



李映新, 黄媛恒, 林兴, 黄仁彬. 昆明小鼠心室肌细胞分离方法及动作电位、L型钙通道电流记录[J]. 中国现代应用药学, 2013, 30(6):581-586

昆明小鼠心室肌细胞分离方法及动作电位、L型钙通道电流记录

Isolation of Calcium-tolerant Cardiomyocytes from KM Mouse and Recording of Action Potential and the Currents of L-type Calcium Channels

投稿时间: 2013-01-10 最后修改时间: 2013-02-27

DOI:

中文关键词: [昆明小鼠](#) [心肌细胞](#) [膜片钳](#) [动作电位](#) [L型钙电流](#)

英文关键词: [KM mouse](#) [ventricular myocyte](#) [patch clamp technique](#) [action potentials](#) [L-type calcium channel currents](#)

基金项目: 广西科学研究与技术开发计划项目(桂科攻0630002-2A); 广西中医药科技专项课题(GZKZ10-122); 2011年广西研究生创新计划项目(2011105981002D26)

作者	单位	E-mail
李映新	广西医科大学, 药学院, 南宁 530021	marchimoro@yeah.net
黄媛恒	广西医科大学基础医学院实验生理学科学实验中心, 南宁 530021	
林兴	广西医科大学医学科学实验中心, 南宁 530021	
黄仁彬*	广西医科大学药学院, 南宁 530021	huangrenbin518@163.com

摘要点击次数: 108

全文下载次数: 111

中文摘要:

目的 探讨耐钙昆明小鼠心室肌细胞的急性分离方法及动作电位、L型钙通道电流的记录。方法 采用三步灌流法, 首先灌流无钙台氏液, 再换成含II型胶原酶 $0.1 \text{ mg} \cdot \text{mL}^{-1}$ 、胰蛋白酶 $0.01 \text{ mg} \cdot \text{mL}^{-1}$ 、牛血清白蛋白 $0.2 \text{ mg} \cdot \text{mL}^{-1}$ 的无钙台氏液灌流, 消化液灌流期间, 每隔5 min加入20 mL的 $20 \text{ mmol} \cdot \text{L}^{-1}$ CaCl_2 , 以观察流出液是否有单个心肌细胞来判断消化终点, 最后灌流含 $1 \text{ mg} \cdot \text{mL}^{-1}$ 牛血清白蛋白的KB液, 采用全细胞膜片钳记录方式记录动作电位及L型钙通道电流。结果 获得80%~90%杆状心肌细胞, 复钙后, 仍有60%细胞保持静止, 细胞表面干净整洁, 折光性强, 边缘和横纹清晰, 立体感强, 获得60%左右的耐钙心室肌细胞, 并记录到典型的动作电位、L型钙通道电流。结论 该分离方法分离的细胞具有耐钙性和正常电生理特性。

英文摘要:

OBJECTIVE To explore and establish simple and reliable method of isolating single calcium-tolerant ventricular myocytes of KM mouse for patch clamping and recording of action potential and the currents of L-type calcium channels. METHODS The three-step enzymatic dissociation method was used to isolate myocytes from ventricular tissue by the Langendorff apparatus. Hearts were perfused retrogradely with Ca^{2+} free Tyrode's solution initially, and then with Ca^{2+} free Tyrode's solution contained $0.1 \text{ mg} \cdot \text{mL}^{-1}$ collagenase II, $0.01 \text{ mg} \cdot \text{mL}^{-1}$ trypsin and $0.2 \text{ mg} \cdot \text{mL}^{-1}$ bovine serum albumin. During the perfusions, $20 \text{ mL } 20 \text{ mmol} \cdot \text{L}^{-1}$ of CaCl_2 was added to the digestive juice every 5 minutes. The terminal of digestion was judged by observing the existence of single myocyte in the efflux solution. KB solution contained $1 \text{ mg} \cdot \text{mL}^{-1}$ bovine serum albumin was ultimately used. The action potential and the currents of L-type calcium channels were recorded by patch clamp in the entire cell mode. RESULTS The 80%-90% cells obtained were rod-shaped myocytes. After the recalcification, 60% cells stayed still, and the action potentials and L-type calcium channel currents could be successfully recorded. CONCLUSION The method is economical and effective, the myocytes obtained in this way are suitable for the recording of patch clamp technique.

[查看全文](#) [查看/发表评论](#) [下载PDF阅读器](#)

关闭

