Ionic Mechanisms Underlying Abnormal QT Prolongation and the Associated Arrhythmias in Diabetic Rabbits: A Role of Rapid Delayed Rectifier K⁺ Current

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Key Words

Diabetes • QT prolongation • Arrhythmias • Ion currents

Abstract

Abnormal QT prolongation with the associated arrhythmias is considered the major cardiac electrical disorder and a significant predictor of mortality in diabetic patients. The precise ionic mechanisms for diabetic QT prolongation remained unclear. We performed whole-cell patch-clamp studies in a rabbit model of alloxan-induced insulin-dependent diabetes mellitus. We demonstrated that heart rate-corrected QT interval and action potential duration (APD) were prolonged by ~20% with frequent occurrence of ventricular tachyarrhythmias. Several K⁺ currents were found decreased in diabetic rabbits including transient outward K⁺ current (I_{to}) that was reduced by ~60%, rapid delayed rectifier K⁺ current (I_{Kr}) reduced by ~70% and slow delayed rectifier K⁺ current (I_{Ks})

reduced by ~40%. The time-dependent kinetics of these currents remained unaltered. The peak amplitude of L-type Ca²⁺ current (I_{CaL}) was reduced by ~22% and the inactivation kinetics was slowed; the integration of these two effects yielded ~15% reduction of I_{CaL} . The inward rectifier K⁺ current (I_{K1}) and fast sodium current (I_{Na}) were unaffected. Simulation with LabHEART, a computer model of rabbit ventricular action potentials, revealed that inhibition of I_{to} or I_{Ks} alone fails to alter APD whereas inhibition of I_{Kr} alone results in 30% APD prolongation and inhibition of I_{CaL} alone causes 10% APD shortening. Integration of changes of all these currents leads to ~20% APD lengthening. Protein levels of the pore-forming subunits for these ion channels were decreased to varying extents, as revealed by immunoblotting analysis. Our study represents the first documentation of I_{Kr} channelopathy as the major ionic mechanism for diabetic QT prolongation.

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Introduction

The most prominent cardiac electrical disturbance in diabetic mellitus (DM) is the abnormality of QT interval. When QT interval is corrected for heart rate (QTc), values longer than 440 ms are considered abnormal, and this limiting value is commonly exceeded in diabetic patients. A prolongation of QT interval has been associated with an increased risk of sudden cardiac death in patients with diabetes due to occurrence of lethal ventricular arrhythmias known as *Torsade de Pointes* or long QT syndrome (LQTS) [1-2]. Particularly noticeable is that in recent years there have been increasing incidences of the appearance of ECG disorders with no association between QT alterations and neuropathy, micro- or macro-vascular diseases, cardiovascular drugs, age or gender, being these situations additional risk factors [2-3]. LQTS occurs in both insulin-dependent type I (IDDM) and insulin-independent type II DM (NIDDM) patients and the prevalence is as high as some 25%. Indeed, QT prolongation has been suggested as a predictor of mortality in both IDDM and NIDDM [2-3].

QT interval reflects the total duration of ventricular depolarization and repolarization or integrated action potential duration (APD) of heart cells. Cardiac APD is determined by the rate of membrane repolarization which is governed by a delicate balance between inward and outward ion currents. Increase in inward currents and/or decrease in outward currents tend to prolong APD thereby QT interval. L-type Ca^{2+} current (I_{CaL}) is a key inward current responsible for maintaining membrane depolarization or duration of plateau phase. The fast Na^{+} current (I_{Na}) is mainly responsible for the initial upstroke of an AP, but also contributes to the length of plateau phase. The outward currents in a ventricular myocyte include a number of K^{+} channels that are of critical importance to determining cardiac repolarization thereby QT interval; they are transient outward K^{+} current (I_{to}) [4], rapid delayed rectifier K^{+} current (I_{Kr}) [5], slow delayed rectifier K^{+} current (I_{Ks}) [5] and inward rectifier K^{+} current (I_{K1}) [6]. I_{to} is mainly responsible for the initial rapid membrane repolarization. I_{Kr} is a key repolarizing current responsible for phase 3 repolarization in cardiac cells of many species such as human [5] and rabbit [7-11], which is carried by the K^{+} channel subunit encoded by the human *ether-a-go-go* related gene (*HERG*). The slow component of delayed rectifier K^{+} current (I_{Ks}) regulates only the late phase repolarization owing to its slow activation kinetics [5]. Inward rectifier K^{+} current (I_{K1}) contributes to the final phase of repolarization [12].

The roles of several of above mentioned ion currents in diabetic APD/QT prolongation have been studied with experimental animal models. I_{to} and I_{ss} (steady-state outward K^{+} current) were found reduced in IDDM animals in most studies [13-18] and unaltered in others [19]. I_{K1} was found unchanged in IDDM [14, 20-21], excluding its contribution to APD prolongation in these animal models of diabetes. I_{CaL} was found smaller in IDMM in some studies [17] but not different in other studies [13, 22]. I_{Na} was also found decreased [23]. These studies contribute significantly to our current understanding of the ionic mechanisms for diabetic QT prolongation, but several important issues remained unresolved. First, previous studies were conducted nearly exclusively in rats and mice, the species in their adulthood not expressing phenotypic and physiologically significant I_{Kr} and I_{Ks} that are otherwise the major repolarizing current determining the plateau phase and total APD in humans. Second, inhibition of I_{to} , though expected to lengthen APD, has been shown to paradoxically shorten APD in many species such as humans and rabbits [24-26]. Third, I_{ss} or the equivalent has not been thus far identified in ventricular cells from species other than rats and mice, such as humans, canines, rabbits, guinea pigs and other $I_{\text{Kr}}/I_{\text{Ks}}$ -bearing species. Therefore, its physiological function and pathological role in diabetic QT prolongation remain questionable. Finally, reduction of I_{CaL} and I_{Na} in diabetic hearts should, if anything, shorten, but not lengthen APD/QT interval. Obviously, our understanding of the ionic mechanisms underlying diabetic QT prolongation is still incomplete and there is at need for further investigation into the issue in an animal model that possesses a similar set of ion current components as humans. This study was designed (1) to have an overall picture of alterations of ion currents/channels in diabetic hearts under identical or similar experimental conditions in rabbit model of diabetes, a species that possesses similar profiles of ion currents as humans and (2) to dissect the relative contribution of these ion currents/channels in diabetic QT/APD prolongation and to shed light on the potential underlying mechanisms.

