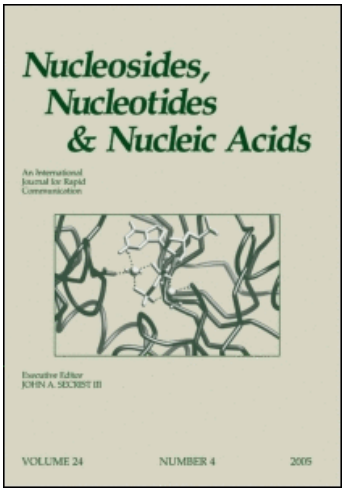

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Adenylosuccinate Lyase Deficiency: Study of Physiopathologic Mechanism(s)

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ABSTRACT

Nucleotide concentrations were normal in adenylosuccinate lyase-deficient fibroblasts, and the succinylpurines were not toxic for cultured neuronal cells.

Key Words: Adenylosuccinate; Succinylpurines; Physiopathologic mechanisms; Neurotoxicity.

INTRODUCTION

Adenylosuccinate lyase (ADSL) catalyses the scission of succinylaminoimidazolecarboxamide (SAICA) ribotide into AICA-ribotide, and the formation of AMP from adenylosuccinate (S-AMP). ADSL deficiency, first described in 1984,[1] provokes accumulation in body fluids of the succinylpurines, SAICA-riboside and succinyladenosine (S-Ado), the dephosphorylated derivatives of the substrates of the enzyme. ADSL-deficient patients display variable but most often profound psychomotor delay,

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with often epilepsy and/or autistic features.[2] In severely retarded patients, S-Ado:SAICA-riboside ratios are close to 1. In moderate or mildly retarded children, concentrations of SAICA-riboside are similar but S-Ado:SAICA-riboside ratios reach 2–4. These observations have led to the hypothesis that SAICA-riboside is the neurotoxic compound, and that S-Ado could counteract its noxious effects. To unravel the physiopathologic mechanisms in ADSL deficiency we have measured nucleotide concentrations in patients' fibroblasts and evaluated whether the succinylpurines are neurotoxic on primary rat cortical neurons.

MATERIALS AND METHODS

Skin fibroblasts (passage number 6 to 30) were cultured for 10 days in Dulbecco's modified Eagle's medium with 1 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% fetal bovine serum (FBS), dialysed or undialysed. For nucleotide measurements, cells from 1 flask (175 cm^2) were harvested by trypsinisation and centrifugation, the cell pellet washed in PBS and disrupted with 10% PCA. The neutralized supernatant was analysed by HPLC. Primary cultures of cortical neurons were prepared from 17-day-old Wistar rat embryos. Cells were plated either in 96-well culture dishes or on glass coverslips pretreated with poly(L-lysine) (10 μg/ml in PBS) and cultured for 10 days in NEUROBASAL^{TM} medium supplemented with 2% B-27 and 0.5 mM L-glutamine. Neuronal cultures (up to 98% of neurons) displayed high differentiation and survival rates, measured by the MTT assay. For calcium measurements, neurons were loaded for 1 h at room temperature with 2 μM Fura-2 AM. Rinsed coverslips were then mounted in a perfused microscope chamber (1 ml). Images of fluorescence emitted at 510 nm after excitation at 340 nm and 380 nm were saved every 10 to 20 msec, and presented as changes in the fluorescence ratio R340/380 against time.

RESULTS AND DISCUSSION

Nucleotide concentrations in standard culture conditions, i.e. in the presence of 10% undialysed FBS, and in dialysed serum, were similar in patient and in control fibroblasts (Table 1). This might be explained by the partial deficiency of ADSL in

Table 1. Nucleotide concentrations (nmol/mg protein) in a mildly affected patient and in 7 severely affected ADSL-deficient patients.

	Undialysed serum		Dialysed serum	
Patient	AMP + ADP + ATP	GDP + GTP	AMP + ADP + ATP	GDP + GTP
Control	25.9 ± 1.4	5.7 ± 0.3	27.4 ± 1.6	5.9 ± 0.4
Mildly retarded	22.7	5.3	23.2	4.9
Severely retarded	24.7 ± 2.3	5.3 ± 0.4	24.6 ± 1.7	5.5 ± 0.4

these cells, still allowing sufficient de novo synthesis in the absence of salvage pathway substrates, removed by dialysis.

On the basis of structural analogy of the succinylpurines with N-methyl-D-aspartate (NMDA), we tested their neurotoxicity on rat cortical neurons after 10 days of differentiation. After a 48 h treatment, glutamate 100 and 300 μM induced a neuronal loss of 30% and 66%, respectively. MK801, an antagonist of NMDA receptors had no effect alone but reversed the toxicity of glutamate. In these conditions, neither SAICA-riboside nor S-Ado affected neuronal viability, and did not modify glutamate excito-toxicity. Intracellular calcium, similar to viability, was found to respond to glutamate but not to the succinylpurines. This indicates that the succinylpurines do not exerce direct neurotoxic effects in these conditions.

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