PIVOTAL ROLE OF NCX1 IN GLUTAMATE-ENHANCED ATP SYNTHESIS AND IMPROVEMENT OF CELL SURVIVAL IN RAT HEART-DERIVED H9C2 MYOBLASTS EXPOSED TO HYPOXIA/REOXYGENATION INJURY

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Myocardial ischemia culminates in ATP production impairment, ionic derangement and cell death (Kalogeris et al., 2012). The provision of metabolic substrates during the reperfusion may significantly limit cell injury by improving mitochondrial performance. Different approaches have been explored to limit the damage occurring during myocardial ischemia/reperfusion (Ibanez et al., 2015). One of the most promising is the resumption of the aerobic metabolism through the provision of energy substrates to the ischemic tissue, and in this regard, glutamate (glut) is a good candidate. Glut is a key molecule in cellular metabolism, both in normoxia and under ischemic conditions (Kristiansen et al., 2008; Safer, 1975). In a previous study, we showed that, under normoxic conditions, glut can be used as intermediary metabolite for ATP synthesis, and that both the Na+/Ca2+ exchanger 1 (NCX1) and the Na+ dependent Excitatory Amino Acid Transporters (EAATs) play a critical role in this pathway (Magi et., 2012; Magi et al., 2013). Specifically, we reported a functional interaction between NCX1 and the Excitatory Amino Acid Carrier 1 (EAAC1), both at plasma membrane and mitochondrial level, where they cooperate in order to favor glut entry into the cytoplasm and then into the mitochondria, enhancing ATP synthesis. In this study, we investigated the potential ability of glut to improve cell survival in rat heart-derived H9c2 myoblasts subjected to hypoxia/reoxygenation (H/R) and the involvement of NCX1 and EAAC1. To this aim, we used two H9c2 clones, H9c2-WT cells (devoid of any detectable endogenous NCX1) and H9c2-NCX1 cells (stably expressing a functional NCX1). In these models, H/R challenge produced a significant cell damage and a dramatic drop in ATP cellular content. Compared to H9c2-WT, H/R-induced cell death was much greater in NCX1-transfected cells. Under normoxia, 1 hour exposure to 1 mM glut induced a significant raise in intracellular ATP levels in H9c2-NCX1 cells but not in H9c2-WT, confirming that this metabolic response relies upon NCX1 activity. Noteworthy, in H9c2-NCX1 cells, the administration of 1 mM glut during the first hour of reoxygenation evoked a raise in ATP production up to the levels observed in normoxia and significantly improved cell survival. Interestingly, glut supplementation during the reoxygenation phase prevented the H/R-induced increase of the NCX1 reverse-mode activity, assessed by monitoring intracellular Ca2+ rises through confocal imaging in Na+-free conditions. Conversely, glut failed to elicit any significant amelioration in terms of ATP synthesis and cell viability in H9c2-WT cells. These responses were counteracted by both EAATs (300 µM DL-TBOA) and NCX (1 µM SN-6) blockers, suggesting the need of an interplay between these proteins in order for glut to exert its protective action.

Collectively, these data provide clear evidence that glut supplementation from the beginning of the reoxygenation phase can positively affect cell viability by sustaining the oxidative metabolism and increasing ATP content, with NCX1 and EAATs playing a critical role. As for normoxia, we propose an alternative and regulated mechanism whereby EAATs activity would stimulate NCX1

reverse mode, leading to an increase in mitochondrial Ca2+ concentration, to a higher physiological steady-state level that could stimulate Ca2+-sensitive dehydrogenase activity, and the rate of ATP synthesis.

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