Materials and Methods

Preparation of Rabbit Model of Type I Insulin-Dependent Diabetes Mellitus (DM)

Male New Zealand white rabbits weighing 1.6~2.0 kg (Charles River Canada Inc) were housed individually in stainless steel wire-bottomed cages in a room with a 12:12-hrs light-dark cycle and standard laboratory rabbit chow and drinking water

ad libitum. The animals were randomly assigned to control (Ctl) and IDDM groups. The control animals were untreated age-matched rabbits studied in parallel to IDDM animals. To establish diabetes, a single injection of 140 mg/kg (body weight) of pre-warmed (37°C) alloxan monohydrate (Sigma-Aldrich), freshly dissolved in saline at a concentration of 100 mg/ml, was administered via marginal ear vein under local anesthesia. To prevent fatal hypoglycemia from massive insulin release, 10% glucose solution (100 mg/kg, s.c.) was administered 4 and 6 hrs after alloxan treatment. The blood was collected via marginal ear vein after local anesthesia for determining the plasma level of glucose using a glucometer (TheraSense, USA) and the blood glucose level was monitored weekly thereafter until 10 weeks when the animals were sacrificed for further experiments. Only those animals with serum glucose concentrations =15 mM were considered diabetic and were used for further studies. The plasma insulin was measured by an ELISA kit (Yanaihara Inc, Insulin ELISA Kit; Cat# YK060). All procedures were in accordance with the guidelines set by the Animal Ethics Committee of the Montreal Heart Institute.

Implantation of Telemeters and ECG Recording in Conscious Rabbits

Rabbits were anesthetized with ketamine (Vetalar®, BioNiche Animal Health Canada, Belleville, ON, Canada) and xylazine (Rompun®, Bayer Inc, Toronto, ON, Canada) mixture (7:1) at a dosage of 1.2 ml/3 kg (i.m.). Abdominal hair was shaved and skin was cleaned and sterilized with antiseptic. A small incision was then made on the skin for subcutaneous implantation of ECG telemeter (EMKA Technologie, Paris, France) and the probes of the telemeter were fixed to the right and left underarm positions. Antibiotic cream (Polytopic®, Sabex Inc, Boucherville, QC, Canada) was applied to the closed skin wounds followed by adherent surgical dressing. Bandages were then used to protect the wounds. Antibiotic solution (0.5 ml, Longisil®, Vétroquinol N.-A. Inc) containing penicillin G benzathine (150,000 IU/ml) and penicillin G procaine (150,000 IU/ml) was applied i.m. daily for 5 days after the surgery. Seven days after implantation, the transducer was activated to record the real-time ECG in conscious rabbits before induction of diabetes as the basal measurement. The ECG signal was acquired and analyzed by the EMKA Technologies's IOX acquisition software and ECG-Auto, respectively. ECG was monitored continuously for 24 hrs immediately after treatment with alloxan and from day 2 after alloxan, ECG was recorded for 20 min at an interval of every 3 hrs. ECG recorded in this way is equivalent to the standard lead II ECG.

Surface ECG Recording in Anesthetized Rabbits

Standard lead II ECG was recorded before implantation of telemeters and right before being sacrificed for experiments (10 weeks after establishment of diabetes). Sedation and induction of anesthesia were obtained with ketamine (65 mg/kg) and xylazine (13 mg/kg, i.m.). Three-lead surface ECG was recorded with silver electrodes placed under the skin at optimized positions to obtain maximal amplitude recordings, enabling accurate measurements of QT intervals. The QT measurements and simultaneously recorded RR intervals were used to derive

heart rate corrected QT intervals using Carlsson's formula ($QT_c = QT - 0.175(RR - 300)$) [27].

Isolation of Rabbit Ventricular Myocytes

Myocytes were isolated from rabbit left ventricular endocardium via enzymatic digestion with the procedures similar to previously described [28]. Rabbits were anesthetized by sodium pentobarbital (60 mg/kg, i.v.). Hearts were rapidly excised and mounted on a Langendorff apparatus and perfused retrogradely with the following four solutions in a sequential order: 1 mM Ca^{2+} Tyrode (2 min), Ca^{2+} -free Tyrode (3-5 min), Ca^{2+} -free Tyrode containing collagenase (Worthington type II) for 25-35 min. The left ventricular wall was shaved to obtain endocardial layer (=1.5 mm thick) and the samples were minced in the storage solution and filtered. For I_{Kr} recordings, cells from the apical region and for I_{Ks} recordings cells from the basal area were used to enable better recordings as it has been reported I_{Kr} density is larger in the apex and I_{Ks} is larger in the base [29]. The freshly isolated myocytes were gently centrifuged and resuspended in the storage solution for patch-clamp studies. The solution for cell storage contained (mM): 20 KCl, 10 KH_2PO_4 , 25 glucose, 70 K-glutamate, 5 β -hydroxybutyric acid, 20 taurine, EGTA, 40 mannitol, and 0.1% albumin (pH 7.4).

Whole-Cell Patch-Clamp Recording

Patch-clamp techniques have been described in detail elsewhere [5, 28, 30, 31]. Currents were recorded in the whole-cell voltage-clamp mode and action potentials (APs) were recorded in the current-clamp mode, with an Axopatch-200B amplifier (Axon Instruments). Borosilicate glass electrodes had tip resistances of 1-3 M Ω when filled with the internal pipette solution. The pipette solution for K^+ current recordings contained (mM): 130 KCl, 1 $MgCl_2$, 5 Mg-ATP, 10 EGTA, and 10 HEPES (pH adjusted to 7.25 with KOH). The pipette solution used for recording I_{Na} contained (mM) 135 CsF, 5 NaCl, 10 EGTA and 5 Mg-ATP, 5 HEPES, and for I_{CaL} contained (mM): 20 CsCl, 110 Cesium aspartate, 1 $MgCl_2$, 5 Mg-ATP, 10 EGTA, and 10 HEPES (pH 7.25 with CsOH). The internal pipette solution for AP recording contained the same components as for K^+ currents recording, except that EGTA was reduced to 0.05 mM. The extracellular (normal Tyrode's) solution for I_{to} and I_{K1} currents and AP recording contained (mM): 136 NaCl, 5.4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 HEPES (pH 7.4 with NaOH). The extracellular solutions used for recording I_{Na} contained (mM) 132.5 CsCl, 5.0 NaCl, 1.0 $MgCl_2$, 1.0 $CaCl_2$, 11 glucose, and 10 HEPES. For I_{CaL} recordings, the solution contained (mM): 136 TEA-Cl, 5.4 CsCl, 1 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 HEPES (pH adjusted to 7.25 with CsOH). For I_{Kr} and I_{Ks} recordings, the superfusate was changed to an NMG solution composed of the following (in mM): 149 N-methyl-D-glucamine, 5 $MgCl_2$, 0.9 $CaCl_2$, and 5 HEPES (pH adjusted to 7.4 with HCl). In addition to the use of different solutions to optimize recordings of the currents of interest and to avoid unwanted currents, ion channel blockers were also used to prevent the contaminating currents. For studies on K^+ currents, I_{Na} was inactivated by holding the membrane at -50 mV and I_{CaL} was blocked by $CdCl_2$ (200 μ M) in the bathing solution. 4-Aminopyridine (1 mM) was used to inhibit I_{to} for recording other currents and glyburide (10 μ M)

