mechanisms of benefical effects of insulin on electrical activity of cardiomyocytes from MetS rats are not known yet, under the literature data, one can suggest that external insulin induction may regulate cellular glucose metabolism, thereby preventing increased oxidative stress induced changes in sarcolemmal membrane ion channels such as voltage-dependent Na^+- and K^+-channel currents. (Supported by grants from Tübitak SBAG-2124S254)

1346-Pos Board B323

Both Hyperglycemia and Hyperinsulinemia Induce Changes in Voltage-Dependent K^+ Channel Currents in H9c2 Ventricular Cells

Sinan Degirmenci, Yusuf Olgar, Aysegul Toy, Belma Turan.
Department of Biophysics, Ankara University, Ankara, Turkey.

The diabetic patients, generally with overweight or obesity, face to heart diseases, while insulin resistance is one of reasons that underlies abnormal cardiac electrophysiology. Insulin is a crucial hormone which regulates physiological processes such as energy metabolism, contractile activity, protein expression and ionic transports into/out of the cells. Besides, insulin provides glucose uptake to the cell via facilitating the localization of the glucose transporter, GLUT4, on the surface of the membrane. It has been proposed that this mechanism is deteriorated and therefore the electrical and mechanical activities of the heart are deteriorated on individuals who have insulin resistance and/or insulin deficiency. It is also proposed that insulin can activate different types of voltage-gated cation channels. We tested this hypothesis by studying the H9c2 cell line, derived from embryonic rat ventricular cells under both hyperglycemia and hyperinsulinemia. Basically, currents were recorded via whole cell configuration of the patch clamp technique. These cells were investigated on physiological, hyperglycemic (25-mM) and hyperglycemic (25-mM) plus hyperinsulinemic (100-nM) conditions. According to our findings on the cells, incubated 24-h with 25-mM glucose, both the peak values of inward and outward K^+-currents were significantly lower compare to the currents on the normo-glycemic condition. On the other hand, currents recorded after 24-hour of 25-mM glucose and 30-min of 100-nM insulin incubation (hyperglycemic+hyperinsulinemic) were significantly increased compare to hyperglycemic condition ($p<0.05$). Furhermore, 30 and 120-min of 100-nM insulin incubation to cells on normo-glycemic condition deteriorates K^+-currents and leads to hyperglycemia like effect. As a consequence, our data suggests that hyperglycemia-induced prolonged action potential in mammalian cells via reduction of the K^+-currents can underly the apperance of arrithymias in diabetic patients. (Supported by grant from Tübitak SBAG-2124S254)

1347-Pos Board B324

CaMKII Inhibitor KN-93 Directly Blocks IKr in Cardiac Myocytes

Bence Hegyi[1], Ye Chen-Izu[1,2], Zhong Jian[1], Rafael Shimkunas[1,2], Leighton T. Izu[1], Tamas Banyasz[1,3].
[1]Department of Pharmacology, University of California, Davis, Davis, CA, USA, [2]Department of Biomedical Engineering, University of California, Davis, Davis, CA, USA, [3]Department of Physiology, University of Debrecen, Debrecen, Hungary.

Rationale: KN-93, an inhibitor of the calcium/calmodulin-dependent protein kinase II (CaMKII) is widely applied in cardiovascular research fields to study the mechanisms of cardiomyopathy and cardiac arrhythmias. Whereas KN-93 is a potent inhibitor of CaMKII, several off-target effects have also been reported in expression cell systems and smooth muscle cells, but there is a lack of information on the KN93 side effects in mammalian ventricular myocytes. Hence, the goal of this study was to examine the effect of KN-93 on IKr, the rapid component of delayed rectifier potassium current which significantly influences cardiac arrhythmogenesis.
Method: IKr was recorded by using the action potential-clamp and conventional voltage-clamp techniques in the ventricular myocytes from rabbit and guinea pig hearts.
RESULTS:
(1)KN-93 inhibited IKr with an IC50 value of 102.57 ± 9.28 nM, which is significantly lower than the previously reported IC50 value of KN-93 (>300 nM) for inhibiting CaMKII.
(2)The inhibitory effect of KN-93 on IKr developed within 1 minute, a time period too short to be attributed to CaMKII inhibition.
(3)The inhibitory effect of KN-93 was also present when cytosolic calcium was chelated by 10 mM BAPTA which should lower the CaMKII activity.
(4)KN-92 also inhibited IKr, even though it does not inhibit CaMKII.
Conclusion: Taken together, these data demonstrate for the first time that KN-93 exerts a direct inhibitory effect on IKr in cardiomyocytes, which is not mediated by CaMKII. This off-target effect of KN-93 should be taken into account when interpreting the data from using KN-93 to investigate the role of CaMKII in cardiac function.

