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Neurosteroids enhance the viability of staurosporine and doxorubicin treated differentiated human neuroblastoma SH-SY5Y cells

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Abstract:
Previously, we found that neurosteroids inhibited hydrogen peroxide- and staurosporine-induced damage of undifferentiated human neuroblastoma SH-SY5Y cells. However, differentiated neuroblastoma cells morphologically and functionally resemble neuronal cells, and are thus considered to be a model system for studying neuronal apoptotic processes. In the present study, we examined the effects of dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and pregnenolone (PGL) on the viability of retinoic acid-differentiated human neuroblastoma SH-SY5Y cells. Mitochondrial and extracellular apoptotic processes in these cells were induced by staurosporine and doxorubicin, respectively. Calcein viability assays showed that doxorubicin (0.5 μM for 24 h) decreased cell viability by ca. 20% as compared to control cultures. DHEA and DHEAS at 0.1 and 1 μM concentrations, respectively, significantly inhibited the doxorubicin toxicity. PGL showed a neuroprotective effect only at 0.1 μM, whereas it was inactive at a higher concentration (1 μM). Staurosporine (1 μM for 24 h) decreased SH-SY5Y cell viability by ca. 50%. DHEA (0.1 and 1 μM) and DHEAS (0.1 and 1 μM) significantly antagonized the toxic effects of staurosporine, whereas these compounds showed no activity at the lowest concentration (0.01 μM). PGL inhibited the staurosporine-induced decrease in cell viability only at the concentration of 0.1 μM. Since staurosporine generated a stronger detrimental effect on SH-SY5Y cell viability than doxorubicin, we studied the mechanisms of neurosteroid action only in the former model. Staurosporine (1 μM for 24 h) enhanced lactate dehydrogenase (LDH) release by ca. 40% and this effect was inhibited by DHEA (0.01, 0.1, and 1μM), DHEAS (0.1 and 1 μM) and PGL (0.01 and 01 μM). In order to verify an involvement of phosphatidylinositol-3-kinase (PI3-K) in the antiapoptotic action of neurosteroids, a specific inhibitor of this protein kinase (LY 294002 at 10 μM) was used. Pretreatment of the cells with LY 294002 antagonized the ameliorating effects of DHEA, DHEAS, and PGL on staurosporine-induced LDH release.
These data indicated that at physiological concentrations, DHEA, DHEAS, and PGL prevented RA-differentiated SH-SY5Y cell damage produced by activation of both mitochondrial and extracellular apoptotic pathways. Furthermore, this study confirmed that the neuroprotective effects of neurosteroids in a staurosporine model of cytotoxicity appeared to be dependent upon PI3-K activity.

Key words:
dehydroepiandrosterone, pregnenolone, staurosporine, doxorubicin, calcein, lactate dehydrogenase, phosphatidylinositol-3-kinase, differentiated SH-SY5Y cells