plus Mg-ATP (5 mM) in the pipette solution to prevent ATP-sensitive K^+ current. Dofetilide (1 μ M) and HMR1556 (1 μ M) (Avanti Polar Lipid Inc., Alabaster, AL, USA) were used to block I_{Kr} and I_{Ks} , respectively, for I_{to} recordings. Experiments were conducted at $36 \pm 1^\circ\text{C}$ except that I_{Na} was recorded at $18 \pm 1^\circ\text{C}$. Junction potentials were zeroed before formation of the membrane-pipette seal. Series resistance and capacitance were compensated and leak currents were subtracted.

The voltage protocols for current recordings are shown in the insets of the respective figures. The currents were all recorded immediately after membrane rupture and series resistance compensation in order to minimize the possible time-dependent rundown, run-up, or negative shift of currents. Individual currents were normalized to the membrane capacity to control for differences in cell size, being expressed as current density pA/pF. No significant difference in cell capacitance was found between the two groups: 189 ± 17.9 pF for Ctl and 209.9 ± 8 pF ($p=0.08$) for IDDM. The amplitude of I_{to} was measured as the difference between the initial peak of I_{to} and the current level remaining at the end of the pulse. I_{K1} was measured as the magnitude of the current at the end of the pulse relative to zero reference. I_{Kr} was expressed as dofetilide-sensitive currents by subtracting the currents recorded 10 min after dofetilide (1 μ M) from the baseline currents before dofetilide. I_{Ks} was taken as HMR1556-sensitive currents in the presence of dofetilide. The amplitude of I_{Kr} and I_{Ks} was measured from both step currents at various test potentials (the difference between the current level at the end of the pulse and zero level) and tail currents (the difference between the peak tail current and zero level) at a repolarizing potential of -40 mV for I_{Kr} or at -20 mV for I_{Ks} . The amplitude of I_{CaL} and I_{Na} was measured as the difference between the peak inward currents and the currents remaining at the end of the pulse.

Western Blot

The membrane protein samples were extracted from rabbit ventricles for immunoblotting analysis of ion channel proteins, with the procedures essentially the same as described in detail elsewhere [28]. The protein content was determined with Bio-Rad Protein Assay Kit (Bio-Rad, Mississauga, ON, Canada) using bovine serum albumin as the standard.

Membrane protein sample (~ 150 μ g) was fractionated by SDS-PAGE (7.5%-10% polyacrylamide gels) and transferred to PVDF membrane (Millipore, Bedford, MA). The sample was incubated overnight at 4°C with the primary antibodies in 1:50~1:200. Affinity purified polyclonal primary antibodies against C-termini of human $Na_v1.5$ (the pore-forming α -subunit of I_{Na}), $Ca_v1.2$ (for the α_{1c} subunit of I_{CaL}), $Kv4.3$ (the pore-forming α -subunit of I_{to}), $Kir2.1$ (for I_{K1}), HERG (the pore-forming α -subunit of I_{Kr}), and KCNQ1 ($KvLQT1$, the pore-forming α -subunit of I_{Ks}), and against N-terminus of KCNE1 (minK, the β -subunit of I_{Ks}), were all raised in goat. HERG is used for rHERG throughout the manuscript for simplicity as the rabbit ERG channel sequence (Genbank accession U87513) is 93% and 96% homologous to human ERG at the nucleotide and amino acid levels, respectively. Inhibitory peptide for each antibody was used to test the antibody specificity. Next day, the membrane was washed in TTBS three times (10 min/each) and

incubated for 2 hrs with the HRP-conjugated donkey anti-goat IgG (H+L) (1:600) in the blocking buffer. Both primary and secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Bound antibodies were detected using the chemiluminescent substrate (Western Blot Chemiluminescence Reagent Plus, NEN Life Science Products, Boston, USA). GAPDH was used as an internal control for equal input of protein samples, using anti-GAPDH antibody purchased from RDI (Flanders, NJ). Coomassie staining was also performed to verify the sample quantity. Western blot bands were quantified using QuantityOne software by measuring the band intensity (Area $\times$ OD) for each group and normalizing to GAPDH. The final results are expressed as fold changes by normalizing the data to the control values.

Data Analysis

Group data are expressed as mean $\pm$ S.E. Statistical comparisons (ANOVA followed by Dunnett's method) were carried out using Microsoft Excel. A two-tailed $p < 0.05$ was taken to indicate a statistically significant difference. Nonlinear least square curve fitting was performed with CLAMPFIT in pCLAMP 8.0 or GraphPad Prism.

Results

Diabetic QT Prolongation and the Associated Arrhythmias

A total of 55 rabbits were used in this study, among which 22 were in the control group, and 33 in the IDDM group. Twenty-nine out of 33 rabbits (29/33, 88%) developed typical characteristics of type I diabetes 12-24 hrs after single injection of alloxan, as indicated by the elevated non-fasting blood glucose level (22.6 ± 1.1 mM, $p < 0.05$ vs control) as compared with the normal value (5.4 ± 0.7 mM) in control animals and by the reduced plasma insulin level (from 42 ± 14 for Ctl to 0.08 ± 0.05 for IDDM IU/ml, $p < 0.05$). The induced IDDM stabilized from day 3 after the onset until 10 weeks when the animals were used for cellular and molecular biology studies. Four of 33 rabbits of IDDM group (4/33, 12%) were resistant to the alloxan treatment, either failed to develop diabetes (blood glucose < 12 mM) or recovered from diabetes shortly after the onset. In addition, among the 29 IDDM rabbits, 10 died before complete data collection.

