中国药理学与毒理学杂志 2012 26 (1): 52-57 ISSN: 1000-3002 CN: 11-1115/R

论著

大黄酸对体外大鼠皮质神经元突起长度及微管相关蛋白2 mRNA表达的影响

鄢 黎[1], 周晓雯[1], 周 星[1], 赖永长[1], 罗焕敏[1,2,3]

（暨南大学 1. 医学院药理学系, 2. 脑科学研究所, 广东 广州 510632; 3. 暨南大学 香港大学脑功能与健康联合实验室, 广东 广州 510632）

收稿日期 2011-9-19 **修回日期** **网络版发布日期** 2012-2-23 **接受日期** 2011-12-22

摘要 **目的** 研究大黄酸（RH）对大鼠皮质神经元的营养作用，并初步探讨其相关机制。**方法** 体外DMEM/F12+0.4%B27培养新生大鼠皮质神经元，应用神经元特异性烯醇化酶（NSE）和微管相关蛋白2（MAP2）免疫细胞化学染色法鉴定神经元。神经元细胞加入RH 2，4和8 μmol·L^{-1} 作用72 h，计算神经元平均突起长度；或分别同时加入Trk受体拮抗剂K252a 50 nmol·L^{-1} 和PI3K抑制剂LY294002 10 μmol·L^{-1}测量神经元平均突起长度。MTT法检测细胞存活，并测定培养液中乳酸脱氢酶（LDH）的含量。逆转录-聚合酶链反应（RT-PCR）半定量检测MAP2 mRNA表达。**结果** NSE及MAP2免疫荧光染色结果显示，绝大多数细胞呈阳性反应，所培养的细胞90%以上为神经元。MTT和LDH检测结果表明，与溶媒对照组相比， RH 2，4和8 μmol·L^{-1}能明显提高神经元存活率（*P*<0.01）；平均突起长度明显增加（*P*<0.01）。与RH 8 μmol·L^{-1}组相比，同时加入K252a 50 nmol·L^{-1}或LY294002 10 μmol·L^{-1}，平均突起长度明显缩短（*P*<0.01）。与溶媒对照组相比，RH 2，4和8 μmol·L^{-1}组MAP2 mRNA表达量明显增加（*P*<0.01）。**结论** RH对新生大鼠皮质神经元具有神经营养作用，能促进神经元突起的生长和提高神经元的存活率。RH神经营养作用可能部分通过激活Trk受体，继而激活Ras/PI3K/PKB通路而发挥的。

关键词 大黄酸 神经营养 碱性成纤维细胞生长因子 酪氨酸受体激酶受体 磷脂酰肌醇3激酶

分类号 R285

Effect of rhein on neurite outgrowth and microtubule-associated protein 2 mRNA expression in primary cultured rat cortical neurons

YAN Li[1], ZHOU Xiao-wen[1], XING Zhou[1], LAI Yong-chang[1], LUO Huan-min[1,2,3]

(1. Department of Pharmacology, School of Medicine, 2. Institute of Brain Sciences, Jinan University, Guangzhou 510632, China; 3. The Joint Laboratory of Brain Function and Health of Jinan University-University of Hong Kong, Jinan University, Guangzhou 510632, China）

Abstract

OBJECTIVE To investigate the neurotrophic effects of rhein (RH) on rat cortical neurons and explore possible mechanisms. **METHODS** Cortical neurons were cultured in serum-free medium *in vitro*. The neurons were identified by immunofluorescence staining of two related proteins: neuron-specific enolase (NSE) and microtubule-associated protein 2 (MAP2). Neurons were treated with RH 2, 4 and 8 μmol·L^{-1} for 72 h. RH merely or co-treated with K252a (a tyrosine kinase receptor inhibitor) or LY294002 (a specific inhibitor of PI3K) was added before the average length of neurite outgrowth was measured by Image-Pro software for morphological analysis. Neuronal survival by MTT assay and LDH assay was investigated. The expression of MAP2 mRNA was determined by RT-PCR. **RESULTS** NSE and MAP2 immunofluorescence staining of cultures suggested that most of the cultured cells were neurons. Compared with vehicle control group, RH 2, 4 and 8 μmol·L^{-1} could raise the neuron survival rate (113.5±1.5)%, (112.4±0.5)% and (115.7±2.5)% (*P*<0.5), respectively; and increase the lengths of neurites to 157±34, 158±38 and (160±36)μm (*P*<0.01), respectively. Compared with RH 8 μmol·L^{-1} group, average length of neurites was reduced in K252a 50 nmol·L^{-1} +RH 8 μmol·L^{-1} group and in LY294002 10 μmol·L^{-1}+RH 8 μmol·L^{-1} group to 127±20 and (136±30)μm (*P*<0.01), respectively. Compared with vehicle control group, the expression of MAP2 mRNA increased in RH 4 and 8 μmol·L^{-1} groups. **CONCLUSION** RH can significantly increase neurite lengths and neuronal survival in the primary cultured rat coritcal neurons. The neurotrophic effect of RH may depend on activating the Trk receptor and subsequently the Ras/PI3k/PKB pathway.

Key words rhein neurotrophic factor bFGF Trk tyrosine kinase receptor PI3K

DOI: 10.3867/j.issn.1000-3002.2012.01.011

通讯作者 罗焕敏 tlhm@jnu.edu.cn