INVOLVEMENT OF NCX IN GLUTAMATE-INDUCED NEUROPROTECTION IN AN IN VITRO MODEL OF ISCHEMIA/REPERFUSION INJURY

1)Castaldo P. 2)Macrì ML. 3)Piccirillo S. 4)Bastioli G. 5)Magi S. 6)Lariccia V. 7)Amoroso S.

Pasqualina Castaldo

Healthy neurons require adequate energy supply from mitochondria to meet their high-energy demands (Szydlowska et al., 2010). In brain ischemia, reduction of oxygen and substrates af-fects mitochondrial respiratory chain and aerobic metabolism, which in turn may culminate in ATP production impairment, Ca2+ buffering derangement and cell death (Katsura et al., 1994; White et al., 2000). Of note, energy dysfunction may induce adaptations in cerebral metabo-lism, including the utilization of alternative energy supplies, such as amino acids. Thus, the provision of metabolic substrates to ischemic neurons may have significant effects in limiting injury by improving mitochondrial performance during reperfusion. In this regard, it has been shown that glutamate (Glut) and glutamine can be used as metabolic fuels to recover from mi-tochondrial failure after ischemia/reperfusion injury (Pascual et al., 1998). Indeed, as an in-termediate for the replenishment of citric acid cycle metabolites, glutamate is a key molecule in cellular metabolism. In a previous study, we found that, under normoxic conditions, glut can be used as intermediary metabolite for ATP synthesis, and that both the Na+/Ca2+ exchanger (NCXs) and the Na+ dependent Excitatory Amino Acid Transporters (EAATs) play a critical role in this pathway. In particular, we found that EAATs localize also within brain mitochondria and mediate Glut entry into the matrix (Magi et al., 2012). This new Glut transport pathway is sus-tained by the functional interaction with NCX and operates both in mitochondria and on the surface of neuronal cells (Magi et al., 2012-13).

On the basis of these findings, the aim of the present study was to investigate the potential role of Glut in limiting neuronal damage using oxygen-glucose deprivation and re-oxygenation (OGD-R) as an in vitro model of ischemia/reperfusion injury.

As neuronal model, we used SH-SY5Y neuroblastoma cells differentiated into neuron-like cells by treatment with retinoic acid (10 µM) for 7 days. Cells were subjected to 16 h hypoxia followed by 24 h reoxygenation. In this model, hypoxia/reoxygenation (H/R) challenge produced a significant cell damage, evaluated by measuring lactate dehydrogenase activity, and a dramatic drop in ATP cellular content. Under H/R condition, exposure to 500 µM Glut during the reoxygenation phase induced a significant raise in intracellular ATP level and an improvement of cell survival. These effects were reverted by exposure to specific NCX1 and EAATs inhibitors (SN6 1 µM and DL-TBOA 300 µM, respectively). Interestingly, Glut supplementation induced an increase of the NCXs reverse-mode activity, assessed by monitoring intracellular Ca2+ rises through confocal imaging in Na+-free conditions. The presence of SN6 counteracted these re-sponses. In order to better clarify the specific involvement of the different members of NCXs and EAATs families, we used specific small interfering RNA (siRNA). Our experiments demon-strated that NCX1 and EAAC1 siRNA were able to counteract the protective effect induced by Glut, suggesting the need of an interplay

between these proteins in order for Glut to exert its protective action. Accordingly, the same condition prevented the increase of NCX1 reverse mode activity induced by Glut.

Collectively, our data provide evidence that Glut supplementation during the reoxygenation phase can improve cell viability by sustaining the oxidative metabolism and increasing ATP content. Moreover, NCX1 and EAATs seem to play a critical role in this phenomenon.

Szydlowska et al. (2010) Cell calcium 47, 122-129.

Katsura Ket al., (1994) Biochemical Society transactions 22:991-996.

White et al., (2000) Journal of the neurological sciences 179:1-33.

Magi et al, (2012). PlosOne 7 e34015.

Magi et al., (2013) Mol Pharmacol 84:603-614.

Pascual et al. (1998) Stroke 29:1048–1056