Guanidino Compounds in Guanidinoacetate Methyltransferase Deficiency, a New Inborn Error of Creatine Synthesis

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The first inborn error of creatine metabolism (guanidinoacetate methyltransferase [GAMT] deficiency) has recently been recognized in an infant with progressive extrapyramidal movement disorder. The diagnosis was established by creatine deficiency in the brain as detected by in vivo magnetic resonance spectroscopy and by defective GAMT activity and two mutant GAMT alleles in a liver biopsy. Here, we describe characteristic guanidino-compound patterns in body fluids of this index patient with GAMT deficiency. Concentrations of guanidino compounds (creatine and guanidinoacetate) and creatinine were determined by cation-exchange chromatography and by color reaction with picric acid, respectively, in urine, plasma, and cerebrospinal fluid (CSF). Creatine concentrations were low in plasma, CSF, and urine while guanidinoacetate concentrations were markedly elevated. Daily urinary creatinine excretion was low, whereas creatinine concentrations in random urine samples were not always discriminative. Guanidino compound to creatinine ratios were not informative, as low creatinine concentrations resulted in high values for all determined compounds. During a 22-month period of oral treatment with creatine-monohydrate, plasma and urinary creatine concentrations increased to levels high above the normal range, and daily urinary creatinine excretion—proportional to total body creatine—became normalized. Guanidinoacetate concentrations remained elevated even during additional substitution of ornithine, which inhibits guanidinoacetate synthesis in vitro. The results indicate that GAMT deficiency can be recognized noninvasively by determination of guanidino compounds (creatine and guanidinoacetate) in body fluids. A deficiency of creatine, but not an accumulation of guanidinoacetate, can be corrected by treatment with oral creatine substitution.
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IN HUMANS, creatine is synthesized in the liver and pancreas,[1] involving arginine:glycine amidinotransferase and guanidinoacetate methyltransferase ([GAMT] EC 2.1.1.2) as enzymes. GAMT catalyzes the final step in the biosynthesis of creatine by transferring a methyl group from S-adenosylmethionine to guanidinoacetate.[2] Creatine is used in muscle and nerve tissue, where the pool of creatine/creatine phosphate together with creatine kinase and adenosine triphosphate/monophosphate play an important role in the storage and transmission of phosphate-bound energy. Creatine is converted by nonenzymatic cyclization to creatinine, with a daily turnover of 1.5% of body creatine.[3] For maintenance of the body pool, 1 to 2 g (7.6 to 15.2 mmol) creatine—equivalent to the daily urinary excretion of creatinine—must be provided from endogenous synthesis or from dietary sources.[1]

GAMT deficiency is the first inborn error of creatine synthesis in man, and its clinical[4] and molecular[5] features, as well as the favorable response to oral creatine replacement therapy,[6] have recently been described in the first patient with this disorder. Here, we report characteristic guanidino-compound (creatine and guanidinoacetate) and creatinine patterns in the body fluids from this same patient diagnosed with GAMT deficiency.

SUBJECTS AND METHODS

Urine, plasma, and cerebrospinal fluid (CSF) were available from a male infant with GAMT deficiency. Samples obtained before treatment with creatine monohydrate were evaluated at ages 14 and 22 months. In addition, samples obtained during a 22-month course of treatment with creatine monohydrate (4 and 8 g/d) and during a 12-week period of additional substitution with ornithine HCl (8 g/d) were evaluated. Both substances were administered orally in eight single doses with 2-hour intervals between 6 AM and 10 PM, and with an 8-hour interval between 10 PM and 6 AM. Details about the clinical features of the patient and the favorable clinical response to creatine substitution have been described elsewhere.[4,6]

Determination of Metabolites in Urine, Plasma, and CSF

Creatine, guanidinoacetate, and other guanidino-compounds were assayed using a Biotronic LC 5001 (Biotronic, Maintal, Germany) amino acid analyzer adapted for guanidino-compound determination. Guanidino compounds were separated over a cation-exchange column using sodium citrate buffers, and were detected with the fluorescence ninhydrin method as previously reported in detail.[7]

Serum and urinary creatinine were determined using a Beckman 2 (Irvine, CA) creatinine analyzer by a color reaction with picric acid. With this method, there was no unspecific reaction with standard solutions of guanidinoacetate or creatine.

