinase, the rate-limiting enzyme in creatine synthesis. Serum arginine concentration curves after administration were similar for the two groups. The increment in serum ornithine was more than three times as great in patients as in controls. The mean half-times in plasma ornithine were 360 and 97 min, respectively. In the patients, the metabolic clearance of ornithine from the extracellular fluid was significantly delayed. Urinary guanidinoacetate excretion rose markedly in all controls, the excretion rate being higher in females. The patients always excreted less than the controls, the differences within the sexes being highly significant. Differences in creatine excretion after administration were less marked.

We conclude that in gyrate atrophy patients, formation of guanidinoacetate, creatine, and possibly phosphocreatine is inhibited at the transamidination step by the high concentrations of ornithine. Deficiency of the high-energy phosphates may underlie the pathogenesis of the eye and muscle atrophies.

INTRODUCTION

Gyrate atrophy of the choroid and retina is an autosomal recessive tapetoretinal dystrophy that begins clinically by the age of 5–9 yr as night blindness, myopia, and constriction of the visual fields (1). Patchy atrophic areas appear at the midperiphery of the ocular fundi and spread towards the optic disc. The visual fields narrow down to practical blindness by age 20–40 yr, progression being faster in males. The patients are otherwise healthy but rapid muscular performance may be subnormal for age. The number of type II muscle fibers is decreased, and parts of the remaining fibers contain atrophic areas, seen in electron microscopy to be filled with tubular aggregates (2). Among the tapetoretinal dystrophies gyrate atrophy is not uncommon, for numerous cases have been reported from several countries. However, our 41 Finnish homozygous patients seem to represent a concentration in a population of 4.7 million.

In 1973 we found 10- to 20-fold increases in ornithine concentration in the body fluids of these patients (3). Lymphocytes and fibroblast cultures confirmed a deficiency of the main catabolic enzyme of ornithine, ornithine ketoacid transaminase (ornithine transaminase, EC 2.6.1.13) in these patients (4–7). However, the mechanism of the tapetoretinal and muscular atrophy in the disease is obscure. Ornithine per se obviously is not the cause, as ornithine concentration is similarly increased in another disease without any eye or muscle changes (8). Arginine-glycine amidinotransferase (EC 2.1.4.1.), the rate-limiting enzyme in creatine production, is effectively inhibited by ornithine in rat kidney (9). To investigate this inhibition in vivo we administered arginine intravenously to patients with gyrate atrophy and found subnormal formation of guanidinoacetate, suggesting failure of creatine and subsequent phosphocreatine production as the cause of atrophy in the muscles and eyes.

METHODS

Experimental. 1.1 mmol/kg body wt arginine as a 5% (wt/vol) aqueous solution was administered intravenously in 5 min to each of six patients with gyrate atrophy (aged 15–43 yr, three females) and six healthy controls (aged 30–42 yr, two females) after a 10-h fast. Before arginine, the base-line values

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Received for publication 27 March 1980 and in revised form 5 June 1980.

J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/80/10/0684/04 $1.00*
Volume 66 October 1980 684–687

were measured during a 0.5–2-h infusion of 5% mannitol, 12 ml/kg per h. Adequate diuresis after the arginine administration was ensured by giving a similar infusion of mannitol for 4–6 h. Plasma samples for measurement of amino acids were taken at 15–30-min intervals for 4–6 h, and urine was collected in 30-min periods by voiding. Two male patients were unable to void at the expected intervals; for them, longer collection periods were used. The subjects remained in bed for the first 4 h and were then allowed to move. All subjects had given informed consent to the experiment, and they all remained symptom-free during the test.

