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La^{3+} Transmembrane Research in Guinea Pig Ventricular Cells by Fura-2 Fluorescence

Rong CHEN, Pin YANG*, Chun Ying WEI

Institute of Molecular Science, Shanxi University, Taiyuan 030006

Abstract: Binding of La^{3+} to Fura-2 can change 340/380 nm fluorescence intensity ratio. Whether La^{3+} cross ventricular cell membrane was detected by this fluorescent probe technique. Fura-2 loaded isolated guinea pig ventricular cells were exposed to 0.01-0.1mM extracellular Lanthanum ion concentration, 340nm/380 nm fluorescence ratio was not changed. Using calcium channel agonist BAY K8644, KCL (35mM) depolarization to open the voltage-dependent calcium channel (VDCC); Adrenoceptor agonist to excite adrenoceptor, 340/380 ratio was not changed, suggesting that La^{3+} can not enter guinea pig ventricular cells in this case.

Keywords: Ventricular cells, adrenaline, La^{3+}, transmembrane, Fura-2, BAY K8644.

The safety of rare earth fertilizer was paid more attention now. Whether RE^{3+}(rare earth ion)cross membrane or not is the key problem of rare earth element biological effect. It was not solved until now. Ventricular cells which have different type of ions channel and adrenoceptor played very important role in biological function[1]. To our knowledge, trans-ventricular cells membrane behavior of rare earth ions has not been researched by Fura-2 fluorescent probe technique. Guinea pig ventricular cells were chosen to detect La^{3+} influx by Fura-2 fluorescence in our work.

The trans-ventricular cells membrane behavior of rare earth ions

Suspensions of isolated guinea pig ventricular cells were produced by collagenase treatment of ventricular fragments[2]. The resulting cell suspension was diluted at $10^{8\text{-}9}$/L in culture medium. The ventricular cells were incubated at 37℃ in culture media containing 1 μ M Fura-2/AM for 45 min. After loading was accomplished, cells were placed in hepes-buffered physiological solution for fluorescence measurement at both 340 and 380 nm wavelengths using LS-50B spectrofluorometer Fast Filter, with emission wavelength 510 nm,slit 10 nm.

We exposed Fura-2 loaded ventricular cells to 0.01-0.1mM extracellular lanthanum concentration ($[La^{3+}]_0$) and simultaneously measured the 340/380 nm fluorescence intensity ratio. After exposure to La^{3+}, the 340/380 fluorescence ratio did not increase. Because *in vitro* studies confirmed that the sensitivity of Fura-2 for La^{3+} is greater than for Ca^{2+} and we avoided influence of extracellular calcium concentration ($[Ca^{2+}]_0$) influx by keeping $[Ca^{2+}]_0$ zero, our results indicated that La^{3+}(0.01-0.1mM) can not cross guinea pig ventricular cell membrane .Under the same experimental conditions, we tested the trans-ventricular cell membrane behavior of other rare earth ions Y^{3+}, Gd^{3+}, Eu^{3+}, Ho^{3+}, Yb^{3+} and Sm^{3+}, 340/380 fluorescence ratio did not increase, indicating no

RE^{3+} transmembrane.

Effect of BAY K8644, KCl on trans-ventricular cells membrane behavior of La^{3+}

BAY K8644 is a selective VDCC agonist which can open VDCC, induce Ca^{2+} influx and dramatically prolong the Ca^{2+} channel open-time[3]. To test the importance of La^{3+} influx *via* the Ca^{2+} channel, we exposed Fura-2 loaded ventricular cells to 5×10^{-7}M BAY K8644 for 1 min to open VDCC before addition of 0.1mM La^{3+}, 340/380 ratio did not increase. It shows that BAY K8644 did not promote La^{3+} influx.

High concentration KCl depolarization to open VDCC has different mechanism with that of BAY K8644[4]. Result with KCl (35mM) depolarization pretreatment shows that high concentration KCl did not promote La^{3+} influx.

Under the same experiment conditions, BAY K8644, KCl (35mM) did not promote the other rare earth ions Y^{3+}, Sm^{3+} *etc*. transmembrane.

Effect of adrenalin on *trans*-ventricular cells membrane behavior of La^{3+}

Adrenaline is an adrenoceptor agonist[5]. It is confirmed that adrenalin can induce Ca^{2+} influx through exciting adrenoceptor in ventricular cells, which is an active ions transmembrane way. To test the importance of La^{3+} influx *via* the α, β-adrenoceptor. We exposed Fura-2 loaded ventricular cells to 20μM adrenaline before addition of 0.1mM[La^{3+}], 340/380 ratio did not increase. It shows that adrenoceptor agonist can not promote La^{3+} influx.

Under the same experiment conditions, adrenaline did not promote the other rare earth ions Y^{3+}, Sm^{3+} *etc*. transmembrane.

Discussion

In summary, our findings indicate that La^{3+} can not cross the guinea pig sarcolemma through the calcium channel and adrenoceptor. The results support that La^{3+} affected heart cells function by extracellular method of action[6]. It could be explained that La^{3+} has a crystalline ionic radius similar to that of Ca^{2+}, but the charge density is higher, La^{3+} can effectively displace Ca^{2+} from an ionic binding sites and influence crossing-membrane transport of Ca^{2+}, Na^{+} *etc*., which have caused the change of cells function.

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References and notes

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