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ApoE亚型蛋白对神经元轴突生长的影响

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Title: Effect of ApoE isoforms on growth of neuronal axons in mice

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关键词: 神经元; 轴突; 载脂蛋白E; 微管蛋白; 细胞分裂周期蛋白

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摘要: 目的 观察不同亚型ApoE蛋白（apolipoprotein E，ApoE）对神经元轴突生长的影响，探讨其可能机制。 方法 取新生C57野生鼠和APOE基因敲除鼠大脑皮质，进行神经元原代培养；在培养第1天，在APOE基因敲除鼠神经元培养基中加入重组人类ApoE2、3、4蛋白，将所培养细胞分为ApoE(-)组、ApoE2组、ApoE3组、ApoE4组和野生组5组。倒置相差显微镜观察神经元生长情况，测量其轴突长度和突起数量。免疫荧光对神经元轴突进行染色，并对神经元轴突荧光强度进行测定；Western blot检测不同亚型ApoE对细胞分裂周期蛋白42（cell division cycle 42，cdc42）表达的影响。结果 培养第1、3、5天，野生组、ApoE2组、ApoE3组神经元平均轴突长度较ApoE(-)组及ApoE4组长(P<0.05)；培养第5天，ApoE(-)组、ApoE4组神经元突起数量分别为(1.80±0.45)个和(1.90±0.84)个，较野生组（3.80±0.84）个、ApoE2组（3.60±0.55）个、ApoE3组（3.40±1.14）个少（P<0.05）；野生组、ApoE2组、ApoE3组神经元轴突荧光强度分别为（54.10±7.32）、（52.40±6.33）、（50.50±8.21），较ApoE(-)组（37.20± 9.30）和ApoE4组（39.00±8.32）强（P<0.05）；野生组、ApoE2组、ApoE3组cdc42蛋白表达高于ApoE(-)组、ApoE4组（P<0.05）。而在所有实验结果中野生组、ApoE2组与ApoE3组组间以及ApoE(-)组与ApoE4组组间比较差异均无统计学意义（P>0.05）。 结论 不同亚型ApoE蛋白对神经元轴突生长的影响不同，对tubulinIII及cdc42表达的影响也各不相同，后者可能是其影响神经元轴突生长的机制之一。

Abstract: Objective To determine the effect of different genotypes of apolipoprotein E

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(APOE) on the axonal growth and investigate the possible underlying mechanism. Methods Cortical neurons from new born wild type mice or APOE gene knockout mice (APOE$^-$) were isolated and cultured primarily *in vitro*. The recombinant human ApoE2, ApoE3 and ApoE4 proteins were added into the culture medium of APOE$^-$ neurons respectively, thereby the cultured neurons were divided into 5 groups, namely WT group (wild type mice), ApoE$^-$ group, ApoE2 group, ApoE3 group and ApoE4 group. The length of axons and neurites number was measured by phase contrast microscopy. The axons were labeled by immunofluorescence staining to measure the fluorescence intensity of axons. The effect of different ApoE proteins on the expression of cell division cycle 42 (cdc42) was determined by Western blotting. Results The average length of axons was significantly longer in the WT, ApoE2 and ApoE3 groups than ApoE$^-$ and ApoE4 groups (P<0.05). The average number of neurites was 3.80±0.84, 3.60±0.55, and 3.40±1.14, respectively for WT, ApoE2 and ApoE3 groups, markedly larger than those of ApoE$^-$ group (1.80±0.45) and ApoE4 group(1.90±0.84, P<0.05). The axonal fluorescence intensity of WT, ApoE2 and ApoE3 groups was 54.10±7.32, 52.40±6.33, and 50.50±8.21, respectively, obviously higher than the ApoE$^-$ (37.20±9.30) and ApoE4 groups (39.00±8.32, P<0.05). The expression of cdc42 was significantly higher in WT, ApoE2 and ApoE3 groups than ApoE$^-$ and ApoE4 groups (P<0.05). There was no significant difference among WT, ApoE2 and ApoE3 groups, and between ApoE$^-$ and ApoE4 groups（P>0.05）. Conclusion The growth of neurons axons is differently affected by ApoE isoforms, and so is the expression of tubulinⅢ and cdc42, which maybe one of the mechanisms of effect of ApoE isoforms on the growth of neurons axons.

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