ANOXIC PRECONDITIONING-INDUCED NEUROPROTECTION IN CORTICAL NEURONS IS MEDIATED BY NO• VIA Mn-SOD

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Many studies *in vivo* and *in vitro* have demonstrated that neurons exposed to brief periods of sublethal anoxia develop resistance to subsequent more prolonged and lethal anoxic insults. This phenomenon known as anoxic preconditioning (APC) was firstly described in the myocardium and only recently in the brain. The molecular mechanisms responsible for the induction and maintenance of ischemic tolerance in the brain are complex and remain largely undefined.

In this study we investigated the effect of APC on survival of cortical neurons exposed to oxygen and glucose deprivation (OGD) followed by reoxygenation and the transductional mechanisms responsible for the neuroprotective effect of APC, with particular regard to the effects of NO• on mitochondrial Mn-superoxide dismutase (Mn-SOD) expression and activity. Preconditioning was obtained by exposing neurons to a sublethal insult of 30 minute OGD in the presence of 95% N_2 and 5% CO_2. After a 24-hr interval, neurons were exposed to a severe insult consisting of 3 hr of OGD followed by 24 hr of reoxygenation. The results obtained show that APC induces an increase of nNOS expression and NO• production that is paralleled by an increase in Mn-SOD expression and activity that persisted also during the second OGD phase. These effects were associated to a reduction in cytochrome c (cyt c) release into the cytosol, an improvement in mitochondrial function, and a reduction in free radical production. Interestingly, the treatment of neurons with L-NAME, a well known NOS inhibitor, reduced the increase in Mn-SOD expression occurring during APC and reverted APC-induced neuroprotection, thus suggesting that NO• may be responsible for these effects.

APC induces also an increase of the phosphorilated extracellular-regulated kinase expression (pERK), the activated form of ERK 1/2. A treatment of cortical neurons with PD98059, a selective inhibitor of ERK1/2, prevents the increase of Mn-SOD expression and reverted APC-induced neuroprotection. This findings suggest that ERK1/2 pathway is involved in APC-induced neuroprotective activity.

Collectively, the present results suggest that the neuroprotective role played by NO• during APC may occur through the stimulation of Mn-SOD expression and activity and that this effect is mediated by ERK1/2 cascade.