Coordinated down-regulation of KCNQ1 and KCNE1 expression contributes to reduction of I_{Ks} in canine hypertrophied hearts

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Abstract

Objective: In animal models of hypertrophy, electrical remodeling giving rise to QT prolongation occurs rapidly and is associated with the development of torsade de pointes (TdP) arrhythmias and sudden death. Chronic AV block (CAVB)-induced hypertrophy in dogs has been associated with a reduction in the slow component (I_{Ks}) of the delayed rectifier potassium current (I_{K}), which contributes to a prolongation of ventricular repolarization, the development of an acquired form of long QT, and the substrate for triggered activity and TdP. The present study was designed to probe the molecular basis for the decrease in I_{Ks} by studying the characteristics of KCNE1 and KCNQ1, the putative genes responsible for formation of the channel.

Methods and Results: Using a combination of Northern blot, competitive multiplex PCR and immunoblot assays, we found that CAVB reduces KCNE1 and KCNQ1 RNA in the canine ventricles by 70 and 80%, respectively. Protein levels of KCNE1 and KCNQ1 were reduced by 60 and 50%, respectively. We also demonstrate at the molecular level the basis for inter-ventricular difference in I_{Ks} density previously reported in hearts of normal dogs and show the basis for reduction of this difference in the CAVB dog.

Conclusions: Our results indicate that the CAVB-induced reduction in I_{Ks} is due to a down-regulation of KCNE1 and KCNQ1 transcription. The data suggest that electrical remodeling of the cardiac ventricle during hypertrophy involves regulation of the gene expression through modulation of transcriptional and translational regulatory pathways. The reduction in KCNE1 and KCNQ1 expression increases the dependence of ventricular repolarization on the rapid component of I_{K} and may potentiate the action of Class III antiarrhythmic agents.

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1. Introduction

Cardiac hypertrophy secondary to hypertension is associated with a 6-fold increase in the risk of sudden death. Prolongation of the repolarization time has been a consistent finding in hypertrophied hearts. This electrical remodeling has often been observed before fibrosis or clinical signs of heart failure become evident in animal models [1,2].

In the canine chronic atrio-ventricular block (CAVB) model, biventricular hypertrophy results from adaptation to volume overload induced by long-term bradycardia and AV asynchrony [2,3] and develops before observable changes in the tissue collagen content or capillary/fiber ratio [2]. Electrical remodeling is accompanied by the development of cellular hypertrophy [4] and predisposes the heart to the development of torsade de pointes arrhythmias [5–7]. CAVB significantly increases ventricular action potential duration (APD), more so in the left ventricle (LV) than in the right (RV), and steepens the APD–rate relationship [2]. CAVB-induced changes in repolarization are due in part to a decrease in the amplitude of the slow component of the voltage-gated delayed rectifier potassium current (I_{Ks}) [8]. In dogs with normal sinus rhythm (SR), I_{Ks} is normally

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larger in RV [9–12]. Reduction of $I_{Ks}$ density during CAVB [13] may contribute to an increase in inter-ventricular and transmural dispersion of repolarization, potential indices [14] for the development of torsade de pointes [5].

The $I_{Ks}$ channel is formed by the association of the KCNQ1 and KCNE1 gene products [15,16]; one or both are involved in LQT1, LQT5 and Jervell-Lange-Nielsen forms of congenital long QT syndrome (LQTS) [17]. The molecular basis for the inter-ventricular distribution of $I_{Ks}$ and its reduction during CAVB is unknown. In this study we examine the hypothesis that the inter-ventricular distribution of $I_{Ks}$ in sinus rhythm dogs and its reduction in density during CAVB are linked to altered expression of either KCNQ1, KCNE1 or both genes.

2. Methods

At three time points (sinus rate, and at the idioventricular rhythm at acute and 4–6 weeks chronic AV block), a six-lead surface ECG was serially recorded together with two endocardially placed monophasic action potential catheters. To correct for the slowing of the heart rate, the anesthetised, adult mongrel dogs (20–40 kg) were paced at AAVB and CAVB at a cycle length which was comparable to the cycle length of the previous sinus rhythm. For details about placement of the MAP catheters and the analysis of the signals, we refer to previous publications [2,5].

The average area of the cardiac myocytes was obtained under brightfield microscopy by measuring the width and the length of rod shape myocytes.