Analytical. Plasma and urinary amino acids were measured with a Beckman 121 M amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.), and serum urea, creatinine, and glucose by routine methods. Serum creatine was measured by repeating the Jaffe creatinine measurement after acid hydrolysis. For quantitation of urinary guanidinoacetate, 50-ml portions of collections were deproteinized by acidification and the supernate lyophilized. The pellet was suspended in 99.5% alcohol to remove the mannitol, and the supernate relyophilized and suspended in 1.5 ml of 0.1 M citrate buffer, pH 3.5. A 1-ml portion was pipetted onto a 19 × 0.9-cm column, packed with Beckman Spherical Ion Exchange Resin, type M81. The guanidino derivatives were eluted with 0.1 M citrate buffer, 50 ml/h, pH 3.5. 4-ml fractions were collected, and guanidinoacetate (fractions 35–37) and creatine (fractions 17–19) were measured by the Voges-Proskauer reaction. The recoveries of creatine and guanidinoacetate were 90 and 85%, respectively.

RESULTS

The fasting plasma arginine concentration (mean±SD) in patients with gyrate atrophy (83.4±27.9 μmol/liter) did not differ significantly from the controls (72.5±12.8 μmol/liter). Arginine infusion caused an equal rise in plasma arginine in the two groups (Fig. 1). The hyperbolic disappearance curves were also similar, and in both groups at 6 h after the infusion, mean plasma arginine was still threefold higher than during fasting. Urinary arginine excretion behaved like the plasma curves, with two differences. In the basal state the patients excreted significantly more than the controls, and their excretion returned to the starting levels later (Table I). Thus during the 6-h collection period the patients lost more of the load into the urine (17.3±3.1%) than did the control subjects (10.1±3.5%).

Characteristically, the mean plasma ornithine concentration during fasting was 19.7 times as high in patients as in controls (Table I). After the arginine infusion, plasma ornithine peaked in both groups at 30–45 min. The molar increment in the patients was more than three times that in the controls. The disappearance curves from the plasma of patients and controls apparently initially followed first order kinetics, but in the controls a change was seen after 210 min, i.e., slightly before the starting plasma concentration was reached. The half-times for ornithine in plasma during the period of first order kinetics were 360 min (K = 0.0019) and 97 min (K = 0.0071) in patients and controls, respectively. In the controls, plasma arginine was

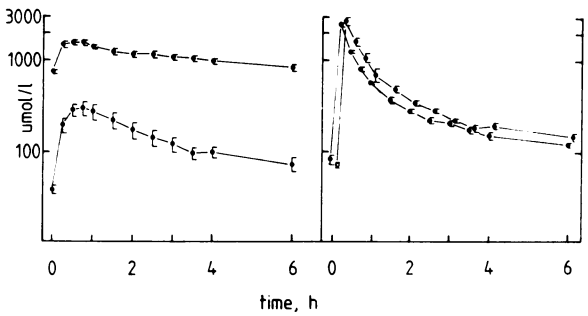

FIGURE 1 Plasma arginine and ornithine curves after a 5-min i.v. arginine infusion, 1.1 mmol/kg body wt in six patients with gyrate atrophy (●) and six controls (○). Plasma ornithine on the left and plasma arginine on the right. Mean±1 SEM is shown. Note the logarithmic scale on the ordinate.

consistently far above ornithine, but in the patients arginine concentration exceeded ornithine only at the 15-min measurement, i.e., immediately after the arginine infusion. Even during fasting the patients lost large amounts of ornithine into the urine but the arginine infusion led to massive ornithinuria, which in molar units accounted for a significant part of the amino acid infused (Table I). In the controls ornithine excretion was only slightly increased by the load.

The ornithine concentration needed for equal disappearance rate of the extracellular fluid ornithine was 5–10 times as high in the patients as in the controls. However, the disappearance rate of ornithine in the patients exceeded clearly the amount excreted into the urine.

Guanidinoacetate was constantly excreted into the urine by the controls, but only negligible amounts were detectable in the urine of the patients (Fig. 2). After the infusion, guanidinoacetate excretion increased rapidly in both groups. However, the rise in the patients was only a fraction of that in the controls, in whom the excretion rate was continuously 10- to 20-fold higher. In both groups urinary guanidinoacetate excretion was more rapid in the females. For plasma creatine, changes after the load were insignificant in all test subjects. Urinary creatine excretion seemed to be sex dependent, the females again having higher values. After the infusion, most controls had a brief peak in creatine excretion (Fig. 3). In the patients, either the peak was delayed and less marked, or excretion did not change at all. The values for patients and controls clearly overlapped.