Remarkably, heart rate-corrected QT interval (QTc interval) were consistently prolonged in rabbits after treatment with alloxan (183 ± 5 ms) for 10 weeks compared with the baseline values obtained before treatment (155 ± 2 ms, $p < 0.05$, $n=19$), but not in control animals (159 ± 3 ms for baseline vs. 158 ± 4 ms for time-matched comparison, $n=22$). These data indicate a 21% prolongation of QTc interval in the IDDM rabbits over

Fig. 1. Electrical disorders in rabbits with insulin-dependent diabetes mellitus (IDDM). (A) Abnormal prolongation of QT interval in IDDM rabbits. Shown are representative ECG recordings from a control (Ctl) and an IDDM (10 weeks) rabbits, and mean data (n=22 rabbits for Ctl and n=19 for IDDM) of heart rate-corrected QT interval (QTc interval). The data were obtained from anesthetized rabbits. The dash lines define the measurements of QT interval. * $p < 0.05$ IDDM vs. Ctl. (B) Ventricular arrhythmias in IDDM rabbits. The examples of ventricular tachycardia (VT) and ventricular fibrillation (VF) were recorded by an ECG telemeter from an IDDM rabbit 2 min before sudden death. (C) and (D) Blood glucose concentration and QTc interval, respectively, as a function of time after alloxan administration in weeks (wk). * $p < 0.05$ vs. 0 wk; + $p < 0.05$ vs. Ctl at the same time point (n=22 rabbits for Ctl and n=19 for IDDM). * $p < 0.05$ vs. Ctl. (E) Prolongation of action potential duration (APD) in IDDM rabbits. The action potentials were elicited by a train of 10-consecutive stimuli of 2-ms duration and twice threshold strengths at a frequency of 2 Hz. The recordings obtained from 10th pulses were taken as steady-state value and used for data analysis. Mean data are from n=12 cells from 4 (Ctl) and 5 (IDDM) rabbits. * $p < 0.05$ vs. Ctl.

the control animals. Also noticeable is that in the rabbits that failed to develop IDDM, their QTc interval was normal (167 ± 3 ms) and comparable to that of control rabbits ($p > 0.05$). The QTc data are shown in Figure 1A. In the present study, the heart rate of diabetic rabbits (205 ± 6 bpm) was only slightly slower than that of the control ones (211 ± 8 bpm, $p > 0.05$).

Excessive QTc prolongation creates the substrates for arrhythmogenesis. This was indeed demonstrated in our experiments with the IDDM rabbits. As depicted in Figure 1B, arrhythmias, mainly of ventricular tachycardia

(VT) defined as a run of rapid ventricular deflections (QRS complex) lasting longer than 30 s, were observed in the IDDM rabbits, which was otherwise absent in control animals. Of the 19 IDDM rabbits used for data analyses, 12 developed VT (12/19, 63%). The VT often predisposed to ventricular fibrillation (VF) leading to sudden death. We were able to record ECG with telemetry from 4 out of 10 rabbits right before they died from five days after development of IDDM. The ECG clearly showed runs of polymorphic VT and VF.

To delineate the cellular mechanism underlying the

Fig. 2. Comparison of various K^+ currents in ventricular myocytes between healthy and IDDM rabbits. Currents were recorded with the voltage protocols shown in the insets. Left panels: representative recording of I_{to} (A), I_{K1} (B), I_{Kr} (C), and I_{Ks} (D) in healthy (Ctl) and IDDM rabbit hearts, and right panels: averaged I (current density)- V relationships (right panels). The inset in the I_{K1} I-V curves shows the outward portion of I_{K1} . I_{Kr} is defined as the dofetilide ($1 \mu M$)-sensitive currents and I_{Ks} the dofetilide-resistant currents. The I-V curves for I_{Kr} and I_{Ks} were constructed from step currents and similar results were obtained with tail currents. The values in the brackets indicate the number of cells used for data analysis (the same below). * $p < 0.05$ IDDM vs. Ctl.

QTc prolongation in our IDDM model, single cell action potentials (APs) were recorded in enzymatically dispersed myocytes from left ventricular endocardium. As illustrated in Figure 1E, APD at 50% repolarization (APD_{50}) and 90% repolarization (APD_{90}) was approximately 35% and 24%, respectively, longer in IDDM than in healthy subjects, which are somewhat longer than the 21% lengthening of QTc interval.

Functional Alterations of Ion Currents in Diabetic Hearts

To unravel the changes of ion currents that may account for the QTc/APD prolongation and the associated arrhythmias in our IDDM animals, we performed whole-cell patch-clamp studies of the ion currents operating under physiological conditions in ventricular myocytes, including I_{to} , I_{K1} , I_{Kr} , I_{Ks} , I_{CaL} , and I_{Na} . Our results revealed

Fig. 3. Comparison of L-type Ca^{2+} current (I_{CaL}) and fast Na^{+} current (I_{Na}) in ventricular myocytes between healthy and IDDM rabbits. Currents were recorded with the voltage protocols shown in the insets. (A) The inactivation time constants (τ) of I_{CaL} were obtained by the single exponential fit to the decaying phase of the currents at potentials from -10 to +30 mV. To integrate the changes of amplitude and inactivation kinetics, the areas under the I_{CaL} traces were calculated for more rational comparison of I_{CaL} sizes between healthy and IDDM rabbits. * $p < 0.05$ IDDM vs. Ctl.

reduction of multiple ion currents (I_{to} , I_{Kr} , I_{Ks} , and I_{CaL}) in cells isolated from IDDM rabbits, compared with healthy rabbits.

I_{to} current density was approximately 60% smaller in diabetic myocytes than in control ones and similar percentage of reduction was seen at all test potentials ranging from -40 mV to +60 mV (Fig. 2A). The activation and inactivation kinetics remained unaltered (data not shown).

I_{K1} current density was found smaller in IDDM myocytes only at non-physiological potentials negative to -90 mV and no difference was seen at potential between -90 mV and +10 mV, where I_{K1} channels conduct outward currents (the inset of Fig. 2B).

I_{Kr} was substantially depressed in IDDM cells, as illustrated in Figure 2C. Intriguingly, the depression of I_{Kr} appears to be inversely voltage-dependent with greater reduction at more negative potentials. For instance, at -40 mV I_{Kr} was some 80% smaller in IDDM than in control animals, whereas at +10 mV, I_{Kr} was only 60% smaller in IDDM than in control animals. The steady-state voltage-dependent activation property of I_{Kr} was not changed and

the kinetics of I_{Kr} did not seem to differ between control and IDDM either (data not shown).

I_{Ks} was also reduced in IDDM rabbits relative to healthy control animals (Fig. 2D), but to a much less extent compared to I_{Kr} . Similar to other K^{+} currents, the gating properties of I_{Ks} were unaltered by pathological conditions of IDDM (data not shown).