Plasma ornithine concentrations were measured by ion-exchange chromatography on a Biotronic LC3000 amino acid analyzer.

RESULTS

Guanidino Compounds in Plasma, CSF, and Urine Before Treatment With Creatine Monohydrate

In plasma, the most pronounced findings were extremely low concentrations of creatine and creatinine and extremely high concentrations of guanidinoacetate, with values more than 10-fold higher than normal. Homoarginine was constantly increased, besides various less pronounced abnormalities, including low arginine and high γ-guanidinobutyrate concentrations. The abnormal plasma patterns were also reflected in CSF; however, for guanidinoacetate and creatine, the abnormalities in CSF were even more pronounced, with values more than

100-fold higher (guanidinoacetate) or lower (creatine) than normal. In random urine samples, an extremely high guanidinoacetate concentration (2,224 to 3,987 μmol/L; normal, 63.4 to 429) was the most specific abnormality, whereas creatine concentrations were within the low-normal range (56.9 to 106 μmol/L; normal, 46 to 5,250). Due to consistently low urinary creatinine concentrations (1,060 to 2,060 μmol/L; normal, 1,800 to 4,400), guanidino-compound/creatinine ratios were unspecifically high for all measured metabolites (Table 1).

Guanidino Compounds in Plasma and Urine During Treatment With Creatine Monohydrate and Ornithine HCl

As a consequence of GAMT deficiency, endogenous synthesis of creatine is defective. On the assumption that exogenous (intestinally absorbed) creatine is not degraded in the liver and is as accessible to muscle and brain as the endogenous compound, we began oral replacement therapy at age 23 months with creatine monohydrate 4 g/d (305 to 370 mg/kg body weight/d) and doubled the creatine monohydrate dose (8 g/d, 600 to 640 mg/kg body weight/d) at age 35 months.

Plasma creatine concentrations increased from low (0.39 to 1.24 μmol/L; normal, 86.9 ± 18.5) to extremely high (270 to 763 μmol/L) values, indicating sufficient intestinal absorption of creatine monohydrate (Fig 1). The increase of plasma creatine was paralleled by normalization of low plasma creatinine concentrations (plasma creatinine before treatment, 7 μmol/L [0.08 mg/dL]; during treatment, 35.4 μmol/L [0.4 mg/dL]; normal,[8] 17.7 to 35.4 [0.2 to 0.4]). Guanidinoacetate decreased gradually from 12.9 to 25.7 μmol/L to 5.76 to 4.32 μmol/L, but values remained increased at all times (normal, 0.832 ± 0.315) (Fig 1). The increased homoarginine concentrations (2.43 to 3.73 μmol/L) also decreased (minimum, 1.21 to 1.48 μmol/L), but also did not return to normal (normal, 0.66 ± 0.18). These patterns were not changed when the creatine monohydrate dose was doubled.

After 4 weeks of therapy, the low urinary creatinine excretion (8.9 to 12.4 μmol [1.0 to 1.4 mg]/kg body weight/d; normal,[9] 71 to 177 [8 to 20]/kg body weight/d) normalized (147 μmol [16.6 mg]/kg body weight/d), indicating restoration of the body creatine pool (Fig 2A). Simultaneously, urinary creatine increased to values far above the upper-normal range (65,613 to 111,810 μmol/L), indicating renal overflow due to high-dose oral creatine substitution. Urinary guanidinoacetate concentrations decreased gradually from extremely high values (2,224 to 3,987 μmol/L) before creatine substitution to lower but still elevated values (1,120 μmol/L; normal, 63.4 to 429) (Fig 2B).

On the rationale that guanidinoacetate synthesis should be reduced by inhibition of arginine:glycine amidinotransferase with high-dose ornithine,[10] a 12-week trial (42 to 45 months of age) with additional substitution of ornithine HCl (8 g/d, 600 to 640 mg/kg/body weight/d) was performed. Plasma ornithine concentrations increased from 74 to 89 μmol/L before ornithine substitution to 250 to 400 μmol/L during ornithine substitution (normal, 10 to 163), but there was no parallel decrease in plasma (Fig 1) and urinary (Fig 2B) guanidinoacetate concentrations.