1348-Pos Board B325

Modulation of hERG 1a Trafficking by hERG 1b Subunits in Heart

Kelly A. Aromolaran[1], Donald D. Chang[2], R. Suzanne Zukin[1], Henry M. Colecraft[2], Mohamed Chahine[3], Mohamed Boutjdir[4], **Ademuyiwa Aromolaran**[2].
[1]Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA, [2]Physiology and Cellular Biophysics, Columbia University, New York, NY, USA, [3]Medicine, Laval University, Quebec City, QC, Canada, [4]Medicine, Cell Biology and Pharmacology, SUNY Downstate, New York, NY, USA.

In human heart the rapid component of the delayed rectifier potassium current (IKr), is an important contributor to repolarization. IKr exists as heterotetrameric channel complexes composed of hERG 1a/1b subunits. hERG 1b is defined by a shorter and unique N-terminus that lacks the Per-Arnt-Sim or ether-à-go-go, domain with implications for its role in the faster deactivation of hERG 1a/1b currents compared to homomeric hERG 1a currents. Decreases in IKr, due to either congenital mutations in hERG 1a/1b subunits or acquired in diabetes, delay repolarization, leading to long QT syndrome (LQTS), a condition that increases the likelihood of fatal arrhythmias. The mechanism by which hERG 1a/1b subunits traffic in heart is unknown. Here, we tested the hypothesis that hERG 1b subunits modulate the surface expression of hERG 1a in heart. hERG 1a/1b subunits were tagged intra-cellularly with YFP/CFP and extra-cellularly with a 13-residue high affinity α-bungarotoxin binding site (BBS). BBS-tagged hERG 1a-YFP expressed alone or with 1b-CFP generated functional currents. Cell surface BBS-tagged channel subunits were selectively detected with quantum dot. Tagged hERG 1a/1b subunits expressed in rat ventricular myocytes using adenovirus were co-localized and targeted to the surface sarcolemma, t-tubules and intercalated disks, similar to native channels. Optical pulse chase assays revealed for the first time that co-expression of hERG 1b increases the surface expression of hERG 1a in heart. Our data emphasize that hERG 1b is an important modulator of IKr. Understanding this process is likely to provide crucial insights for the development of targeted therapies for LQTS.

1349-Pos Board B326

Blockade of Calcium/Calmodulin-Dependent Kinase II (CaMKII) Induces Heterogeneous Depression of Conduction and Promotes Ischemic Ventricular Fibrillation (VF)

Mark Warren, Katie J. Sciuto, Junko Shibayama, Alexey V. Zaitsev.
The Nora Eccles Harrison Cardiovascular Research and Training Institute, Salt Lake City, UT, USA.

CaMKII regulates amongst many targets the sodium channel (I_{Na}), the primary determinant of cardiac excitability and conduction. Cellular studies diverge on the downstream effect of CaMKII on I_{Na}. Successful pharmacological therapy targeting CaMKII requires understanding how it regulates excitability and conduction in intact tissue, especially during myocardial ischemia when reduced sodium channel availability causes unsafe conduction. We recorded fluorescence of voltage-sensitive dye Di-4-ANEPPS in the left (LV) and right (RV) ventricles of Langendorff-perfused rabbit hearts (n=11) before and during 30 minutes of global ischemia, with and without perfusion of CaMKII blocker KN-93 (2.75 microM). We computed the local conduction delay (LCD, measured in ms/mm) and compared the 10-90% range of LCD values in the RV and LV at baseline and 4 minutes of ischemia. KN-93 caused heterogeneous slowing of conduction at baseline, which was further exacerbated by ischemia. Without major effect on the 10% level, KN-93 extended the 90% level of the LCD distribution (at baseline: 63% increase in LV; 211% increase in RV. At 4 minutes of ischemia: 230% increase in LV; 344% increase in RV; all $p<0.05$). During ischemia, KN-93 prompted the initiation (at 5.6 ± 0.47 min of ischemia vs. 17.4 ± 1.89 min in control, $p<0.05$) of VF through recurrent conduction block at a basal RV site of marked conduction depression. The ''chemical phosphatase'' butanedione monoxime (BDM, 20 mM) reduced almost threefold the KN-93 concentration required for depression of conduction and early ischemic VF. **Conclusions:** Constitutive activity of CaMKII is essential to maintain robust and uniform conduction in normal and acutely ischemic heart. CaMKII block is pro-arrhythmic during ischemia. The increased sensitivity to KN-93 in the presence of BDM suggests a delicate balance between kinase and phosphatase activity in regulation of cardiac conduction.

1350-Pos Board B327

Concurrent PDE3 and PDE4 Activation Suppresses Local Ca^{2+} Releases (LCR) to Regulate Normal Spontaneous Firing of Sinoatrial Node Cells (SANC)

Tatiana M. Vinogradova, Yevgeniya Lukyanenko, Kirill V. Tarasov, Syevda Sirenko, Alexey E. Lyashkov, Yue Li, Edward G. Lakatta.
GRC, NIA, NIH, Baltimore, MD, USA.