I_{CaL} was only slightly reduced in IDDM hearts. The reduction was voltage-independent with around 20% decreases at potentials ranging from -40 mV to +50 mV. Noticeably, the inactivation process of I_{CaL} was moderately but significantly slowed in IDDM, relative to control, myocytes (Fig. 3A). For example, at +10 mV the inactivation time constant, obtained by the mono-exponential fit to the decaying phase of I_{CaL} , was 29.0 ± 1.2 ms ($n=5$ cells) for control myocytes and 24.0 ± 1.3 ms ($n=7$ cells) for IDDM myocytes ($p < 0.05$). Since slowing of I_{CaL} inactivation tends to maintain Ca^{2+} entry through the channels so as to counteract the reduction of I_{CaL} amplitude, we calculated the charge amount by integrating the area under I_{CaL} traces at 0 and +10 mV in order to better quantify the changes of I_{CaL} (Fig. 3A). In

Fig. 4. Relative contributions of various ion currents to APD prolongation in ventricular myocytes from IDDM rabbits. (A and B) Comparison of various ion currents at selected test potentials (+10 mV and -40 mV or -10 mV for I_{Ks}). * $p < 0.05$ IDDM vs. Ctl. (C) Simulation of action potentials based on the changes of ion currents in IDDM hearts, using LabHEART software. The labels in each panel indicate the simulation with the inhibition of a given current and the percent reduction of currents is indicated in the brackets. Bottom left panel (IDDM) represents AP simulated with combinational changes of all ion currents examined.

this way, we found that I_{CaL} was reduced by ~15% in IDDM hearts.

I_{Na} density, by comparison, was not significantly changed by the pathological process of IDDM (Fig. 3B), neither was the inactivation kinetics (Data not shown).

Relative Contributions of Various Ion Currents to APD Lengthening in IDDM

To estimate the relative contributions of various ion currents to diabetic QTc/APD prolongation, we performed the following analyses. First, we compared the relative changes of the ion currents that demonstrated differences between IDDM and control cells at the selected test

potentials: +10 mV and -40 mV. The voltage of +10 mV was chosen for it roughly represents the phase 2 plateau level, and -40 mV was chosen because this potential falls roughly into the middle of phase 3 repolarization, of a rabbit ventricular AP. As illustrated in Figure 4A and 4B, at +10 mV the current densities of I_{to} , I_{Kr} and I_{Ks} are within the same range, around 1.5 pA/pF, and the degrees of depression of I_{to} and I_{Kr} in IDDM cells are also quite comparable (around 60%) and I_{Ks} was decreased by approximately 37%. At the same potential, I_{CaL} density (4.2 pA/pF) was roughly the sum of the three K^+ currents mentioned above and the reduction of I_{CaL} (charge) was around 15% when the slowing of inactivation was taken

into consideration by using the area under I_{CaL} traces (Fig. 3A). Evidently, the total decreases in K^+ currents were much greater than the decrease in I_{CaL} at +10 mV, which is expected to result in net lengthening of the plateau phase or APD_{50} . At -40 mV, the overall density of all currents studied was considerably smaller than at +10 mV; for I_{Ks} , -10 mV was used for analysis because I_{Ks} was minimal at -40 mV. Among the various ion currents, I_{Kr} density was the greatest and more importantly, I_{Kr} depression in IDDM cells was also to the greatest extent, ~80%. By comparison, the reduction of I_{Ks} at -10 mV was smaller than at +10 mV (~27% at -10 mV vs. 37% at +10 mV). Decreases in I_{to} and I_{CaL} at -40 mV were to a similar degree as at +10 mV, ~60% and 23%, respectively. Obviously, the total reduction of outward K^+ currents tremendously exceeded that of the inward current and slowing of phase 3 repolarization is expected; hence, a decrease in I_{Kr} seems to be a major contributor.

To get further insight into the above analysis, we investigated the changes of APD by simulation of AP with LabHEART, an interactive computer model designed for ion channels rabbit ventricular myocyte [32]. The model allows us the opportunity for predicting changes of APD based on the changes of ion currents, by inputting the percent changes of each individual current or of any combinations of different currents. In this way, we were able to obtain the predicted APs as shown in Figure 4C. Reduction of I_{to} (60%) alone hardly changed APD. 40% decrease in I_{Ks} failed to alter APD either. I_{Kr} suppression by 70% (roughly the average between 60% and 80% at +10 mV and -40 mV, respectively), however, produced remarkable lengthening (~30%) of APD_{50} and APD_{90} . In contrast, depression of I_{CaL} by only 22% resulted in abbreviation of APD (changes of I_{CaL} amplitude, instead of the integrated area, was used for maximum effect), approximately 10% shortening of APD_{50} and APD_{90} . APD prolongation caused by I_{Kr} inhibition and APD shortening caused by I_{CaL} inhibition should partially cancel out each other. This was indeed verified when changes of all currents were incorporated into the simulation; APD was less prolonged than when only I_{Kr} was taken into account: APD_{50} was lengthened by 19% and APD_{90} by 23%.

Altered Protein Levels of Various Ion Channel Subunits

To investigate whether the reduced densities of the ion currents tested in our experiments were due to decreases in expression levels of the responsible channel proteins, we went on to carry out Western blot analysis

Fig. 5. Alterations of protein levels of K^+ ion channel subunits revealed by Western blot analysis. The relative quantification of protein levels was attained by normalizing the band densities to GAPDH, followed by further normalization to the values from control hearts. The data were averaged from experiments in triplicate with 7 hearts of healthy and IDDM rabbits, respectively, and are expressed as fold changes over control. * $p < 0.05$ IDDM vs. Ctl.

and the results are depicted in Figure 5. Kv4.3 (75 kDa), the major pore-forming α -subunit of I_{to} in humans and rabbits [28], was reduced by ~30% in IDDM rabbits. Kir2.1 (110 kDa), the major component of the inward rectifier K^+ channels I_{K1} [33], remained unchanged in IDDM hearts. HERG, the pore-forming β -subunit of I_{Kr} [34], demonstrated two discrete bands with molecular mass of 155 kDa and 135 kDa, respectively. The former represents the N-glycosylated form and the latter is non-N-glycosylated form of the channels. The N-glycosylated HERG was reduced by around 45% and the non-N-

Fig. 5. Alterations of protein levels of K^+ ion channel subunits revealed by Western blot analysis. The relative quantification of protein levels was attained by normalizing the band densities to GAPDH, followed by further normalization to the values from control hearts. The data were averaged from experiments in triplicate with 7 hearts of healthy and IDDM rabbits, respectively, and are expressed as fold changes over control. * $p < 0.05$ IDDM vs. Ctl.

glycosylated HERG was reduced by some 55%, in IDDM relative to those in healthy rabbits. Unexpectedly, KCNQ1 (80 kDa), the pore-forming α -subunit of I_{Ks} [35], decreased by approximately 25% in IDDM hearts. Moreover, minK (14 kDa), the auxiliary β -subunit of KCNQ1, decreased by as high as 85%. However, despite the fact that minK protein was more pronouncedly decreased than KCNQ1 protein, we did not observe marked differences in the activation or deactivation kinetics of I_{Ks} (data not shown). The pore-forming α_{1c} -subunit of I_{CaL} , $Ca_v1.2$ (210 kDa) was reduced by some 15%. No difference was found for $Na_v1.5$ (200 kDa), the α -subunit of cardiac I_{Na} [36] between IDDM and healthy hearts (Fig. 6).

