中国药理学与毒理学杂志 2011 25 (3): 320-326 ISSN: 1000-3002 CN: 11-1115/R

实验方法

在Sf9昆虫细胞-杆状病毒系统中表达毒蕈碱型M_2及M_5受体重组突变体

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收稿日期 2010-9-8 **修回日期** **网络版发布日期** 2011-7-15 **接受日期** 2011-4-19

摘要 **目的** 为乙酰胆碱毒蕈碱(M)受体亚型特异性的变构调节剂及基因工程的研究提供实验平台。**方法** 用PCR及搭桥PCR法对乙酰胆碱M_2及M_5受体作以下突变：① 将N-糖基化位点Asp突变为Asn；② 删除对蛋白酶敏感的M受体的第三个细胞内环；③ 在C端添加凝血酶识别位点（CMV）和6- His标记。将PCR扩增出重组嵌合蛋白基因亚克隆到杆状病毒转移载体，制备重组杆状病毒并感染昆虫细胞表达M_2/M_5受体蛋白。Western印迹及放射性配体受体结合实验验证受体的正确表达及功能。**结果** 通过搭桥PCR，成功扩增出1018 bp的重组M_2受体和1041 bp重组M_5受体核酸序列；使用pUC/M13的扩增引物成功构建M_2/M_5重组转移载体。将重组载体质粒与线性化病毒DNA共转染昆虫细胞Sf9，制备重组杆状病毒并感染昆虫细胞，见细胞空泡样病变。Western印迹分析确定重组杆状病毒感染昆虫细胞M_2/M_5蛋白表达，放射性配体受体饱和实验结果表明，表达的重组受体蛋白与[^{3}H] *N*-甲基-东莨菪碱具有特异性结合能力。**结论** Sf9昆虫细胞能够表达M_2及M_5重组受体蛋白，M_2及M_5重组受体蛋白的病毒样颗粒可用于M受体的新药研究。

关键词 乙酰胆碱 毒蕈碱受体 昆虫细胞 杆状病毒 基因克隆 基因表达

分类号 R966，Q78

Expression of recombinant M_2 and M_5 muscarinic receptors in the Sf9-baculovirus system

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Abstract

OBJECTIVE To study the expression of human muscarinic receptors (M_2 and M_5 recombinant receptors in the baculovirus expression system. **METHODS** The mutation of human wild type M_2 and M_5 receptors was constructed by PCR or/and overlap PCR as follows: ① The putative glycosylation residues Asp 2, 3, 6, and 9 were replaced with Asn to prevent molecular heterogeneity; ② The central part of the protease-susceptible third intracellular loop was deleted; ③ A hexa- histidine tag and a thrombin cleavage site were added at the C terminus for purification. The recombinant receptor gene was confirmed and amplified by PCR, and subcloned to baculovirus pFastBac 1vector. Then the recombinant vector was co- transfected with the linearized virus DNA into sf9 cells by Lipofectamine. The recombinant M_2 and M_5 receptor protein was prepared and purified. The expression level of M_2 and M_5 receptors was evaluated by Western blotting, and pharmacological characteristics were confirmed by radio-legend binding assay. **RESULTS** The target DNA fragment of M_2 (1018 bp) and M_5 (1041 bp) recombinant receptors was amplified by overlap PCR. The recombinant plasmid pfastbac1/M_2(M_5) vector was successfully constructed, and transfected to Sf9. Vacuolus pathological changes were observed within cells compared to non- transfection of Sf9. The baculovirus particle protein was prepared and purified from these infected cells. The expression of M_2/M_5 was further confirmed by Western blotting. The specific binding character of recombinant M_2/M_5 receptors was detected by radio-legend binding assay. **CONCLUSION** The expression of M_2 and M_5 recombinant receptors in the baculovirus expression system will facilitate studies on new drugs from M receptor or genetic engineering.

Key words acetylcholine muscarinic receptor insect cell baculovirus gene cloning gene expression

DOI: 10.3867/j.issn.1000-3002.2011.03.020

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