Molecular pathways involved in NO formation in myenteric neurons after in vivo ischemia/reperfusion injury in the rat small intestine

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Nitric oxide (NO) plays a fundamental role in the alterations of myenteric motor neurons during ischemia/reperfusion (I/R) injury in the gut (Rivera et al., 2009). In this condition, the activity of neuronal and inducible nitric oxide synthase isoforms (nNOS and iNOS, respectively) may be differentially regulated. After I/R, up-regulation of iNOS has a detrimental action on intestinal motility, contributing to a decrease of the intestinal transit (Hassoun et al, 2001). Conversely, nNOS has been hypothesized to have a neuroprotective role during a metabolic damage in the gut. We have recently demonstrated that both NOS represent sources for NO overproduction in the rat myenteric plexus during I/R, although iNOS seems to play a major role (Giaroni et al., 2013) The aim of this study is to further investigate the possible molecular cascades involved in nNOS and iNOS activation after an I/R injury in the rat small intestine. In particular, the possible involvement of homeobox gene pathways, OTX1 and OTX2, will be investigated. Homeobox proteins represent nuclear transcription factors involved in neuronal development and differentiation (Karen B et al., 2010) and are also closely related to neurotoxicity (Mihara M. et al, 2003). Conditions of in vivo I/R in the rat were obtained by clamping the superior mesenteric artery for 60 min followed by 24 and 48 hours of reperfusion. Controls were represented by sham operated animals undergoing laparatomy without clamping of the superior mesenteric artery. mRNA levels of genes related to ischemia, i.e. VEGFalpha and HIF1alpha significantly increased after 24h and 48h of I/R, as an index of ischemic damage. Myeloperoxidase activity significantly increased after 24 and 48 hours of I/R and returned to control values in the presence of 1400W (10 μM) and N(ω)-propyl-L-arginine (NPLA, 1μM), selective iNOS and nNOS inhibitor, respectively. Intestinal transit was significantly reduced 48 hours after I/R ($P<0.05$) and returned to control values in presence of 1400W, but not of NPLA. nNOS immunopositive neurons were unchanged 24 and 48h after I/R with respect to control values, while iNOS immunopositive neurons significantly increased. Accordingly, iNOS, but not nNOS, mRNA significantly increased, after I/R. The number of OTX immunopositive neurons was 10.48±0.7% (n=5) and 10.92±0.6% (n=5) in preparations obtained 24h and 48h after sham operation, respectively. Both values significantly increased 24 and 48 hours after I/R (17.10±0.7%, n=6; 17.44±1.13%, n=6, $p<0.001$, respectively). OTX colocalized with a subpopulation of both iNOS and nNOS myenteric neurons. OTX protein levels significantly increased in longitudinal muscle myenteric plexus preparations after I/R. OTX1 mRNA expression significantly increased 24h and 48h after I/R, displaying a higher enhancement at 48h I/R. OTX2 mRNA levels significantly increased after 24h I/R, but not after 48h I/R. The modulation of the ischemic damage on OTX1 and OTX2 mRNA isoform expression was significantly reduced by both NOS inhibitors, NPLA and 1400W. On the whole the present data suggest that in the myenteric plexus of the rat small intestine a close interplay occurs between NO produced by both iNOS and nNOS OTX pathways after and I/R injury. Further investigations are needed to elucidate a possible different role of OTX genes, which may be either neuroprotective (OTX2) of neurodamaging (OTX1) in these conditions.

Rivera et al, (2009) Acta Neuropathol. 118, 261-270.
Hassoun et al, (2001) J Surg Res 2001. 97, 150–4.
Giaroni et al, (2013) Neurogastroenterol Motil 25, e114–e126
Karen B et al, (2010) J Histochem Cytochem 58, 669–678.
Mihara M. et al. Mol Cell 2003, 11: 577-90;