DISCUSSION

Guanidinoacetate excretion in patients with gyrate atrophy of the choroid and retina was significantly slower than in controls, both during fasting and after an infusion of arginine. Even after an unphysiologic arginine infusion, the concentration of arginine in the

TABLE I

Characteristics of the Patients with Gyrate Atrophy and Controls

Subjects	Sex	Age	Weight	Fasting plasma		Fasting serum		Urinary creatinine	Renal clearance of		Fraction of arginine infusion* excreted in 6 h as	
				Ornithine	Arginine	Creatinine	Creatine		Ornithine	Arginine	Ornithine	Arginine
		yr	*kg*	*μmol/liter*				*mmol/24 h*	*ml/min/1.73 m²*	*ml/min/1.73 m²*	*%*	
Patients												
H.S.	m	11.7	29	717	88.0	25	0	5.3	1.27	0.68	18.4	16.5
R.K.	m	15.0	51	811	107.9	53	0	12.0	2.95	1.53	11.9	10.8
P.E.	m	26.8	58	915	67.0	49	0	8.1	0.55	0.48	26.2	16.3
R.P.	f	30.3	68	548	54.1	33	10	6.7	5.93	1.97	15.2	16.2
A.H.	f	31.3	56	678	51.7	43	6	5.7	1.71	0.92	28.9	21.8
A.T.	f	42.3	57	729	126.8	47	0	7.0	2.16	0.79	24.5	14.9
Mean		26.2	53.2	733	82.6	41.7	2.7	7.4	2.43	1.06	20.8	16.1
±SD		±11.3	±13.1	±124	±30.4	±10.6	±4.3	±2.4	±1.90	±0.57	±6.7	±3.5
Controls												
T.T.	m	25.6	69	39.7	78.8	104	6	13.3	0.50	0.21	1.0	12.1
J.R.	m	28.9	71	20.3	50.2	85	3	14.3	0.15	0.14	1.9	14.2
U.L.	f	30.6	55	39.4	72.7	70	15	12.3	0.29	0.14	1.8	17.1
O.S.	m	33.0	74	36.8	86.9	79	59	19.6	0.29	0.18	1.4	6.0
A.J.	f	35.1	66	42.3	67.1	74	16	12.3	0.28	0.18	1.9	13.0
J.H.	m	41.4	85	50.9	79.4	60	20	16.8	0.61	0.24	1.9	12.1
Mean		32.4	70.0	38.2	72.5	78.7	19.8	14.8	0.44	0.18	1.7	12.4
±SD		±5.5	±9.8	±10.0	±12.8	±15.0	±20.2	±2.9	±0.24	±0.04	±0.4	±3.6
P‡				≪0.0001	NS	<0.001	<0.1	<0.001	<0.1	<0.01	<0.0001	<0.1

* 1.1 mmol/kg body wt of L-arginine-HCl, as a 5% (wt/vol) aqueous solution, was infused in 5 min. Adequate diuresis before and after the load was ensured by infusion of 12 ml/kg per h of 5% mannitol. For details, see Methods.

‡ *P* for difference between the patients with gyrate atrophy and controls.

patients only temporarily exceeded that of ornithine in the plasma and presumably elsewhere in the body. Thus, one product of the action of arginine-glycine amidino-transferase was constantly present in abnormally high concentrations. We have shown that this amidino-transferase is competitively inhibited by ornithine, with an inhibition constant for ornithine in rat kidney homegenate of 0.253 mM (9). Our present results show that endogenous ornithine is also an effective inhibitor of guanidinoacetate production in man in vivo: the activity of the enzyme, which is rate-limiting for the vital synthesis of creatine, is also effectively

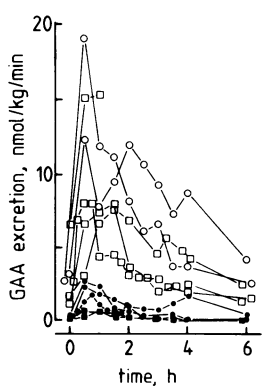

FIGURE 2 Guanidinoacetate (GAA) excretion after an infusion of 1.1 mmol/kg body wt of arginine i.v. in (■) male and (●) female patients and (□) male and (○) female controls.