DISCUSSION

GAMT deficiency results in defective endogenous creatine biosynthesis with depletion of body creatine and accumulation of guanidinoacetate, which is a substrate to the defective enzyme. Accordingly, in this first patient with GAMT deficiency, high concentrations of guanidinoacetate and low concentrations of creatine were characteristic findings in urine, plasma, and CSF. In addition, daily (24-hour) urinary creatinine excretion was extremely low, reflecting depletion of the body creatine pool. Creatinine excretion is directly related to body creatine,[11] and assessment of it in 24-hour urine may be helpful in the diagnosis of defects in creatine biosynthesis. However, this test may not be reliable in newborns and very young infants, since animal experiments indicate that the maternal intrauterine

Table 1. Guanidino-Compound Levels in a Patient With GAMT Deficiency Before Treatment With Creatine Monohydrate in Plasma (μmol/L), CSF (μmol/L), and Random Urine Samples (μmol/g creatinine; μmol/L)

	Plasma			CSF			Urine					
	Patient		Control	Patient		Control	Patient				Control (n = 17)	
Guanidino Compound	14 Mo	22 Mo	(n = 17)	11 Mo	22 Mo	(n = 15)	14 Mo		22 Mo			
							μmol/g	μmol/L	μmol/g	μmol/L	μmol/g	μmol/L
α-K-δ-GVA	<0.035	0.07	<0.035-0.215	<0.018	<0.018	<0.018-0.050	40.2	5.63	26.1	7.04	<DL-40	<DL-10
GSA	0.150	0.230	0.283 ± 0.136	0.150	0.150	0.052 ± 0.022	266	37.3	231	62.4	24-120	2.90-27.3
CT	0.39	1.24	86.9 ± 18.5	0.280	0.530	38.8 ± 4.76	407.0	56.9	394	106	250-15,000	46-5,250
GAA	12.9	20.7	0.832 ± 0.315	10.6	12.7	0.055 ± 0.032	15,889	2,224	14,767	3,987	400-1,600	63.4-429
α-N-AA	<0.015	<0.015	<0.019-0.270	<0.007	<0.007	<0.007-0.045	212	29.7	535	117	18-70	3.16-14.1
ArgA	<0.015	<0.070	<0.019-0.068	<0.007	<0.007	<0.007-0.035	53.7	7.52	94.4	25.5	4-30	0.440-7.81
β-GPA	<0.013	<0.013	<0.013	<0.007	<0.007	<0.007	14.0	1.96	3.96	1.07	<DL	<DL
CTN	5.21	3.33	20.2 ± 7.49	<1.0	<1.0	34.9 ± 5.59		1,060		2,060		1,800-4,400
γ-GBA	0.080	0.050	<0.019	0.080	0.050	<0.006-0.065	594	83.2	486	131	15-60	2.65-10.7
Arg	19.1	43.9	97.5 ± 26.6	11.4	16.9	21.3 ± 4.37	255	35.7	302	81.5	20-120	3.64-20.6
HArg	2.43	3.73	0.660 ± 0.178	0.800	0.640	0.205 ± 0.072	19.8	2.77	46.3	12.5	<DL-15	<DL-3.42
G	0.260	0.160	<0.200-0.390	<0.030	0.060	<0.030-0.100	175	24.5	90.7	24.5	10-90	2.17-17.4
MG	<0.02	<0.02	<0.06	<0.010	<0.010	<0.010	9.7	1.36	8.93	2.41	<DL-10	<DL-3.33

NOTE. Plasma and urine samples were taken at ages 14 and 22 months; CSF samples were obtained at 11 and 22 months. Normal values were obtained from respective samples of age-matched controls.

