Roles of ionotropic glutamate receptors in early developing neurons derived from the P19 mouse cell line

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摘要

Abstract

We cultured a P19 mouse teratocarcinoma cell line and induced its neuronal differentiation to study the function of ionotropic glutamate receptors (GluRs) in early neuronal development. Immunocytochemical studies showed 85% neuronal population at 5 days in vitro (DIV) with microtubule-associated protein 2-positive staining. Thirty percent and 50% of the cells expressed the -amino-3-hydroxy-5-methyl-4-isopropinonate (AMPA) receptor subunit, GluR2/3, and the kainate (kainic acid; KA) receptor subunit, GluR5/6/7, respectively. In Western blot analysis, the temporal expression of GluR2/3 began to appear at 3 DIV, whereas GluR5/6/7 was already expressed in the undifferentiated cells. P19-derived neurons began to respond to glutamate, AMPA and KA, but not to the metabotropic GluR agonist trans-1-aminocyclopentane-1,3-decarboxylic acid, by 5 DIV in terms of increases in intracellular calcium and phospholipase C-mediated poly-phosphoinositide turnover. Furthermore, KA reduced cell death of P19-derived neurons in both atmospheric and hypobaric conditions in a phospholipase C-dependent manner. The common AMPA/KA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione, but not the AMPA receptor antagonist, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium, profoundly increased hypobaric insult-induced neurotoxicity. In a flow cytometry study, the nerve growth factor-mediated antiapoptotic effect was facilitated by AMPA, with an induction of TrkA, but not p75NTR expression. Therefore, AMPA and KA receptors might mediate neurotrophic functions to facilitate neurotrophic factor signaling to protect neurons against hypoxic insult in early neuronal development.