and electrical function of the heart. Although there is reduction of Ca^{2+} transients during ischemia, the underlying mechanism of this Ca^{2+} mishandling is not fully understood. Currently, no reports exist in which Ca^{2+} currents and SR Ca^{2+} release are measured simultaneously *in situ* during global ischemia. Recently, we found that global ischemia induces large increases in diastolic Ca^{2+} leading to an increase in sarcoplasmic reticulum (SR) Ca^{2+} content. Contrary to what we expected there was a smaller fractional SR Ca^{2+} depletion during an action potential (AP). However, Ca^{2+} sparks measured in intact hearts during ischemia are kinetically undistinguishable from preischemic recordings. Our idea is that depressed Ca^{2+} transients during ischemia may result from a reduction of L-type Ca^{2+} currents and/or coupling between plasma membrane Ca^{2+} influx and SR Ca^{2+} release. Recently, we found using loose patch photolysis that Ca^{2+} dependent currents during an AP showed a fast early component driven by an L-type Ca^{2+}influx and a slower late component mediated by a Na^{+}-Ca^{2+} exchanger (NCX) forward current. To understand why Ca^{2+} transient decreases, we performed experiments to address if ischemia induces changes in Ca^{2+} dependent currents. Our results indicate that ischemia produced a decrease of the early Ca^{2+} current and the late NCX current. However, the reduction of the NCX current occurs before the L-type current attenuation. Although both Ca^{2+} mediated currents recover during reperfusion after ischemia, L-type Ca^{2+} currents recuperate faster. We conclude that the impairment of NCX during ischemia is an early event in both the contractility reduction and diastolic Ca^{2+} increase and that this impairment is maintained during postischemic diastolic dysfunction.

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Down Regulation of L-Type Calcium Current in Rat Atrial Myocytes during Heart Failure

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Heart failure causes down-regulation of the atrial L-type Ca^{2+} current (ICaL) that has consequences on contractile function and susceptibility to atrial fibrillation. The reduction in atrial ICaL density has been suggested to involve changes in both the expression and phosphorylation of channel protein. The objective of this project was to examine the mechanism of the decrease in atrial ICaL in a surgical model of heart failure. Heart failure was induced in adult male Wistar rats by surgical ligation of the left anterior descending coronary artery (CAL) under general anesthesia. Control animals were subject to equivalent sham operation (Sham). Left atrial myocytes were isolated ~18 weeks after surgery and superfused with a Tyrode's solution (37 °C). Whole-cell L-type Ca currents (ICaL) were recorded during depolarisation to 0 mV from a holding potential of −80 mV and normalised to whole-cell capacitance, which was also used as an index of cell size. CAL cells were hypertrophied (94.7 ± 5.2 pF, n=63) compared to Sham (56.6 ± 2.1 pF, n=47; $P \leq 0.0001$). ICaL density at 0 mV was reduced in CAL compared to Sham (3.3 ± 0.4 pA/pF; n=31 vs 7.3 ± 0.7 pA/pF; n=33; $P \leq 0.001$), but the response of ICaL to 1 μmol/L norepinephrine was greater in CAL (x6.8) than in Sham (x3.2) so that there was no difference in ICaL density between the two groups in the presence of norepinephrine. These data indicate a change in channel protein phosphorylation without a change in channel density. The regulation of L-type Ca^{2+} channel phosphorylation may be a potential therapeutic target for atrial arrhythmias in heart failure.

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Roscovitine as the Archetypal Member of a Novel Class of Antiarrhythmics Targeting Late $I_{Ca,L}$

