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Short Communication

Upregulation of respiratory chain enzymes in guanidinoacetate methyltransferase deficiency

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In guanidinoacetate *N*-methyltransferase (GAMT) deficiency (McKusick 601240) synthesis of creatine is compromised. This leads to intracellular depletion of creatine phosphate, which serves as a short-term 'energy buffer', especially in organs with high, fluctuating energy demand like the brain. Therefore, cells from GAMT-deficient patients have to rely almost exclusively on ATP produced directly by the mitochondrial respiratory chain. This increased metabolic strain on the respiratory chain may lead to compensatory upregulation of respiratory chain enzymes. We therefore examined activities of respiratory chain enzymes in fibroblasts from a patient with GAMT deficiency.

MATERIALS AND METHODS

Skin fibroblasts were taken from the first patient diagnosed with GAMT-deficiency (Stöckler et al 1996). Cells were cultured in MEM medium with 10% fetal calf serum FCS) or, for creatine-free incubations, in Waymouth medium (Life Technologies) without FCS for 21 days. Fibroblasts were incubated in Waymouth medium supplemented with 1 mmol/L creatine, which is the approximate concentration reached *in vivo* in patients supplemented with creatine for 14 days in an attempt to reverse the effect of creatine depletion.

Respiratory chain enzyme activities, including active regulation of the mitochondrial ATP synthase (complex V) in response to metabolic conditions of the cells, were determined spectrophotometrically after sonication as described in detail in a previous study (Das and Kohlschütter 1999).

Creatine phosphate, ATP and ADP were measured in the cell homogenate quenched in DMSO (+3 mmol/L EDTA) by bioluminescence using a luciferin/luciferase test kit (Bioorbit Turku, Finland) according to published methods (Harris and Slater 1975; Holm-Hansen and Karl 1978; Lundin 1978).

Table 1 High-energy phosphates (nmol/mg protein) and respiratory chain enzymes (nmol/mg protein per min) in control (healthy) fibroblasts and fibroblasts from GAMT-deficient patients incubated with and without creatine

Fibroblasts	*ATP*	*Creatine phosphate*	*Complex I + III*	*Complex V*
Controls				
+creatine (MEM + 10% FCS)	52	6	105(58–125)	181(140–230)
−creatine (Waymouth)	48	6	66	223
GAMT-deficient				
Waymouth + creatine	43	6	111	143
Waymouth only	42	0*	230*	327*

Four Petri dishes from each cell line were incubated with and without creatine with the exception of healthy + creatine, where 31 Petri dishes were assayed for enzyme activities. Values in parentheses refer to range
* Significant difference versus control + creatine ($p < 0.05$)

RESULTS

No differences in fibroblasts from GAMT-deficient patients and healthy controls incubated under the same conditions could be seen by light microscopy. Biochemical results are summarized in Table 1.

In GAMT-deficient fibroblasts incubated without creatine, creatine phosphate was absent. When the incubation medium was supplemented with 1 mmol/L creatine for 1 week, creatine phosphate concentration increased to 50% of normal (results not shown); after 2 weeks creatine phosphate content was normalized. ATP content in GAMT-deficient cells was slightly lower than in normal cells irrespective of the incubation conditions. No differences in ADP content could be found between GAMT-deficient and normal cells (results not shown).

Activities of respiratory chain complexes II + III and complex IV were normal in GAMT-deficient cells incubated in MEM with or without creatine supplementation (results not shown). In GAMT-deficient cells incubated in a medium free of creatine (Waymouth), activities of complexes I + III and of mitochondrial ATP synthase (complex V) were increased. When the incubation medium was supplemented with 1 mmol/L creatine (or 10% FCS, results not shown) for 2 weeks, enzyme activities were restored to normal. When GAMT-deficient fibroblasts were incubated for 1 week in the presence of creatine complexes I + III and ATP synthase, activities were still increased (results not shown). Incubation of normal fibroblasts in a medium devoid of creatine did not lead to elevation of respiratory chain activities above normal values.

DISCUSSION

In cells from patients with GAMT-deficiency, the function of creatine phosphate as an 'energy (ATP) buffer' is lost. Therefore, the demand on the mitochondrial respiratory chain to supply ATP in aerobic tissues is increased. This increased metabolic load is reflected in upregulation of respiratory chain complexes I + III and of mito-

chondrial ATP synthase (complex V) in GAMT-deficient fibroblasts. These results are in line with previous observations in rats fed with guanidinopropionic acid, which inhibits creatine uptake into cells, leading to intracellular creatine phosphate depletion. These animals were reported to have an increased respiratory chain activity of mitochondria from different tissues (Freyssenet et al 1994; O'Gorman et al 1996). In our experiments, supplementation of the GAMT-deficient cells with 1 mmol/L creatine led to complete restoration of normal respiratory chain activities. Restoration of enzyme activities took about 2 weeks, which may mean that down-regulation of enzyme activities involves changes in protein synthesis and/or protein degradation of respiratory chain complexes rather than action of small regulatory proteins like the naturally occurring inhibitor protein IF_1 (Lippe et al 1988) or the calcium binding inhibitor protein (Yamada and Huzel 1988).

It is not clear what causes the reversible upregulation of respiratory chain enzymes in GAMT-deficient cells. Increased intracellular levels of guanidinoacetate or decreased concentrations of creatine inside the cell may mediate the transition in enzyme activities.

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