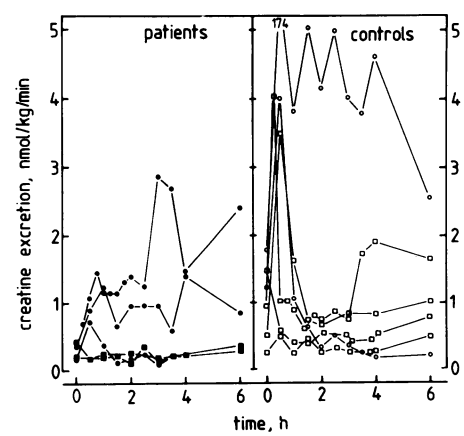

FIGURE 3 Creatine excretion after an intravenous infusion of 1.1 mmol/kg of arginine. The exceptionally high excretion in one control may reflect dietary factors or be caused by excessive exercise before the infusion. Symbols as in Fig. 2.

controlled by creatine concentration via an endproduct negative feedback inhibition, as both endogenous and exogenous creatine quickly repress transamidinase activity (10). We suggest that in patients with gyrate atrophy the inadequate guanidinoacetate production and further deficiency of creatine and phosphocreatine may be linked directly with the eye and muscle atrophies. In another disease with similar plasma ornithine concentrations, the transport of ornithine into the mitochondria is probably defective (11) and intramitochondrial ornithine concentration remains normal. In this situation the intramitochondrially located L-arginine-glycine amidinotransferase would not be inhibited by ornithine and endogenous creatine synthesis would not be restricted.

We did not find a good correlation between the excretion rates of guanidinoacetate, the rate limiting component of the creatine synthesizing reaction, and of creatine, although the patients, if compared with controls of their own sex, showed a clear tendency to low rates of creatine excretion. The guanidinoacetate formed is methylated effectively (12) and the creatine formed is rapidly trapped by the muscles (13). Thus both serum and urinary creatine concentrations probably better reflect the creatine lost from the muscles either during normal renovation of creatine stores, or as result of pathologic leakage, as in some myopathies or after trauma.

The five-carbon unit of arginine is mainly incorporated into proteins, recycled in the urea cycle, used in the synthesis of quantitatively unimportant amounts of polyamines, or degraded via ornithine, mainly through the ornithine ketoacid amino transferase. This enzyme is deficient in patients with gyrate atrophy. In the present experiments, the bulk of the arginine was rapidly converted into ornithine in both patients and controls. The metabolic clearance rate of ornithine from plasma and extracellular fluid was significantly reduced in the patients (Table I and Fig. 1). Because no other important routes for ornithine catabolism are known, our findings imply that the ornithine ketoacid aminotransferase in the patients functions efficiently at these elevated ornithine concentrations.

Because an arginine load in the patients seems to lead to a higher than normal increase in ornithine, any attempt to improve guanidinoacetate and creatine synthesis by feeding extra arginine to these patients seems to be inefficient, if not deleterious. If our hypothesis is valid, i.e., if deficient guanidinoacetate synthesis is the mechanism of the eye and muscle changes, two approaches remain for treatment of these patients. Stringent restriction of all protein in the diet would presumably result in a decrease in ornithine concentration and allow the arginine-ornithine ratio to revert to normal, so tending to normalize creatine production. On the other hand, exogenous creatine could be efficiently administered to the patients and would lead to adequate availability of the creatine moiety in the muscles and eyes, which are assumed to be critically dependent for their energy supply on phosphocreatine.

ACKNOWLEDGMENTS

The skillful technical assistance of Miss Marjatta Viikari and Mrs. Ritva Nurmi is acknowledged.

This work was supported by grants from the Foundation for Pediatric Research, Finland, the Sigrid Jusélius Foundation, and the Association of the Finnish Life Insurance Companies.

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