Abbreviations: α-K-δ-GVA, α-K-δ-guanidinovalerate; GSA, guanidinosuccinate; CT, creatine; GAA, guanidinoacetate; α-N-AA, α-N-acetylarginine; ArgA, argininic acid; β-GPA, β-guanidino propionate; CTN, creatinine; γ-GBA, γ-guanidinobutyrate; Arg, arginine; HArg, homoarginine; G, guanidine; MG, methylguanidine; DL, detectable limit.
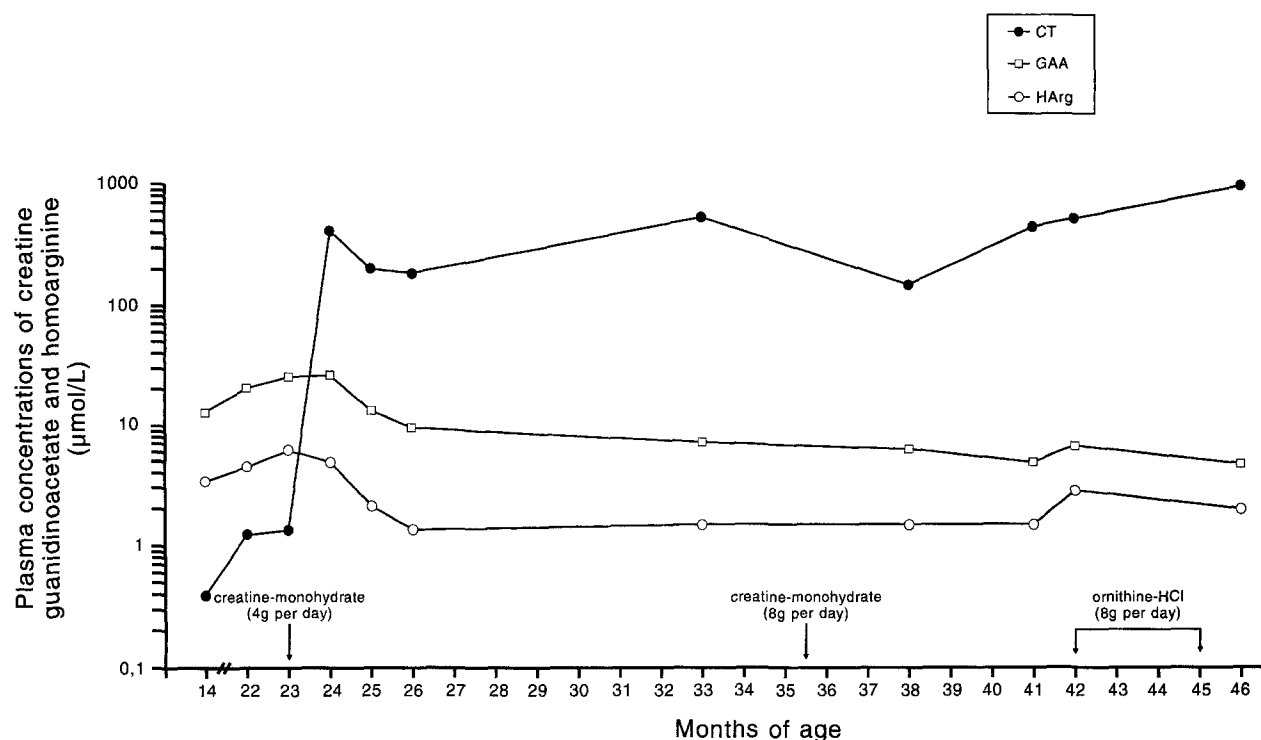
Fig 1. Plasma concentrations of creatine (●), guanidinoacetate (□), and homoarginine (○) in a patient with GAMT deficiency before treatment with creatine monohydrate (samples taken at 14, 22, and 23 months of age) and during a 22-month time course (23 to 46 months of age) of treatment with creatine monohydrate (4 and 8 g/d). Ornithine HCl was additionally substituted between 42 and 45 months of age. Normal range: creatine, 86.9 ± 18.5 μmol/L; guanidinoacetate, 0.832 ± 0.315 μmol/L; homoarginine, 0.660 ± 0.178 μmol/L.

creatine supply[12] provides the newborn with a full creatine pool, and depletion will follow a slow process with a daily reduction of the creatine pool equal to 1.5% of creatine turnover.[3]

In random urine samples, an elevated guanidinoacetate concentration was specific. Calculation of guanidino-compound to creatinine ratios showed elevated values for all metabolites. These artificially high ratios are explained by the fact that in primary creatine deficiency, creatinine excretion is consistently low independently of the concentration of the renal urinary filtrate. Therefore, determination of urinary guanidino compounds, especially when related to urinary creatinine concentrations, are less informative than the concentrations measured in plasma and CSF.

