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论著

淀粉样β蛋白片段25～35下调大鼠海马PI3K/Akt/p70S6K信号传导通路

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摘要 目的 探讨阿尔茨海默病（AD）的淀粉样β蛋白(Aβ)的沉积是否损害神经细胞存活的信号传导通路。方法 实验分为生理盐水对照组；$A\beta_{25-35}$组；$A\beta_{25-35}$ +布洛芬组；$A\beta_{25-35}$+布洛芬+LY294002组；$A\beta_{25-35}$+LY294002组。大鼠分别灌胃给予布洛芬7.5或15 mg·kg^{-1}，每日1次，连续3周后，左侧脑室内注射$A\beta_{25-35}$（10 μL, 1 mmol·L^{-1}，之后继续灌胃给予布洛芬1周。PI3K特异性阻断剂LY294002（5 μL, 4 mmol·L^{-1}在注射$A\beta_{25-35}$前1 h左侧脑室内注射。注射$A\beta_{25-35}$后1周，取海马CA1区，Western免疫印迹法观察P53, Bax, FasL, Bcl-2, Akt和p70S6K的蛋白表达水平。应用半胱氨酸天冬氨酸蛋白酶(caspase)3活性测定试剂盒分析caspase 3活性变化，RT-PCR方法观察p70s6k mRNA表达水平。结果 脑室内注射$A\beta_{25-35}$可引起大鼠海马CA1区磷酸化Akt/PKB和磷酸化p70S6K表达明显降低，分别从对照组1.32±0.14和0.769±0.028下降到0.69±0.08和0.479±0.032。同时,海马CA1区促凋亡蛋白P53, Bax和FasL表达及caspase 3活性明显增加，抗凋亡蛋白Bcl-2表达明显降低。预先注射LY294002可使caspase 3活性较单独注射$A\beta_{25-35}$组进一步增加。给$A\beta_{25-35}$前后连续给予布洛芬4周可明显对抗$A\beta_{25-35}$引起的上述变化。LY294002可明显减弱布洛芬上调磷酸化Akt/PKB和磷酸化p70S6K表达的作用。结论 $A\beta_{25-35}$引起抗凋亡通路PI3K/Akt/p70S6K下调可能参与AD的神经元损伤。布洛芬具有较好的对抗作用，这可能与上调PI3K/Akt/p70S6K通路中的一些蛋白有关。

关键词 淀粉样β蛋白 Akt/蛋白激酶B p70S6K 海马 信号传导

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Amyloid β-protein fragment 25－35 down-regulates PI3K/Akt /p70S6K pathway in rat hippocampus *in vivo*

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Abstract

AIM To investigate whether Aβ deposit in Alzheimer disease(AD) impairs signal transduction pathway responsible for neuronal survival. METHODS The rats were randomly divided into six groups: control group and $A\beta_{25-35}$ group, $A\beta_{25-35}$+ibuprofen groups (7.5 and 15 mg·kg^{-1}, respectively), $A\beta_{25-35}$ +ibuprofen+LY294002 group, and $A\beta_{25-35}$+LY294002 group. Rats were given ibuprofen (7.5 and 15 mg·kg^{-1} daily, ig) for 3 weeks prior to and 1 week after icv single dose of $A\beta_{25-35}$ (10 μL, 1 mmol·L^{-1}). LY294002 was injected icv 1 h before the injection of $A\beta_{25-35}$. Seven days after $A\beta_{25-35}$ injection, the hippocampal expressions of P53, Bax, Fas ligand (FasL), Bcl-2 proteins, phospho-Akt/PKB, and phosphorylated 70 ku ribosomal protein S6 kinase (p70S6K) and caspase 3 were determined in the brain tissue preparations from CA1 area with Western blot. The activity of caspase 3 was measured using a caspase 3 colorimetric activity assay kit. RT-PCR was used to show the change of p70s6k mRNA level. RESULTS $A\beta_{25-35}$ icv injection significantly down- regulated phosphorylated Akt/PKB from 1.32±0.14 to 0.69±0.08 and p70S6K from 0.769±0.028 to 0.479±0.032 in hippocampal CA1 region. These changes were accompanied by increased expressions of the proapoptotic proteins P53, Bax, and FasL and decreased expression of the anti apoptotic protein Bcl-2 in rat hippocampus. In addition, caspase 3 activity was significantly enhanced in hippocampal CA1 region in $A\beta_{25-35}$-treated rats compared with control rats. Ibuprofen can reverse these $A\beta_{25-35}$-induced changes. CONCLUSION Down regulated anti-apoptotic

Akt/p70S6K signaling pathway induced by $A\beta_{25-35}$ in rat hippocampus may contribute to the neuronal damage in AD. Ibuprofen prevents $A\beta_{25-35}$-induced down-regulation of PI3K/Akt/p70S6K signaling pathway.

Key words amyloid beta protein Akt/protein kinase B p70S6K hippocampus singal transduction

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