Complete AV block was produced by injection of 37% formaldehyde into the AV groove. Details of the procedure were previously described [2,5]. All animals were euthanized by pentobarbital injection and the hearts were rapidly excised and placed in a cold cardioplegic solution [18]. Dogs had AV block for 102±60 days before euthanasia. Tissue or biopsies were snap frozen in liquid nitrogen and stored at −70°C if RNA extraction or protein isolation was not immediately performed. DNA templates for the KCNQ1 (517 bp) and KCNE1 (99 bp) probes were generated from a dog cDNA library by PCR using primers with homologous sequence in human, *Xenopus laevis*, mouse and rat. Each amplicon was sequenced and ligated into the PCR-Script AMP SK(+) expression vector (Stratagene, Menasha, WI). The cDNA probes were then amplified by PCR and radioactively labeled by incorporation of trace amounts of [α-32P]dCTP. Total RNA was extracted using the TRIZOL Total RNA extraction kit (Gibco BRL Life Technologies, MD) according to the manufacturer’s protocol. The quality and quantity of RNA were, respectively, assessed by visual inspection on denaturing gels and measured by optical density (OD) reading at 260 and 280 nm. Total RNA (20 μg/lane) was fractionated on a 1% denaturing agarose gel and transferred to a nitro-cellulose membrane (Hybond NX, Amersham, Piscataway, NJ) for hybridization with the KCNQ1 and KCNE1 probes, respectively. Radiolabeled 18S rRNA ([α-32P]dCTP) was used as loading control. Blots were pre-hybridized (blocked) with salmon sperm ssDNA. Probes were diluted to an activity of 10^7 cpm/ml and allowed to hybridize on the membrane at 57–60°C overnight. Blots were exposed to a phosphor screen for 3–4 days and analyzed on a phosphor imager (Kodak, NJ). Following analysis of the first hybridization (KCNQ1), blots were stripped and re-probed for KCNE1 RNA.

2.2. Competitive multiplex RT-PCR

Cardiac tissue (2 mg) was dissected from the base of the anterior wall of each ventricle. Total RNA was extracted as described above and reverse transcribed (RT) using Superscript II (Gibco BRL, Gaithersburg, MD) according to the manufacturer’s protocol. Yields were quantified by OD_260/280 and gel densitometry. A fragment of v-erB (viral erB) was amplified with composite primers having short terminal sequences identical to their respective target indices [14] for the development of torsade de pointes [5]. The signal was detected using FAB fragments from sheep

Transmural RV and LV biopsies from CAVB and control dogs were taken from the middle portion of each ventricle.
2.3. Cell dissociation

Cardiac myocytes were dissociated from the left ventricles of adult mongrel dogs as previously described [18]. Following dissociation, the cells were resuspended in a cardioplegic solution containing (in mM): 132 NaCl, 20 HEPES/NaOH, 3.3 MgSO₄·7H₂O, 11.1 r-glucose, 5 KCl, 0.5 CaCl₂, 1.5% BSA and stored at 4°C for 1–3 h. In instances where the yield of viable cells was low, the fraction was enriched by purification on a Percoll gradient as previously described [19].

2.3.1. Electrophysiological recordings

For patch clamp recordings in whole cell configuration, cells were placed in the perfusion chamber of an inverted microscope and superfused with Tyrode solution containing E-4031 (5 μM), a specific Iₖr blocker [20,21] and nifedipine (5 μM), a calcium channel blocker. Pipette solutions contained the following (in mM): K-aspartate 125, KCl 20, MgCl₂ 1, MgATP 5, HEPES 5, EGTA 10, pH 7.1 (KOH). Iₖr was recorded by a depolarization step to 30 mV for 3 s from a holding membrane potential of −50 mV, followed by repolarization to 0 mV (repeated five times every 10 s). Iᵦ was not blocked, but it had little influence on our measurement of Iₖr because of its fast and complete inactivation in this species [22]. Currents were amplified with an Axon Patch 2A, recorded and analyzed using the Axon Pclamp v.8 suite of software (Axon Instruments, Carlsbad, CA).

2.4. Cryosectioning

Thin slices from the heart ventricles were dissected out and immediately placed for 10 min in a relaxing Ca-free Tyrode solution at 4°C containing (in mM): NaCl, 130; KCl,10; CaCl₂, 0.2; MgCl₂, 2; HEPES, 10. The bathing solution was then supplemented with 5 mM caffeine and the tissue was allowed to relax for another 15 min. Tissues were then cryoprotected by incubating the slices for 4–8 h in the relaxing solution supplemented by 25% (w/v) sucrose. The slices were then quickly rinsed in Ca-free Tyrode, placed in cryogenic cassettes, dipped in liquid nitrogen for 2–4 min and stored at −80°C. Sectioning (15 μm) of the tissue block was performed on a Leitz 1720 Digital Kryostat (Leica Microsystems, NJ) according to the manufacturer’s instructions. Hematoxylin-eosin staining followed standard clinical procedures.

