STUDIES IN HIPPOCAMPAL SLICE MODELS OF ISCHEMIC PRECONDITIONING: ROLE OF GLUTAMATE RECEPTORS AND POLY(ADP-RIBOSE) POLYMERASE

Pellegrini-Giampietro Domenico, Gerace Elisabetta, Scartabelli Tania, Landucci Elisa, Chiarugi Alberto & Moroni Flavio

Dipartimento di Farmacologia Preclinica e Clinica, Università di Firenze, Viale Pieraccini 6, 50139 Firenze

Ischemic tolerance is an endogenous neuroprotective mechanism by which neurons are protected from the deleterious effects of brain ischemia, but the underlying cellular events are still unknown (1). The preconditioning response to brief excitotoxic or ischemic episodes involves a complex process that includes activation of glutamate receptors and of poly(ADP-ribose) polymerase (PARP), a nuclear enzyme that is involved in DNA repair but may lead to cell death by energy depletion when DNA damage is excessive. In this study, ischemic tolerance was investigated in organotypic hippocampal slices exposed to 30 min OGD, wich promotes selective pyramidal cell death in the CA1 subregion 24 h later (2). We first developed a model of preconditioning by exposing the slices to increasing concentrations of NMDA (1-100 µM for 15-180 min). Concentrations of NMDA < 10 µM were not toxic, regardless of the period of incubation. When slices were exposed to 3 µM NMDA for 60 min and then, 24 h later, to 30 min OGD, CA1 damage was reduced by approximately 35%. Exposure of slices to the PARP inhibitors PJ34 (10-100 µM) and TiQ-A (0.1-100 µM) during NMDA preconditioning and the subsequent 24 h prevented the development of ischemic tolerance in a dose-dependent manner. We then developed a model of preconditioning by exposing the slices to a low dose (10 µM) of the mGlu1/5 receptor agonist DHPG for 30 min. PARP did not appear to be involved in this paradigm, whereas the selective mGlu1 antagonists LY367385 and 3-MATIDA prevented the toxicity induced by subsequent exposure to 30 min OGD. Finally, we exposed the slices to a brief (10 min) OGD before the toxic OGD insult. Under these conditions, tolerance was prevented by LY367385 but also by the mGlu5 antagonist MPEP. In both DHPG- and OGD-preconditioned slices, the toxic responses to AMPA (3-30 µM, 60 min) and NMDA (10-100 µM, 60 min) were significanty reduced. In conclusion, our study demonstrates that glutamate receptors and PARP are differentially involved in the mechanisms that lead to the development of ischemic tolerance. A better understanding of these processes could be helpful for the refinement of novel strategies for stroke-related neurological diseases.

1. Dirnagl U., Simon R.P., Hallenbeck J.M. (2003) Trends Neurosci. 26: 248-254.
2. Pellegrini-Giampietro D.E., Cozzi A., Peruginelli F., Leonardi P., Meli E., Pellicciari R., Moroni F. (1999) Eur. J. Neurosci. 11: 3637-3647.