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刘红,王晔,汤莹,沈亚峰,杨勇骥,雷长海.膜片钳-激光扫描共聚焦显微镜同步实时控制系统的建立及其在心肌细胞膜钙离子通道研究中的应用[J].第二军医大学学报,2012,33(2):123-129

膜片钳-激光扫描共聚焦显微镜同步实时控制系统的建立及其在心肌细胞膜钙离子通道研究中的应用 (Fulltext)

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摘要：

目的 建立膜片钳-激光扫描共聚焦显微镜同步实时控制系统,并将其应用于体外心肌细胞膜钙离子通道的研究,验证其应用效果。方法 通过在激光扫描共聚焦显微镜上加装膜片钳装置,采用计算机自动控制技术建立膜片钳与激光扫描共聚焦显微镜的同步实时控制系统;将建立好的装置应用于观察体外雄性大鼠心肌细胞膜钙离子通道,并分析观察结果 。结果 成功建立膜片钳-激光扫描共聚焦显微镜同步实时控制系统;当刺激心肌细胞时,激光扫描共聚焦显微镜-膜片钳同步实时控制系统在通过激光扫描共聚焦显微镜观察钙火花的同时可通过膜片钳记录心肌细胞膜钙离子通道电流信号。定量分析结果 表明相邻两个钙火花之间的时间间距分别为(10.055±0.021)、(10.079±0.021)、(10.087±0.021) s,符合膜片钳设定的刺激间隔(10 s);单个钙火花在空间上均局限于2 μm直径范围,在时间上平均经历了约30 ms,从出现至达到最高浓度平均需10 ms,从达到最高浓度到消失平均需20 ms,与钙火花理论吻合。结论 成功建立膜片钳-激光扫描共聚焦显微镜同步实时控制系统,在心肌细胞实现了在利用膜片钳进行全细胞记录观测和测定钙离子通道电流及其开闭时程的同时,利用激光扫描共聚焦显微镜获得了钙火花的显微结构形态图像,测定钙离子的位点变化,有助于进一步了解质膜钙离子通道的内部机制。

关键词：膜片钳术 共焦显微镜检查 同步控制 钙通道

Establishment of patch clamp and laser scanning confocal microscope synchronous real-time control system and its application in study of membranaceous calcium channel

Fulltext

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Abstract:

Objective To establish a patch clamp and laser scanning confocal microscope synchronous real-time control system, and apply it in study of membranaceous calcium channel, so as to verify its effects. Methods By adding the patch clamp on the laser scanning confocal microscope, we established a patch clamp and laser scanning confocal microscope synchronous real-time control system using computer autocontrol technique. The system was used to observe the male rat membranaceous calcium channel in vitro, and the observation results were analyzed. Results We successfully established the patch clamp and laser scanning confocal microscope synchronous real-time control system. When the myocardial cells were stimulated, the system could observe the myocardial calcium spark by laser scanning confocal microscope and at the same time record calcium channel current signal by patch-clamp. Quantitative analysis showed that the time intervals between 2 adjacent calcium sparks were (10.055±0.021), (10.079±0.021) and (10.087±0.021) s, which met the stimulus interval for patch-clamp set (10 s). A single calcium spark had a diameter within 2 μm, and it experienced an average period of about 30 ms. It needed an average of 10 ms from the appearance of the spark to its peak concentrations, and it needed an average of 20 ms from its peak concentration to disappearance, which was consistent with the calcium spark theory. Conclusion A patch clamp and laser scanning confocal microscope synchronous real-time control system has been successfully established. The system is capable of performing whole-cell observation recording and determining the membranaceous calcium channel currents and its closing process by patch clamp; at the same time it can synchronously obtain the micro images of the calcium spark with laser scanning confocal microscope, locating changes of calcium ions and helping to understand the internal mechanism of the membrane calcium channels.

Keywords:patch-clamp techniques confocal microscopy synchronous control calcium channels

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