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L-type calcium current ($I_{Ca,L}$) plays a central role in counterbalancing potassium current during the plateau phase of cardiac action potential (AP). When repolarization reserve is reduced, the aberrant reactivation of $I_{Ca,L}$ during repolarization can cause transient depolarization events called Early Afterdepolarizations (EADs), a recognized trigger of cardiac arrhythmias. Recently we demonstrated that EADs are highly sensitive to the steady-state voltage-dependent properties of $I_{Ca,L}$. Using the dynamic clamp technique in isolated rabbit ventricular myocytes under EAD-favoring condition (*i.e.* oxidative stress or hypokalemia), we found that selectively inhibiting the non-inactivating component (late) of $I_{Ca,L}$ potently suppressed EAD-occurrence. Roscovitine, a purine analog, has been shown to reduce the late $I_{Ca,L}$ component while leaving peak unperturbed. Here we tested the ability of this drug to suppress EADs in isolated rabbit ventricular myocytes induced by exposure to H_2O_2 (600μM). This oxidative stress caused EADs in 81% [95% CI: 66-95%] of the APs, prolonging AP duration (APD_{90}) from 276 ms [97-433 ms] to 840ms [285-1967ms] (n=4). Addition of roscovitine (20μM) completely abolished EADs and reduced the APD_{90} to 189 ms [155-222 ms]. Moreover, roscovitine did not significantly perturb the Ca transient, implying maintenance of normal excitation-contraction coupling. We next exposed isolated aged rat hearts to 0.1 mM H_2O_2 to induce EADs and spontaneous ventricular tachycardia/fibrillation (VT/VF). In all tested hearts (n=4), roscovitine terminated VT/VF and led to resumption of sinus rhythm within 10 min. Our study offers additional proof of concept supporting a pharmacological strategy to target late $I_{Ca,L}$ to prevent EAD-mediated arrhythmias, with roscovitine as an archetypal drug to guide the development of this new class of antiarrhythmics.

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Transient Outward Potassium Current and its Arrhythmogenic Dynamics in Cardiac Myocytes

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The transient outward K^{+} current (I_{to}) is a main contributor to repolarization during the cardiac action potential in cardiac myocytes, which may play important roles in arrhythmogenesis in cardiac diseases including ischemia, Brugada syndrome, and heart failure. Previous experimental and computer modeling studies have shown that I_{to} can cause complex action potential dynamics (alternans and chaos), which can potentiate phase-2 reentry. However, the underlying mechanisms are not well understood. In this study, we carried out computer simulations and developed simplified mathematical models (iterated maps) to reveal the mechanisms of I_{to}-induced complex action potential dynamics. We showed that: 1) I_{to} induces short-term cardiac memory via its interactions with a slowly activating K^{+} current (I_{Ks}) and the L-type Ca^{2+} current ($I_{Ca,L}$), which promotes alternans during the physiological heart rate. This can be a candidate mechanism for T-wave alternans widely seen in patients with Brugada syndrome; 2) I_{to} gives rise to two different types of non-monotonic restitution curves. In the presence of rapidly recovering I_{to}, as the diastolic interval increases, the action potential duration is short at short diastolic intervals (DIs), increases sharply at a certain range of DIs, and saturates at large DIs. In the presence of slowly recovering I_{to}, the action potential duration is normal at short DIs, decreases sharply at a certain range of DIs, and becomes short at large DIs. These restitution curves along with short-term memory induced by I_{to} can give rise to complex action potential dynamics and chaos. In conclusion, using both computer simulation and simplified mathematical models, we can pinpoint the underlying mechanisms for the complex arrhythmogenic action potential dynamics induced by I_{to}.

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An Investigation on Electrical Activity and Sarcolemmal K^{+}-Channels in Cardiomyocytes from Insulin-Resistant Rat Heart

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It is well accepted that metabolic syndrom (MetS) is an important risk factor for cardiac dysfunction whereas no precise mechanism is available for cardiac remodeling. Previously, we have shown that MetS-rats, induced with high-sucrose diet for 16-week, have marked depressed cardiac mechanical activity due to ventricular dysfunction, concomitant with ventricular hypertrophy together with arrhythmia. One major feature in MetS is the insulin resistance that must result from the defective insulin signaling. Insulin resistance is a multifaceted syndrome that causes not only an impaired glucose regulation but also a number of other diseases, including dysfunctional cardiac ventricular contraction. Using 16- week-old over-weighted and insulin-resistant Wistar rats as an animal model, we tested the purpose of this work was to understand cardiac arrhythmogenesis directly linked to the sudden cardiac arrest in patients with MetS. The action potential (AP) duration was markedly prolonged and voltage-dependent K+-channel currents were depressed in left ventricular cardiomyocytes from MetS-group while the amplitude of APs were depressed compared to aged-matched controls. Insulin incubation of MetS cardiomyocytes (100-nM for 30 min) induced significant shortening in AP duration with marked increase in AP amplitude. These benefical effects with insulin in MetS cardiomyocytes were further supported with recovery in the depressed K^{+}-channel currents. Additionally, we observed no arrhythmias during AP recordings in insulin incubated MetS cardiomyocytes. Although the underlying

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)

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

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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)

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CaMKII Inhibitor KN-93 Directly Blocks IKr in Cardiac Myocytes

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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.

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

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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.

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

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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.

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

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