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论著

知母皂苷元改善淀粉样β蛋白引起的体外培养乳大鼠海马神经元的损伤

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摘要 目的 探讨知母皂苷元(Sar)对淀粉样β蛋白片段1-42($A\beta_{1-42}$)引起的海马神经元损伤是否有保护作用。方法 取出生24 h内SD乳大鼠海马神经元, 体外培养7 d, 先加入Sar 10, 30和100 μmol·L^{-1}作用1 h, 然后加入$A\beta_{1-42}$ 50 nmol·L^{-1}作用24 h。应用倒置相差显微镜和微管相关蛋白2(MAP2)免疫荧光染色观察神经元树突的生长;应用Hoechst33258 核染色检测神经元凋亡;Western蛋白质印迹法检测海马神经元突触囊泡蛋白(SYP)、突触后致密蛋白95(PSD95)和活性胱天蛋白酶 3表达。结果 加入$A\beta_{1-42}$作用24 h, 可使培养海马神经元树突静脉曲张样改变和突起回缩, 树突总长度和末梢分枝数明显减少。与正常对照组相比, SYP和 PSD95蛋白表达水平明显降低($P<0.01$), 神经元凋亡细胞百分率和活性胱天蛋白酶3蛋白表达水平明显升高($P<0.01$)。与$A\beta_{1-42}$模型组相比, 先分别加入Sar 30和 100 μmol·L^{-1}可明显对抗$A\beta_{1-42}$引起的这些改变, 培养海马神经元树突总长度(μm)和末梢分枝数从277±76和6±2分别增加到359±144和8±3以及370±158和8±3, 神经元SYP 和PSD95蛋白表达水平明显增加($P<0.01$), 神经元凋亡细胞百分率和活性胱天蛋白酶3表达水平明显降低($P<0.01$)。结论 Sar能够改善$A\beta_{1-42}$引起的海马神经元损伤。

关键词 知母皂苷元 淀粉样β蛋白 海马神经元 突触囊泡蛋白 细胞凋亡
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Protection of sarsasapogenin against amyloid beta-protein induced neurotoxicity in primary cultured hippocampal neurons of neonatal rats

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Abstract

OBJECTIVE To investigate whether sarsasapogenin (Sar) can protect hippocampal neurons from amyloid beta-protein fragment 1-42 ($A\beta_{1-42}$) induced neurotoxicity. **METHODS** Primary neurons were obtained from hippocampi of 0- to 24-h-old Sprague-Dawley rats. After 7 d culture, Sar 10, 30 and 100 μmol·L^{-1} was added to the neurons 1 h prior to incubation with $A\beta_{1-42}$ 50 nmol·L^{-1}. Neuronal dendrite outgrowth was observed using the phase-contrast microscope and immunstaining for the dendritic marker microtubule-associated protein 2(MAP2). Neuronal apoptosis was quantified by scoring the percentage of cells with apoptotic nuclear morphology after Hoechst33258 staining. The cellular extracts were prepared for Western blotting of active caspase 3, synaptophysin (SYP), and post-synaptic density protein 95 (PSD95). **RESULTS** Cultured hippocampal neurons treated with $A\beta_{1-42}$ for 24 h displayed signs of degeneration, including the formation of varicosities and retraction of neuritis. Compared with normal control, $A\beta_{1-42}$ treatment resulted in the decrease in total dendrite branch length and terminal branch number of hippocampal neurons, the decrease of SYP and PSD95 protein expressions($P<0.01$), and the increase of the percentage of apoptotic neurons and active caspase 3 expression ($P<0.01$). Sar 30 and 100 μmol·L^{-1} inhibited $A\beta_{1-42}$-induced neuronal degeneration, increased total dendrite branch length (μm) and terminal branch number respectively from 277±76 and 6±2 to 359±144, 370±158 and 8±3, 8±3, prevented the $A\beta_{1-42}$-induced decrease in SYP and PSD95($P<0.05$), and reduced $A\beta_{1-42}$-induced apoptotic morphology and active caspase 3 protein level($P<0.01$). **CONCLUSION** Sar can protect hippocampal neurons against $A\beta_{1-42}$ toxicity.

Key words sarsasapogenin hippocampal neurons synaptophysin post-synaptic density protein 95 apoptosis

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