

NEUROPROTECTION BY GROUP-II METABOTROPIC GLUTAMATE RECEPTOR AGONISTS REQUIRES THE PRESENCE OF MGLU3 RECEPTORS AND IS MEDIATED BY THE PRODUCTION OF NEUROTROPHIC FACTORS

Giuseppe Battaglia¹, Gemma Molinaro¹, Barbara Riozzi¹, Paola Spinsanti², Anna Traficante¹, Natale Belluardo³, Corrado Corti⁴, Ferdinando Nicoletti^{1,2}, Valeria Bruno^{1,2}

¹I. N. M. Neuromed, Pozzilli; ²Dept. of Human Physiology and Pharmacology, University "La Sapienza", Rome; ³Dept. of Biology, GlaxoSmithKline Medicines Research Centre, Verona; ⁴Dept. of Experimental Medicine, University of Palermo, Palermo, Italy

Agonists of group-II metabotropic glutamate (mGlu) receptors are protective in a variety of *in vitro* and *in vivo* models of neurodegeneration. Whether neuroprotection is mediated by mGlu2 or mGlu3 receptors is unknown at present. We have addressed this question in primary mixed cultures of murine cortical cells prepared from mGlu2 and mGlu3 receptor knockout mice. Mixed cultures of murine cortical cells prepared from mGlu3^{-/-} mice challenged with NMDA did not show a difference in the potency of NMDA-induced excitotoxicity as compared to cultures prepared from wild-type C57Bl mice. The selective mGlu2/3 receptor agonist LY379268 (1 μ M) as well as the selective mGlu5 receptor antagonist MPEP (1 μ M) induced a reduction of NMDA-induced toxicity in mixed neuronal cultures prepared from wild-type mice. Neuronal cell cultures prepared from wild-type mice plated on a confluent layer of astrocytes prepared from mGlu3^{-/-} mice were no longer protected against NMDA toxicity after treatment with LY379268. Conditioned medium collected from glial cells, prepared from wildtype mice and exposed to LY379268 (1 μ M, 20 hrs later) was protective against NMDA toxicity, whereas conditioned medium collected from glial cells prepared from mGlu3^{-/-} mice and exposed to LY379268 lost its neuroprotective effects. Administration of LY379268 (2 mg/kg, i.p., single injection) to wildtype or mGlu2^{-/-} mice induced an increase (about 2 fold) of TGF β levels in the striatum, as assessed 72 hrs later by Western blot analysis. In mGlu3^{-/-} mice, LY379268 failed to affect TGF β levels in the striatum. We extended the study to mice unilaterally injected with toxic concentrations of NMDA in the caudate nucleus or systemically injected with MPTP. In mice lacking mGlu2 receptors, the extent of neuroprotection was greater than in wild-type mice, as indicated by a greater difference in the extent of death compared with the corresponding groups of mice treated with saline. In contrast, the protective activity of LY379268 was lost in mice lacking mGlu3 receptors. In MPTP-injected mice (30 mg/kg, i.p.) the extent of nigro-striatal degeneration was similar in wild-type, mGlu2^{-/-}, and mGlu3^{-/-} mice. Systemic injection of LY379268 (1 mg/kg, i.p.) did not affect nigro-striatal degeneration induced by MPTP in wild-type mice and in mGlu3^{-/-} mice. Interestingly, however, the drug became substantially neuroprotective when injected to mGlu2^{-/-} mice treated with MPTP. These data suggest that mGlu3 receptors are essential for the neuroprotective activity of mGlu2/3 agonists against excitotoxic neuronal degeneration and this effect is likely mediated by TGF β formation.