Oral substitution of creatine monohydrate resulted in a marked increase of plasma creatine concentrations. Final plasma creatine concentrations were markedly higher than normal, with peak values 1 hour after oral creatine administration. The high plasma creatine concentrations and normalization of plasma and urinary creatinine concentrations clearly indicate that substituted creatine was sufficiently absorbed in the intestine and regularly metabolized by muscle and brain, the major sites of creatine utilization.

Guanidinoacetate is synthesized from arginine and glycine by the activity of arginine:glycine amidinotransferase, the regulating enzyme of creatine biosynthesis. The gene expression of this enzyme is mainly controlled by a creatine-dependent negative-feedback mechanism.[13] In our patient, repression of (highly expressed) arginine:glycine amidinotransferase activity by exogenous creatine led to a decrease but not normalization of

guanidinoacetate concentrations in body fluids. Further reduction of guanidinoacetate concentrations via competitive inhibition of arginine:glycine amidinotransferase activity by additional substitution with high-dose ornithine[10] failed. The persistence of guanidinoacetate accumulation despite normalization of body creatine is explained by the block in the main metabolic pathway of guanidinoacetate caused by defective GAMT activity. The parallel changes of plasma guanidinoacetate and homoarginine levels during therapeutic follow-up evaluation confirm previous in vitro studies showing that both homoarginine and guanidinoacetate are synthesized by kidney L-arginine:glycine amidinotransferase.[14]