Discussion

Here we report a study on the electrical disturbances in a rabbit model of type I diabetes (IDDM) and the related ionic and molecular alterations as possible mechanisms. The diabetic animals had abnormal QT prolongation and high incidence of ventricular tachyarrhythmias, resembling the clinical observations in diabetic patients. Our study revealed alterations of multiple ion currents/channels in IDDM hearts. While some of the results reproduced the observations from previous studies by other laboratories, several novel findings are documented in the present study. First, this is the first published study thus far to take I_{Kr} and I_{Ks} into account for diabetic QT prolongation and the associated

arrhythmias. Second, our study is also the first to evaluate the relative contributions of various ion currents to the diabetic APD lengthening under identical or similar experimental conditions. Finally, I_{Kr} /HERG K^+ channel dysfunction in diabetic heart is caused by functional impairment and expression down-regulation. Our study suggests that diabetic QT prolongation results from dysfunction of multiple ion currents/channels and depression of I_{Kr} /HERG is the major ionic contributor.

Dysfunction of I_{Kr} /HERG as a Major Ionic Mechanism for Diabetic QT Prolongation and the Associated Arrhythmias

Here we demonstrated remarkable depression of I_{Kr} and I_{Ks} in IDDM animals, which has not been previously reported in the literature, in addition to depression of I_{to} and I_{CaL} as already known. In agreement with the functional depression, the protein levels of HERG and KCNQ1/minK were also significantly reduced. These findings open up an opportunity for evaluating the relative contributions of the alterations of all these ion currents to diabetic QT/APD prolongation. We used the LabHEART computer model written for rabbit ventricular myocytes [32] to address the issue. With the simulation, we showed that 60% reduction of I_{to} alone does not produce any appreciable alterations of APD. This is not surprising for direct demonstration of I_{to} inhibition causing LQTS has been missing to date, particularly in the clinical settings. While inhibition of I_{to} indeed can result in APD prolongation in species devoid of I_{Kr} such as rats and mice, it paradoxically shortens APD in the species

expressing I_{Kr} like humans and rabbits [24-26]. This phenomenon is commonly interpreted as such that depression of I_{to} elevates the plateau level so as to set the membrane potential to the levels favoring greater activation of I_{Kr} and disfavoring I_{CaL} (at potentials positive to 0 mV, I_{CaL} amplitude is smaller and I_{CaL} inactivation is faster), leading to a briefer APD. Furthermore, a recent elegant study with dynamic clamp techniques provides convincing experimental data and persuading theoretical reasoning for APD shortening in response to I_{to} inhibition in canine myocytes [26]. Therefore, the finding that depression of I_{to} accounts for QT prolongation in diabetic rats and mice may not be directly extrapolated to humans or other I_{Kr} -bearing species like dogs and rabbits.

Most prominently, I_{Kr} was found decreased by around 70% in our study and this depression alone theoretically causes some 30% lengthening of rabbit APD based on the simulation with the LabHEART model [32]. This is expected based on our current understanding of the role of I_{Kr} in cardiac repolarization. I_{Kr} is known to be a critical repolarizing current in cardiac myocytes and drugs which produce acquired LQTS nearly exclusively target I_{Kr} or HERG K^+ channel, the major molecular composition of I_{Kr} . However, the potential role of I_{Kr} in diabetic QT prolongation has somehow been overlooked, mainly owing to the use of animal species lacking of I_{Kr} for studying diabetic electrophysiology.

Depression of I_{Ks} alone by 40% fails to alter rabbit ventricular APD either. This is also understandable since I_{Ks} has been believed to have limited role in cardiac repolarization under physiological conditions, presumably due to its unusual requirement of strong depolarization for activation and its slow activation kinetics as well. I_{Ks} also has the potential to contribute to diabetic APD/QT prolongation although it has limited role in cardiac repolarization under physiologic conditions. I_{Ks} acts as repolarization reserve to limit excessive slowing of repolarization [37-38], and when it is depressed this safety factor is removed so that inhibition of other repolarizing current such as I_{Kr} could lead to more pronounced APD/QT prolongation.

Our study demonstrates that I_{CaL} decreases in its current density and slows in its inactivation kinetics in diabetic rabbits. While the former is expected to shorten APD, the latter tends to lengthen it. To take both factors into account, we analyzed the areas under the I_{CaL} traces to estimate the amount of Ca^{2+} movement across the membrane and this yielded ~15% decrease in I_{CaL} . This reduction alone predicts, by the LabHEART model, <10% shortening of rabbit APD. This is consistent with the role

of I_{CaL} in determining the plateau phase of cardiac myocytes.

The combinational accounts of the altered ion currents in our IDDM rabbits yielded approximately 20% lengthening of APD.

Taken together all these data along with previous studies, there is hardly any doubt that dysfunction of I_{Kr} /HERG is an important ionic pathway for the lengthening of APD/QT in diabetic subjects. The prolongation of APD/QT due to dysfunction of I_{Kr} /HERG and other K^+ currents causes the cell to remain longer at the depolarized plateau phase. During this long plateau phase plus the slowing of inactivation of L-type Ca^{2+} channels in IDDM, Ca^{2+} ions have increased probability to enter the cell and cause APD prolongation. It is conceivable that this prolongation is offset by the shortening due to reduction of I_{CaL} . Decrease in I_{to} shifts the plateau level to more positive potential that might favor activation of L-type Ca^{2+} channels, indirectly leading to APD lengthening. I_{Ks} , when being reduced, should have a weaker ability to limit excessive prolongation of APD and would facilitate the prolonging effects of I_{Kr} depression in diabetic myocytes. In a whole, the diabetic QT/APD prolongation in rabbit is primarily the net outcome between lengthening factor of I_{Kr} /HERG depression and the shortening factor of I_{CaL} depression, with indirect but maybe important contributions from I_{to} and I_{Ks} inhibition to favoring the prolongation. Our study represents the first documentation of I_{Kr} /HERG channelopathy as a major ionic mechanism for, and dysfunctions of other currents as important contributors to, diabetic QT prolongation.