2.5. Immunoblots

Polyclonal antibodies (Ab) against the epitopes SYSEKSKDRGSNITIG and YIRSKKLEHSHDPFN in the COOH termini of KCNQ1 and KCNE1, respectively, were raised in rabbits by intramuscular injection of a 50:50 mixture of eight branch multiple antigenic peptides (MAP) and keyhole limpet hemocyanin (KLH) conjugated linear peptides. Abs were affinity purified on antigen Sephadex columns and their titer was determined by ELISA.

Total cell and tissue proteins were isolated out of the organic phase obtained from the total RNA isolation procedure (AMBION, Austin, TX) by sequential overnight dialysis against 0.1% SDS at 4°C. Membrane proteins were obtained by standard sucrose gradient centrifugation. Briefly, tissues were snap frozen in liquid N₂ and pulverized using a mortar and pestle. Tissue powder (1 g) was homogenized in buffer containing: HEPES (4 mM) pH 7.0, sucrose (320 mM), Pefabloc SC (0.2 mM), pepstatin A (1 μg/ml), leupeptin (1 μg/ml), aprotonin (1 μg/ml), benzamidine (100 μg/ml) and calpain inhibitors I and II (8 μg/ml each) and centrifuged at 2000×g for 10 min to remove debris. The supernatant was set aside and the pellet was resuspended in fresh buffer, re-homogenized and spun a second time at 200×g for 10 min. Both supernatants were pooled and centrifuged at 10 000×g for 1 h and the membrane protein pellet was resuspended in 1 ml buffer. Protein concentration was determined by optical density OD₂₆₈₅₀ readings and bichonionic acid assay (Sigma, St. Louis, MO). Proteins (20–70 μg/lane) were run on SDS–polyacrylamide (PAA) gels (12% PAA for KCNQ1; 20% PAA for KCNE1), and transferred on PVDF membranes (Bio-Rad, Hercules, CA). The blots were incubated overnight with our polyclonal antibodies (anti-KCNQ1; 1:5000 and KCNE1 (Anti-IsK; 1:500). Loading errors were controlled by comparison of the Coomassie blue or Ponceau staining of the PVDF membrane following semi-dry transfer.

The proteins of interest were detected by a secondary goat anti-rabbit Ab conjugated to alkaline phosphatase (AP) and enzymatic reaction with the chemiluminescent AP substrate CDP-Star (NEN, Boston, MA). Blots were exposed to X-ray films and each band intensity was measured using an EDC scanning densitometer (Helena Labs, Fort Worth, TX).

2.6. Statistics

Statistical significance was assessed with the Kruskal–Wallis test for non-parametric ANOVA. A level of P<0.05 was considered significant.

3. Results

We initially monitored electrophysiological parameters in vivo during the adaptation of the canine heart to AV block. Fig. 1 shows that when paced at a cycle length of 600 ms, the QT interval significantly prolonged as the heart adapted (32 days of CAVB). Monophasic action potential recordings showed concomitant prolongation of LV and RV APD and revealed an average increase in inter-ventricular dispersion of repolarization from 20±15 to 45±30 ms (Table 1). With each dog serving as its own
control, chronic AV block significantly increased QTc interval (limb leads) from 285±33 ms in sinus rhythm (SR) to 366±57 ms (Table 1). Structurally, the heart to body weight ratio (g/kg) increased (data±S.D.) from 7.7±1.2 (n=15) in SR [2] to 11.5±1.4 (n=10) after CA VB. Hypertrophy was also evidenced by a thickening of the left ventricle (Fig. 2), resulting from an increase in the average size of myocardial myocytes from 3381±81 μm² (n=1166) to 4753±155 μm² (n=387, P<0.001).

Previous studies [13] have shown CA VB to be associated with a reduction in I钾 density in both ventricles. A similar reduction in I钾 density was also observed in myocytes of our CA VB dogs (Fig. 3).

To link the decrease in I钾 to changes in KCNQ1 and KCNE1 RNA, we performed Northern blot assays. Fig. 4A shows that our probes detected a 3.2- and 0.4-kb band corresponding to the size expected for KCNQ1 (3.2 kb) [15] and KCNE1 (0.39 kb) [23,24] RNA, respectively. In biopsies from three SR dogs, the relative intensity of the bands for KCNQ1 and KCNE1 was similar in RV and LV. CAVB decreased the KCNQ1 RNA signal by 35±10% (vs. SR) in RV and by 38±20% in LV (Fig. 4B). KCNE1 RNA was reduced by 63±8 and 46±27% (vs. SR) in LV and RV, respectively, following CAVB (Fig. 4C). We observed large inter-sample variations in the amount of transcripts within the small number of biopsies performed (n=3). We attributed part of the variation to the low sensitivity of the Northern blot assay, the inherent normalization process.

