ALTERED EXPRESSION AND PHOSPHORYLATION OF NMDA NR1 SUBUNITS IN THE MYENTERIC PLEXUS DURING HYPOXIA-HYPOGLICAEMIA

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Experimental evidences suggest that glutamate is involved in neuronal adaptations occurring in the enteric nervous system during an ischaemic damage (1, 2). In the guinea pig ileum, transient *in vitro* hypoxia/hypoglycaemia evoked a massive increase of spontaneous acetylcholine and glutamate overflow from the myenteric plexus, via NMDA receptor activation (1). The molecular mechanisms underlying enhancement of the excitatory glutamatergic enteric transmission during a metabolic insult have not yet been unravelled. Modifications of specific regulatory sites on NMDA receptors may influence their function during an ischaemic damage in the myenteric plexus, as observed in the central nervous system (3). The aim of the present study was to evaluate the levels of expression of the functional subunit of the NMDA receptor, NR1, and its phosphorylation state in the myenteric plexus of the guinea pig ileum by immunoblotting, after *in vitro* induced hypoxia and hypoglycaemia. The absolute amount of NR1 mRNA was investigated by competitive RT-PCR. Conditions of hypoxia/hypoglycemia were obtained by perfusing isolated longitudinal muscle-myenteric plexus preparations with a physiological Tyrode's solution deprived of oxygen and glucose for 5, 20, 60 min. Levels of expression of NR1 receptor significantly increased in myenteric neurons 20 min after induction of hypoxia/hypoglycaemia and remained elevated thereafter. Absolute amount of NR1 mRNA in control samples was 0.813±0.132 fg NR1 mRNA/pg β actin (n=4) and was not modified by hypoxia/hypoglycaemia. Immunoreactivity levels of NR1 phosphorylated on Ser896 site significantly increased with respect to control values 5 min after application of the metabolic insult and remained elevated thereafter. The expression of NR1 phosphorylated on Ser897 site did not change, at any time after hypoxia/hypoglycaemia. The present data suggest that in the guinea pig ileum *in vitro* hypoxia/hypoglycemia induces alterations in the expression of NR1 receptors, which do not depend upon change in receptor transcription. Such modifications may relay upon post-transcriptional events involving phosphorylation of the protein at Ser896 site, which promotes plasma membrane delivery and stabilization of NMDA receptor. In this view, NR1 Ser896 phosphorylation may represent one of the molecular mechanisms at the basis of NMDA receptor activation in myenteric neurons following *in vitro* ischaemia.

1) Giuliani D et al., Neurochem Int, 2006; 48:191-200; 2) Calcina F. et al., Neuroscience 2005; 134:39-49; 3) Quintana P. et al., Eur. J. Neurosci. 2006; 23: 975-983.