The lack of changes of I_{K1} and I_{Na} is in agreement with the lack of changes in their protein levels and the functional depression of I_{to} , I_{Kr} , I_{Ks} and I_{CaL} is consistent with the decreases in their protein levels. Intriguingly, the degree of reduction at the functional level did not match with the extent of reduction at the protein level, except for $I_{CaL}/Ca_v1.2$ with equivalent changes of function and protein levels. Specifically, I_{to} and I_{Kr} decreased to a greater extent than Kv4.3 and HERG protein levels, respectively, whereas, I_{Ks} decreased to a much smaller extent than KCNQ1 and minK protein levels did. The most plausible explanation for the discrepancy is that a decrease in the protein level may not be the only cause of the current depression and other factors such as altered cellular environment and metabolic status may well serve to modulate the channel function. We have indeed documented that HERG function is critically impaired by hyperglycemia [39], enhanced oxidative stress or accumulation of tumor necrosis factor-alpha (TNF- α)

[40], the conditions encountered in diabetic cardiomyopathy. It should also be mentioned that our Western blot analysis does not allow us to distinguish the channel proteins actually seating on the cytoplasmic membrane from those in endoplasmic reticulum and Golgi. The data therefore indicate changes of the total protein expression level of a given ion channel, but do not lead us to reveal whether protein trafficking is normal or not.

Potential Implications of the Findings

In recent years there is increasing evidence of the appearance of ECG disorders with no association between QT prolongation and neuropathy, micro- or macro-vascular diseases, cardiovascular drugs, age or gender, being these situations additional risk factors. Understanding the exact ionic mechanisms for diabetic QT prolongation has become a timely and imperative task of basic scientists in order to develop more rational approaches for the prevention and treatment of electrical disorders and the resultant sudden cardiac death in diabetic patients. The present study revealed that multiple ion currents/channels are involved in the development of QT/APD prolongation in diabetic subjects with the balance between I_{Kr} /HERG and $I_{CaL}/Ca_v1.2$ as the major determinant. Our finding is the first to document I_{Kr} /HERG as the major ionic pathway leading to the abnormal QT prolongation in diabetic hearts. This implies that I_{Kr} /HERG may be the best target for interventions that enhance the channel function and/or expression to shorten or reverse prolonged QT interval. Indeed, several endogenous signaling molecules and metabolites including insulin (unpublished observation), protein kinase B (PKB) [41], phosphatidylinositol-4,5-bisphosphate (PIP2) and lysophosphatidylcholine have been shown to be able to enhance HERG function and protein level. Among these, PKB may be of the best approach because it is a downstream mediator of the insulin signaling pathway involving glucose metabolism and cell survival and its basal activity is required for the normal function of HERG channels [41]. PKB approach will not only enhance HERG function but also suppress apoptotic cell death that is one of the characteristics of diabetic cardiomyopathy. In diabetic hearts, PKB activity and expression were found decreased in many studies, which might be one of the mechanisms leading to enhanced apoptosis, and I_{Kr} depression as well.

Possible Limitations of the Study

Several issues might limit the clinical application of the results in this study. First, the animal model we used is typical type I insulin-dependent diabetes, and the ionic and metabolic mechanisms for APD/QT prolongation in type II insulin-resistant diabetes may not be exactly the same. In reality, type II diabetes consists of 95% of the total diabetic patients in the clinical settings. Nonetheless, APD/QT prolongation observed in type I diabetes is also known to be present at the similar percentage in type II diabetics. Except for the difference between insulin insufficiency and insulin resistance, the basic metabolic perturbations are quite similar between type I and type II diabetes. Hence, it is not unreasonable to believe that I_{Kr} /HERG dysfunction is also the cause for APD/QT prolongation in type II diabetes. Second, our data obtained from rabbits may not be applicable to humans considering the interspecies difference. Nevertheless, our rabbit model reproduces nearly all the phenotypes pertinent to the electrophysiological alterations seen in clinical diabetes. Also important are that rabbit I_{Kr} shares many similarities to human I_{Kr} in term of their biophysical characteristics and pharmacological properties and that rabbit ERG channel sequence (Genbank accession U87513) is highly similar to human ERG, around 93% and 96% homologous at the nucleotide and the amino acid levels, respectively. Even the expression levels of ERG gene under normal conditions are quite comparable between the two species [42]. Thus, I_{Kr} /HERG dysfunction in our type I diabetic rabbit model may also exist in diabetic patients.

