

论著

## 介导乙酰胆碱诱发内皮依赖性血管舒张反应受体的特性

王莉莉<sup>1\*</sup>, 张锦超<sup>2</sup>, 丁建花<sup>3</sup>, 俞炜源<sup>2</sup>, 汪海<sup>1</sup>, 肖文彬<sup>1</sup>

(军事医学科学院 1. 毒物药物研究所, 2. 生物工程研究所, 北京 100850; 3. 南京医科大学生理学与药理学系, 江苏 南京 210029)

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摘要

目的 针对血管内皮细胞上是否存在M受体的疑点, 从基因水平和信号转导机理上探讨介导乙酰胆碱(ACh)诱发内皮依赖性血管舒张反应受体的特性。方法 以新鲜分离和传代培养的牛主动脉内皮细胞为实验材料, 比较两种内皮细胞M<sub>1</sub>~M<sub>5</sub>受体mRNA的分布状况、ACh诱发细胞内游离钙离子浓度([Ca<sup>2+</sup>]<sub>i</sub>)、环腺苷酸(cAMP)含量变化。①设计高选择性、特异性寡核苷酸探针, 采用点杂交方法对内皮细胞M<sub>1</sub>~M<sub>5</sub>受体mRNA进行分析, 并通过反转录-聚合酶链反应(RT-PCR)、DNA序列测定及同源性分析确证点杂交的实验结果; ②以Fluo-3和Fura-2为荧光探针, 用共聚焦和荧光光度仪测定内皮细胞[Ca<sup>2+</sup>]<sub>i</sub>变化; ③内皮细胞cAMP含量采用<sup>125</sup>I放射免疫分析方法测定。结果 在新鲜分离的牛主动脉内皮细胞存在M<sub>1</sub>~M<sub>4</sub>受体mRNA, 内皮细胞经传代培养后仅检测到M<sub>4</sub>受体mRNA; 1和10 μmol·L<sup>-1</sup> ACh能够引起原代培养内皮细胞[Ca<sup>2+</sup>]<sub>i</sub>增加, 最大增加量为12和19 nmol·L<sup>-1</sup>; ACh激活原代培养内皮细胞的钙离子信号, 表现为单次或连续2次的钙振荡, 具有异时性和快速耐受的特点; 此外, 随ACh浓度增加, 原代培养内皮细胞cAMP含量均呈微弱增加趋势; ACh不引起传代培养内皮细胞[Ca<sup>2+</sup>]<sub>i</sub>浓度的改变, 但能浓度依赖地降低cAMP含量。结论 新鲜分离的牛主动脉血管内皮细胞可能表达M<sub>1</sub>~M<sub>4</sub>受体, 传代培养后仅保留M<sub>4</sub>受体。介导ACh诱发内皮依赖性血管舒张反应的受体可能为M<sub>1</sub>或(和)M<sub>3</sub>受体, 其中钙离子是这种受体激活的重要信使物质。

关键词 [内皮细胞](#) [乙酰胆碱](#) [受体](#) [毒蕈碱性](#) [钙](#) [细胞内](#)

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## Characterization of the receptors mediating acetylcholine-induced endothelium-dependent vascular relaxation in bovine aorta endothelium

WANG Li-Li<sup>1</sup>, ZHANG Jin-Chao<sup>2</sup>, DING Jian-Hua<sup>3</sup>, YU Wei-Yuan<sup>2</sup>, WANG Hai<sup>1</sup>, XIAO Wen-Bin<sup>1</sup>

(1. Institute of Pharmacology and Toxicology, 2. Institute of Biotechnology, Academy of Military Medical Sciences, Beijing 100850, China; 3. Department of Physiology and Pharmacology, Nanjing Medical University, Nanjing 210029, China)

### Abstract

**AIM** To characterize the receptors mediating acetylcholine(ACh)- induced endothelium dependent vascular relaxation (EDVR) in bovine aorta endothelial cell (BAEC) on the molecular level. **METHODS** The M<sub>1</sub>~M<sub>5</sub> acetylcholine receptor(AChR) mRNA in bovine aorta endothelium was identified by high selective probes for m<sub>1</sub>~m<sub>5</sub> and dot blot. As fluorescence probe, Fluo-3 and Fura-2 were used in assaying [Ca<sup>2+</sup>]<sub>i</sub> in endothelial cells by confocus and fluorophotometry. cAMP was analyzed by radioimmunoassay. **RESULTS** Transcripts for the M<sub>1</sub>~M<sub>4</sub> receptors were detected in freshly isolated bovine aorta endothelium, whereas cultured endothelial cells only contain mRNA for M<sub>4</sub> receptor. ACh could activate endothelial cells and elicit the [Ca<sup>2+</sup>]<sub>i</sub> change of endothelium in certain periods of culture, especially in primary stage of culture. The rises of [Ca<sup>2+</sup>]<sub>i</sub> exhibited as oscillation characterized by isotiming and immediate tolerance. In addition, the cAMP concentration was slight elevated in primary cultures of endothelial cells with ACh increased from 0.1 to 10 μmol·L<sup>-1</sup>. However, ACh couldn't affect the [Ca<sup>2+</sup>]<sub>i</sub> level but decreased the cAMP level in dose dependent manner in passage

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cultures of endothelial cells. **CONCLUSION** Bovine aorta endothelium could express  $M_1-M_4$  receptors. The  $M_1-M_3$  receptors were lost during culturing. The receptor mediating ACh- induced EDVR in endothelium may be  $M_1$  and/or  $M_3$  receptor.  $Ca^{2+}$  is an important messenger in their activation.

**Key words** [endothelium](#) [acetylcholine](#) [receptors](#) [muscarinic](#) [calcium](#) [cytosolic](#)

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通讯作者 王莉莉 [Wangll@nic.bmi.ac.cn](mailto:Wangll@nic.bmi.ac.cn)