GUANOSINE AND INHIBITION OF NEURONAL NITRIC OXIDE SYNTHASE ACTIVITY PROMOTE NEUROPROTECTION AGAINST ISCHEMIA IN THE HIPPOCAMPUS.

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Nitric oxide (NO) has been reported either to cause or prevent cell death during an ischemic event. Guanosine, a nucleoside with neuroprotective properties, has been shown to reduce inducible NO synthase (iNOS) expression following oxygen/glucose deprivation (OGD) in hippocampal slices, an in vitro model of cerebral ischemia. The aim of this study was to evaluate the participation of NOS enzymes activities on neuroprotection promoted by guanosine and oxidative damage evoked by OGD.

Male Wistar rats (60-90 days) were used for obtaining hippocampal slices (400 μm) which were subject to 15 min of OGD followed by 120 min of reoxygenation. Guanosine (GUO, 100 μM); L-N³-nitro-L-arginine-methyl-ester (L-NAME – a non-selective NOS inhibitor, 1 mM); N-(3-(Aminomethyl)benzyl)acetamidine (1400W – iNOS inhibitor, 100 μM) or 7-nitroindazole (7-NI – neuronal NOS (nNOS) inhibitor, 50 μM) were added during reoxygenation period. Reactive oxygen species (ROS), NO and peroxynitrite (ONOO⁻) production were assessed with the following probes, respectively, 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA, 80 μM), 4,5-diaminofluorescein diacetate (DAF 2-DA, 10 μM) and dihydrorhodamine 123 (DHR 123, 15 μM).

GUO or L-NAME treatment prevents the increase of ROS, NO and ONOO⁻ production in hippocampal slices subjected to OGD and also prevents the decline of cellular viability. 1400W (iNOS inhibitor) or 7-NI (nNOS inhibitor) did not prevent the increment of ROS production. However, 7-NI prevented the increase of NO and ONOO⁻ levels, whereas 1400W was ineffective. The co-treatment with 1400W plus 7-NI prevented NO and ONOO⁻ levels increase. These results demonstrate that inhibition of nNOS contributes more significantly than iNOS inhibition for neuroprotection in hippocampal slices subjected to ischemia. Furthermore, these results also point to a modulation of nNOS and iNOS activities in the mechanism of neuroprotection evoked by guanosine.