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References

- Casis O, Echevarria E: Diabetic cardiomyopathy electromechanical cellular alterations. *Curr Vasc Pharmacol* 2004;2:237–248.
- Veglio M, Chinaglia A, Cavallo-Perin P: QT interval cardiovascular risk factors and risk of death in diabetes. *J Endocrinol Invest* 2004;27:175–81.
- Sawicki PT, Dahne R, Bender R, Berger M: Prolonged QT interval as a predictor of mortality in diabetic nephropathy. *Diabetologia* 1996;39:77–81.
- Wang Z, Feng J, Shi H, Pond A, Nerbonne JM, Nattel S: The potential molecular basis of different physiological properties of transient outward K^+ current in rabbit and human hearts. *Circ Res* 1999;84:551–561.
- Wang Z, Fermini B, Nattel S: Rapid and slow components of delayed rectifier current in human atrial myocytes. *Cardiovasc Res* 1994;28:1540–1546.
- Wang Z, Yue L, White M, Pelletier G, Nattel S: Differential expression of inward rectifier potassium channel mRNA in human atrium versus ventricle and in normal versus failing hearts. *Circulation* 1998;98:2422–2428.
- Kamiya K, Nishiyama A, Yasui K, Hojo M, Sanguinetti MC, Kodama I: Short- and long-term effects of amiodarone on the two components of cardiac delayed rectifier K^+ current. *Circulation* 2001;103:1317–1324.
- Liu XK, Katchman A, Ebert SN, Woosley RL: The antiestrogen tamoxifen blocks the delayed rectifier potassium current, I_{Kr} , in rabbit ventricular myocytes. *J Pharmacol Exp Ther* 1998;287:877–883.
- Lu Z, Kamiya K, Opthof T, Yasui K, Kodama I: Density and kinetics of I_{Kr} and I_{Ks} in guinea pig and rabbit ventricular myocytes explain different efficacy of I_{Ks} blockade at high heart rate in guinea pig and rabbit implications for arrhythmogenesis in humans. *Circulation* 2001;104:951–956.
- Salata JJ, Jurkiewicz NK, Jow B, Folander K, Guinasso PJ Jr, Raynor B, Swanson R, Fermini B: I_K of rabbit ventricle is composed of two currents evidence for I_{Ks} . *Am J Physiol* 1996;271:H2477–H2489.
- Tsuji Y, Opthof T, Yasui K, Inden Y, Takemura H, Niwa N, Lu Z, Lee JK, Honjo H, Kamiya K, Kodama I: Ionic mechanisms of acquired QT prolongation and torsades de pointes in rabbits with chronic complete atrioventricular block. *Circulation* 2002;106:2012–2018.
- Koumi S, Backer CL, Arentzen CE: Characterization of inwardly rectifying K^+ channel in human cardiac myocytes alterations in channel behavior in myocytes isolated from patients with idiopathic dilated cardiomyopathy. *Circulation* 1995;92:164–174.
- Jourdon P, Feuvray D: Calcium and potassium currents in ventricular myocytes isolated from diabetic rats. *J Physiol* 1993;470:411–429.
- Magyar J, Rusznak Z, Szentesi P, Szucs G, Kovacs L: Action potentials and potassium currents in rat ventricular muscle during experimental diabetes. *J Mol Cell Cardiol* 1992;24:841–853.
- Shimoni Y, Firek L, Severson D, Giles W: Short-term diabetes alters K^+ currents in rat ventricular myocytes. *Circ Res* 1994;74:620–628.
- Tsuchida K, Watajima H: Potassium currents in ventricular myocytes from genetically diabetic rats. *Am J Physiol* 1997;273:E695–E700.
- Wang DW, Kiyosue T, Shigematsu S, Arita M: Abnormalities of K^+ and Ca^{2+} currents in ventricular myocytes from rats with chronic diabetes. *Am J Physiol* 1995;1269:H1288–H1296.
- Shimoni Y: Inhibition of the formation or action of angiotensin II reverses attenuated K^+ currents in type I and type 2 diabetes. *J Physiol* 2001;537:83–92.
- Rusznak Z, Cseresnyes Z, Sipos I, Magyar J, Kovacs L, Szucs G: Characteristics of the ventricular transient outward potassium current in genetic rodent models of diabetes. *Gen Physiol Biophys* 1996;15:225–238.
- Shimoni Y, Ewart HS, Severson D: Type I and II models of diabetes produce different modifications of K^+ currents in rat heart role of insulin. *J Physiol* 1998;507:485–496.
- Shimoni Y, Light PE, French RJ: Altered ATP sensitivity of ATP-dependent K^+ channels in diabetic rat hearts. *Am J Physiol* 1998;275:E568–E576.
- Tsuchida K, Watajima H, Otomo S: Calcium current in rat diabetic ventricular myocytes. *Am J Physiol* 1994;267:H2280–H2289.
- Chattou S, Diacono J, Feuvray D: Decrease in sodium-calcium exchange and calcium currents in diabetic rat ventricular myocytes. *Acta Physiol Scand* 1999;166:137–144.
- Escande D, Coulombe A, Faivre JF, Deroubaix E, Coraboeuf E: Two types of transient outward currents in adult human atrial cells. *Am J Physiol* 1987;252:H142–H148.
- Kenyon JL, Gibbons WR: 4-Aminopyridine and the early outward current of sheep cardiac Purkinje fibres. *J Gen Physiol* 1979;73:139–157.
- Sun X, Wang HS: Role of the transient outward current (I_{to}) in shaping canine ventricular action potential a dynamic clamp study. *J Physiol* 2005;564:411–419.
- Carlsson L, Abrahamsson C, Andersson B, Duker G, Schiller-Linhardt G: Proarrhythmic effects of the class III agent almokalant importance of infusion rate QT dispersion and early afterdepolarisations. *Cardiovasc Res* 1993;27:2186–2193.
- Wang Z, Feng J, Shi H, Pond A, Nerbonne JM, Nattel S: The potential molecular basis of different physiological properties of transient outward K^+ current in rabbit and human hearts. *Circ Res* 1999;84:551–561.
- Cheng J, Kamiya K, Liu W, Tsuji Y, Toyama J, Kodama I: Heterogeneous distribution of the two components of delayed rectifier K current: a potential mechanism of the proarrhythmic effects of methanesulfonanilide class III agents. *Cardiovasc Res* 1999;3:135–147.
- Wang Z, Fermini B, Nattel S: Role of chloride currents in repolarizing rabbit atrial myocytes. *Am J Physiol* 1995;268:H1992–H2002.
- Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch* 1981;391:85–100.
- Puglisi JL, Bers DML: LabHEART an interactive computer model of rabbit ventricular myocyte ion channels and Ca transport. *Am J Physiol* 2001;281:C2049–2060.
- Wang Z, Yue L, White M, Pelletier G, Nattel S: Differential expression of inward rectifier potassium channel mRNA in human atrium versus ventricle and in normal versus failing hearts. *Circulation* 1998;98:2422–2428.
- Sanguinetti MC, Jiang C, Curran ME, Keating MT: A mechanistic link between an inherited and an acquired cardiac arrhythmia HERG encodes the I_{Kr} potassium channel. *Cell* 1995;81:299–307.
- Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MT: Coassembly of K_v LQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. *Nature* 1996;384:80–83.

- 36 Szabo G, Szentandrassy N, Biro T, Toth BI, Czifra G, Magyar J, Banyasz T, Varro A, Kovacs L, Nanasi PP: Asymmetrical distribution of ion channels in canine and human left-ventricular wall epicardium versus midmyocardium. *Pflugers Arch* 2005;450:307–316.
- 37 Jost N, Virág L, Bitay M, Takács J, Lengyel C, Biliczki P, Nagy Z, Bogáts G, Lathrop DA, Papp JG, Varró A: Restricting excessive cardiac action potential and QT prolongation a vital role for I_{ks} in human ventricular muscle. *Circulation* 2005;112:1392–1399.
- 38 Lee SL, Ostadalova I., Kolar F, Dhalla NS: Alterations in Ca^{2+} -channels during the development of diabetic cardiomyopathy. *Mol Cell Biochem* 1992;109:173–179.
- 39 Zhang Y, Han H, Wang J, Wang H, Yang B, Wang Z: Impairment of HERG (Human ether-a-go-go Related Gene) K^+ channel function by hypoglycemia and hyperglycemia: similar phenotypes but different mechanisms. *J Biol Chem* 2003;278:10417–10426.
- 40 Wang J, Wang H, Zhang Y, Gao H, Nattel S, Wang Z: Impairment of HERG K^+ channel function by tumor necrosis factor-a role of reactive oxygen species as a mediator. *J Biol Chem* 2004;279:13289–13292.
- 41 Zhang Y, Wang H, Wang J, Han H, Nattel S, Wang Z: Normal function of HERG K^+ channels expressed in HEK293 cells requires basal protein kinase B activity. *FEBS Lett* 2003;534:125–132.
- 42 Zicha S, Moss I, Allen B, Varro A, Papp J, Dumaine R, Antzelevich C, Nattel S: Molecular basis of species-specific expression of repolarizing K^+ currents in the heart. *Am J Physiol* 2003;285:H1641–H1649.