The Protective Effect of L-Cysteine and Glutathione on the Adult and Aged Rat Brain (Na\(^{+},\)K\(^{+}\))-ATPase and Mg\(^{2+}\)-ATPase Activities in Galactosemia In Vitro

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The aim of this study was to evaluate whether the addition of the antioxidants L-cysteine (Cys) or the reduced glutathione (GSH) could reverse the alterations of brain total antioxidant status (TAS) and the modulated activities of the enzymes (Na\(^{+},\)K\(^{+}\))-ATPase, and Mg\(^{2+}\)-ATPase in adult or aged rat brain homogenates induced by galactosemia in vitro. Mixtures A (mix. A: galactose-1-phosphate (Gal-1-P, 2 mM) plus galactitol (Galtol, 2 mM) plus galactose (Gal, 4 mM) = classical galactosemia) or mixture B (mix. B: Galtol (2 mM) plus Gal (1 mM) = galactokinase deficiency galactosemia) were preincubated in the presence or absence of Cys (0.83 mM) or GSH (0.83 mM) with adult or aged brain homogenates at 37°C for 1 h. TAS and the enzyme activities were determined spectrophotometrically. Mix. A or mix. B preincubation with the adult brain resulted in a significant (Na\(^{+},\)K\(^{+}\))-ATPase inhibition (%30%) and a Mg\(^{2+}\)-ATPase stimulation (%300% and %33%, respectively), whereas lower modifications of the enzyme activities (\(p<0.001\)) were found in the aged brain. Gal mixtures decreased TAS by 40% (\(p<0.001\)) and by 20% (\(p<0.01\)) in adult and aged samples, respectively. The antioxidants significantly increased TAS resulting in the reversion of (Na\(^{+},\)K\(^{+}\))-ATPase inhibition and Mg\(^{2+}\)-ATPase stimulation by mix. B only. The inhibitory effect of Gal and its derivatives on brain (Na\(^{+},\)K\(^{+}\))-ATPase and their stimulatory effect on Mg\(^{2+}\)-ATPase are being decreased with age, probably due to the production of free radicals. Cys and GSH increased TAS resulting in a reversion of the inhibited (Na\(^{+},\)K\(^{+}\))-ATPase in both models of the in vitro galactosemia and the stimulated Mg\(^{2+}\)-ATPase in galactokinase deficiency galactosemia only.

Key words: Adult rat brain; aged rat brain; (Na\(^{+},\)K\(^{+}\))-ATPase; Mg\(^{2+}\)-ATPase; galactosemia; antioxidants.

INTRODUCTION

Three inherited disorders of galactose (Gal) metabolism resulting in galactosemia have been described (Berry and Segal, 2000; Gitzelmann, 1995; Schulpis et al., 1997). The diagnosis is suggested by the detection of Gal and galactose-1-phosphate (Gal-1-P) in blood and is established by demonstration of the enzymes in the peripheral blood cells (Elsas et al., 1995). Moreover, transferase deficiency (classical galactosemia) is associated with the accumulation of high Gal-1-P (4–16 mM) in the tissues in addition to Gal and galactitol

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(Galtol) resulting in mental retardation, seizures, cataract, renal and liver insufficiency, whereas in galactokinase deficiency galactosemia only high levels of Gal and Galtol are observed (Berry and Segal, 2000; Schulpis et al., 1997).

\((\text{Na}^+,\text{K}^+)-\text{ATPase}\) is the enzymatic basis of univalent cation transport (Sweadner and Goldin, 1980). It is implicated in neural excitability (Sastry and Phillis, 1977), metabolic energy production (Mata et al., 1980), uptake and release of catecholamines (Bogdanski et al., 1968), and \(\text{Na}^+\)-dependent tryptophan uptake system (Herrero et al., 1983). Additionally, the role of brain \(\text{Mg}^{2+}\)-ATPase is to control the intracellular \(\text{Mg}^{2+}\) concentration, changes which can modulate the activity of \(\text{Mg}^{2+}\)-dependent enzymes and regulate rates of protein synthesis and cell growth (Sanui and Rubin, 1982).

In our previous studies (Tsakiris et al., 2002a,b), suckling rat brain \((\text{Na}^+,\text{K}^+)-\text{ATPase}\) was inhibited, whereas \(\text{Mg}^{2+}\)-ATPase was greatly activated in the in vitro galactosemia. Furthermore, in our very recent study (Tsakiris et al., 2004), total antioxidant status (TAS) was determined decreased. The addition of the antioxidants \(\text{L}-\text{cysteine (Cys, 0.83 mM)}\) or reduced glutathione (GSH, 0.83 mM) in the above condition resulted in a reversion of the inhibited suckling rat brain \((\text{Na}^+,\text{K}^+)-\text{ATPase}\) in both the in vitro classical galactosemia and galactokinase deficiency galactosemia. On the contrary, the activated suckling rat brain \(\text{Mg}^{2+}\)-ATPase by the latter galactosemic condition was reversed only. Moreover, GSH concentration was found reduced in the aged brains, whereas the production of free radicals was increased (Benzi et al., 1989; Hazelton and Lang, 1980).

Galactosemia appears to be an appropriate disorder for routine newborn screening, as almost normal outcome can be assumed by early neonatal detection and presymptomatic or early postsymptomatic therapy (Schulpis et al., 1997). Since galactosemic patients have been grown elder and they usually discontinue their therapeutic diet (nutrients containing lactose and/or galactose must be avoided), it was of interest to attempt to evaluate, for the first time, the effect of Gal and its derivatives on the adult and aged rat brain \((\text{Na}^+,\text{K}^+)-\text{ATPase}\) and \(\text{Mg}^{2+}\)-ATPase activities with the simultaneous estimation of brain TAS. Furthermore, we tried to investigate whether the addition of Cys or GSH could reverse to normal the above mentioned rat brain modulated enzyme activities by the in vitro galactosemia.

**MATERIALS AND METHODS**

**Animals**

Albino adult (4 months) and aged (25 months) Wistar male rats (Saint Savvas Hospital, Athens, Greece) were used in all the experiments. Body weight was \(220 \pm 15\) g (mean \(\pm\) SD) for adult and \(325 \pm 38\) g for aged rats. The rats were housed four in a cage, at a constant room temperature \((22 \pm 1^\circ\text{C})\) under a 12/12 h (L/D) (light 08:00–20:00 h) cycle and acclimated 1 week before use. Food and water were provided ad lib. Animals were cared for in accordance with the principles of the *Guide to the Care and Use of Experimental Animals* (Committee on Care and Use of Laboratory Animals, 1985).

**Tissue Preparation**

Animals were sacrificed by decapitation. Whole brains from five rats were rapidly removed, weighed, and thoroughly washed with isotonic saline. Tissues were homogenized in