

SODIUM CALCIUM EXCHANGER AND SODIUM DEPENDENT GLUTAMATE TRANSPORTERS INVOLVEMENT IN ENERGY PRODUCTION, IN RAT HEART

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Ca²⁺ homeostasis controls the mitochondrial activity modulating Krebs cycle enzymes. One of the essential regulators of Ca²⁺ homeostasis in cardiomyocytes is the Na⁺-Ca²⁺ exchanger (NCX). This antiporter couples the uphill extrusion of Ca²⁺ to the entrance of Na⁺ and vice versa, depending on membrane potential and intracellular concentrations of Na⁺ and Ca²⁺ ions. In addition, dicarboxylic aminoacid glutamate and aspartate regulate aerobic energy production as participants of the malate-aspartate shuttle. Actually, in rat cardiomyocytes, it has been demonstrated the expression of GLAST (Glutamate/Aspartate transporter), one of the 5 members of the Na⁺-dependent Glutamate Transporter (GluT) family that includes: GLT1 (glutamate transporter 1); EAAC1 (excitatory amino-acid carrier 1); excitatory amino-acid transporter 4 (EAAT4) and excitatory amino-acid transporter 5 (EAAT5). These transporters couple the inward movement of a molecule of glutamate with three Na⁺ and one H⁺ ions, and the outward transport of one K⁺ ion. Both NCX and GluTs activities can be affected by the transmembrane Na⁺ gradients to which these transporters are simultaneously exposed, so that their action may be co-modulated.

The aim of the present study was to characterize, in rat cardiomyocytes, the expression and activity of Gluts and NCX and to evaluate their possible involvement in energy production.

In order to investigate the expression of GluTs and NCX in rat heart, western blotting analyses were performed on heart membrane proteins and isolated heart mitochondria. Results showed that both, membrane and mitochondria, express prevalently GLAST, EAAC1 and NCX1. The possible molecular correlation between NCX and GluTs was investigated by performing coimmunoprecipitation experiments on membrane proteins isolated from rat heart. Preliminary results indicate that NCX coimmunoprecipitate with EAAC1 forming a molecular complex.

To characterize the glutamate-induced ATP production, isolated mitochondria, from rat heart, were exposed for 1 hr to increasing concentration of glutamate (0.1-3 mM). This exposure was able to induce a dose-dependent increase in ATP production. This increase seemed to be inhibited when the mitochondria were preincubated with DL-TBOA, a specific GluTs inhibitor.

Collectively, the preliminary results obtained in the present study suggest that GluTs may be involved in ATP production stimulated by glutamate. In addition, coimmunoprecipitation experiments suggest that EAAC1 and NCX form a functional molecular complex that may participate to control ATP production.