Effects of Isoproterenal on IhERG during K+ changes in HEK293 cells

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Objectives:

The human ether-a-go-go related gene (hERG) encodes the pore forming protein which mediates the rapid delayed rectifier K+ current in the heart (IKr). Together with other ion channels hERG determines the cardiac action potential and regulates the heart beating. Dysfuction of the hERG ion channel will lead to acquired long QT syndrome (LQTS). Therefore, new drug candidates must pass the test for a potential inhibitory effect on the hERG current as a first step in a nonclinical testing strategy. Arrhythmias in patients with LQTS are typically triggered during physical or emotional stress, suggesting a link between sympathetic stimulation and arrhythmias. It is well known that potassium level can affect the QT interval through affecting IhERG both in vivo and in vitro. In this study, we try to find out whether the trigger effect still exist when K+ changes violently in a short time period. In other words, whether the risk of TdP aggravate when patients suffer from acute water electrolyte balance disorder, which is a common symptom in hot weather.

Methods:

HEK293 Cell line stably expressing hERG channel were cultured in DMEM supplemented with 10% of fetal bovine serum. Whole-cell patch-clamp method was applied for ionic current recordings. The compositions of pipette was (in mM) 125 KCl, 5 MgCl2, 5 EGTA-K, 10 HEPES-K and 5 Na-ATP adjusted to pH 7.2 with KOH. The bath solutions for recording the IhERG currents was 136 NaCl, 4 KCl, 1 MgCl2, 10 HEPES-Na, 1.8 CaCl2 and 10 glucose, pH 7.4 with NaOH. The low extracellular K+ solution was 115 KCl, 5 MgCl2, 5 EGTA-K, 10 HEPES-K and 10 Na-ATP adjusted to pH 7.2 with NaOH. Patch-clamp experiments were performed at room temperature (22 ± 1°C). The recording of low K+ current was carried out immediately after the original normal K+ solution has been totally replaced. Isoproterenol (ISO) 100nM was added into both kinds of K+ solution to apply the effect of β1-AR stimulation.

Results:

We found that low K+ solution increased IhERG from 907.39±18.68to 1620.08±249.44pA(n=30,P<0.05); Low K+ also shifted the I-V curve to the left. IC50 in control is 10.31±5.52 mV, low K+ is -6.15±1.58 mV. When adding ISO 100nM to extracellular solution, same effects were shown for both groups. ISO decreased Imax for both group. In control group, Imax reduced from 907.39±18.68to493.16±54.41pA (n=30, P<0.01), while in low K+ group, I max decreased Imax from 1620.08±29.44to 488.48±81.87pA(n=30,P<0.05). At the same time, ISO shifts the I-V curve to the right for the control group and shift the curve to the left for low K+ group. IC50 in control when added ISO is 22.25±3.80 mV, while IC50 in low K+ group after adding 100nM ISO is -31.00±5.73 mV.

Conclusion:

The results from this study is contradict to those in our previous study where low K+ combined with ISO can lead to temporarily increase of QT intervalin vivo. It is reported that an increase in net outward repolarizing current, due to a relatively large increase of IKs, is responsible for the

changes of QT interval in response to beta-adrenergic stimulation in vivo(2). Therefore future studies need to co-transfect IKs channel to confirm this.

References:

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