The extremely high guanidinoacetate concentrations in CSF, exceeding normal values by a factor of 200, deserve special attention. CSF concentrations of guanidinoacetate were approximately the same as the respective plasma values, indicating free passage of guanidinoacetate across the blood-brain barrier. In normal individuals, guanidinoacetate concentrations are significantly lower in CSF than in plasma, suggesting immediate metabolism of guanidinoacetate after its passage across the blood-brain barrier. Immediate metabolism of guanidinoacetate may be effected by its conversion to creatine via cerebral (neuronal) GAMT activity, which has already been proposed in experimental studies.[15,16] Accordingly, in this patient with GAMT deficiency, defective cerebral enzyme activity explains the observed severe accumulation of guanidinoacetate in CSF. Our patient improved drastically on oral creatine substitution,[6] but neurologic symptoms were not completely reversible, despite normalization of the cerebral creatine pool. One reason
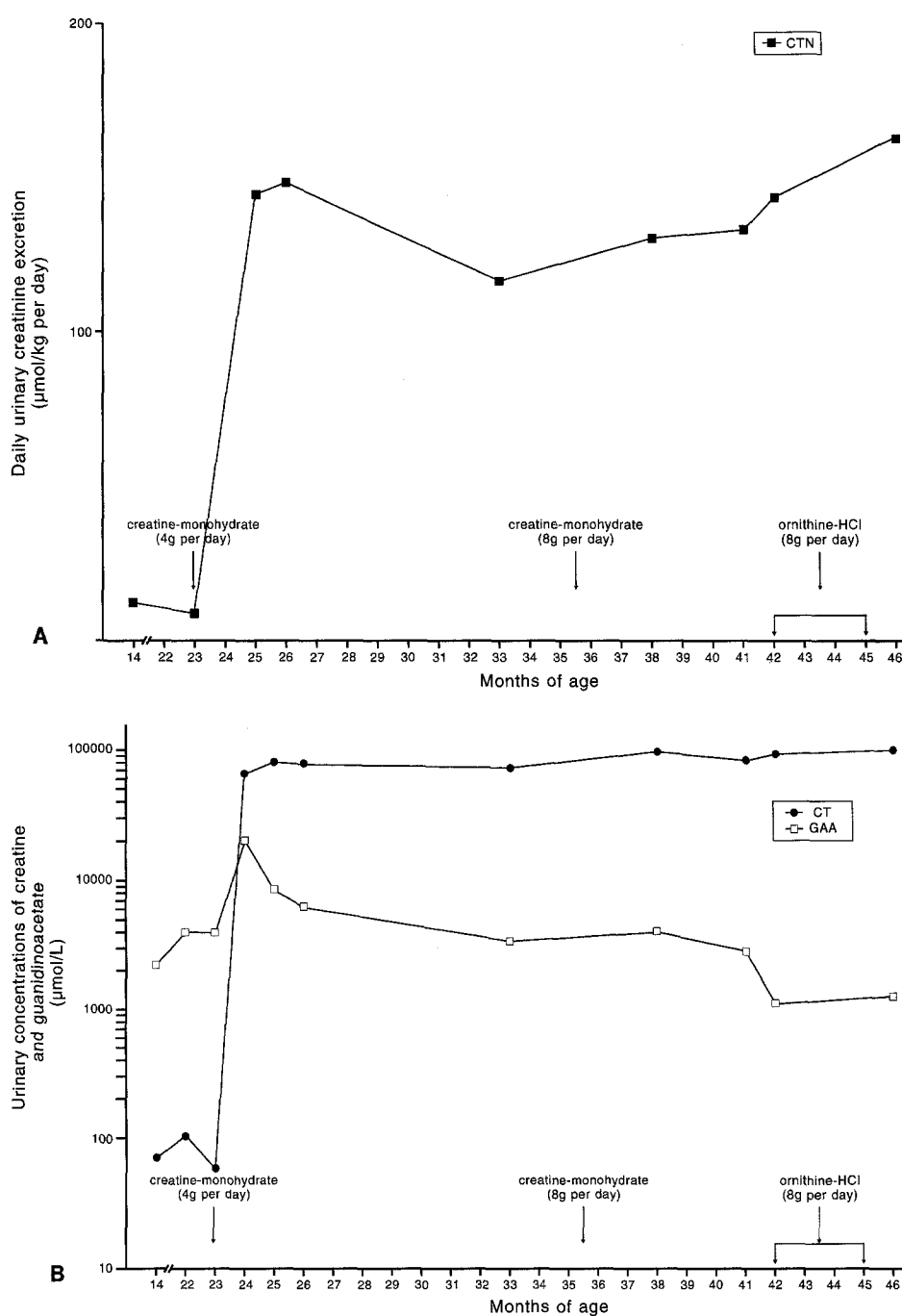
Fig 2.   Daily urinary excretion of creatinine (■) as a measure of total body creatine and (B) urinary concentrations of creatine (●) and guanidinoacetate (□) in a patient with GAMT deficiency. Samples were taken before treatment with creatine monohydrate (at 14, 22, and 23 months of age) and during a 22-month time course of treatment with creatine monohydrate (4 and 8 g/d, 24 to 46 months of age). Normal range: daily urinary creatinine excretion, 71 to 177 μmol/kg body weight/d)[9]; urinary creatine, 46 to 5,250 μmol/L; urinary guanidinoacetate, 63.4 to 429 μmol/L.

for the incomplete reversal of neurologic symptoms during creatine replacement therapy seems to be the accumulation of guanidinoacetate, which is neurotoxic in high concentrations.[17] Another reason may be irreversible brain damage acquired before creatine substitution therapy.

The improvement of biochemical and clinical symptoms during oral substitution of creatine is an indication that GAMT deficiency is a treatable disorder. Therefore, the development of

simple screening methods for early detection is of major importance. As shown in this index patient, accumulation of guanidinoacetate is highly specific for GAMT deficiency, and routine determination of this compound should become possible by chromatography,[7,18] color reaction,[19,20] or gas chromatographic/mass spectrometric methods.[21] The experience in this index patient suggests that plasma and CSF are the best biological samples to be investigated.

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