Table 1
Electrophysiological parameters during sinus rhythm (SR) and chronic AV block (CA VB)

<table>
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<th>RR</th>
<th>QRS</th>
<th>QT</th>
<th>LV APD</th>
<th>RV APD</th>
<th>ΔAPD</th>
<th>QTc</th>
<th>JTc</th>
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<tr>
<td>SR</td>
<td>601±114</td>
<td>67±5</td>
<td>250±40</td>
<td>231±32</td>
<td>210±34</td>
<td>20±15</td>
<td>285±33</td>
<td>218±35</td>
</tr>
<tr>
<td>CA VB</td>
<td>1339±263</td>
<td>86±13</td>
<td>396±59</td>
<td>358±57</td>
<td>313±46</td>
<td>46±28</td>
<td>366±57</td>
<td>280±55</td>
</tr>
</tbody>
</table>

Paired electrophysiological parameters were obtained from the same dogs in sinus rhythm (SR) just before AV block (SR) and after 37±8 days of chronic AV block (CA VB) (n=10). Each dog served as its own control. QT and JT intervals were corrected (QTc and JTc) using the van de Water method [40]. APD, action potential duration at 100% repolarization from MAP recordings (Fig. 1); ΔAPD, difference between LV and RV action potential duration. RR and QT intervals have their standard clinical meanings. All data (±S.D.) are expressed in ms and were significantly increased (P<0.05) after CAVB (unpaired t-test).
Fig. 3. $I_{Ks}$ density is significantly reduced during chronic AVB block. The slowly activating component of the delayed rectifier current ($I_{Ks}$) is significantly smaller in CAVB dog epicardial myocytes compared to those of normal dogs ($P<0.05$). $I_{Ks}$ was recorded in the presence of 5 μM nifedipine and 5 μM E-4031 to block $I_{Ca,L}$ and $I_{Kr}$, respectively. (A) Current traces elicited by step depolarizations from a holding potential of −50 to 30 mV for 3 s and repolarization to 0 mV in a normal myocyte ($C_m$: 170 pF) and a CAVB myocyte ($C_m$: 255 pF). Voltage protocol is shown on the top. (B) In CAVB myocytes, $I_{Ks}$ tail current density was significantly lower (0.17 ± 0.07 pA/pF) compared to normal myocytes (0.70 ± 0.07 pA/pF). $I_{Ks}$ was measured as the tail current on return of membrane potential to 0 from 30 mV. Data shown are mean ± S.E.M ($n=4–6$).

To more accurately determine differences in mRNA, we performed multiplex RT-PCR (Fig. 5). This method was favored over RNase protection assay because of its better reproducibility, its higher sensitivity and ability to avoid the artifactual variations inherent to comparison with housekeeping genes whose abundance may also vary during CAVB. Fig. 5D,E shows that CAVB reduced KCNQ1 reverse transcribed cDNA by 72 ± 25 and 76 ± 29% and KCNE1 by 82 ± 20 and 93 ± 7% in LV and RV, respectively (Fig. 5D,E). Multiplex RT-PCR also revealed that KCNQ1 cDNA was 355 ± 30% more abundant in RV than in LV in SR dogs. CAVB abolished the inter-ventricular difference. In control experiments (Fig.

Fig. 4. Chronic AV block (CAVB) reduces KCNQ1 and KCNE1 RNA levels in canine left (LV) and right ventricle (RV). (A) Representative Northern blots from sinus rhythm (SR) and CAVB dogs. cDNA fragments from KCNQ1 and KCNE1 were obtained by PCR amplification, radiolabelled and hybridized to RNA extracted from tissues of cardiac biopsies taken from the mid region of the left and right ventricular wall (see Methods). Commercially available cDNA probes for 18S ribosomal RNA were used as loading control and reference standards (housekeeping gene). (B,C) The density of the KCNQ1 and KCNE1 bands in arbitrary units (a.u.) was normalized to the density of their respective 18S ribosomal RNA band (relative density). Significance levels: *$P<0.05$, **$P<0.01$, SR versus CAVB ($n=3$) (Kruskal–Wallis test for non-parametric ANOVA).
Fig. 5. Competitive multiplex RT-PCR shows CAVB-induced reduction of KCNE1 and KCNQ1 cDNA reverse transcribed (RT) from messenger RNA and reveals inter-ventricular heterogeneities in mRNA distribution. (A) Representative competitive PCR experiment. An exogenous MIMIC fragment was engineered to have identical primer sequence as the target gene (KCNQ1) and used as internal standard (MIMIC). The same set of primers amplified both the target gene and MIMIC thus eliminating non-linearity in the amplification of the two products. Ten-fold dilutions of MIMIC and 2 μg of RT-cDNA were used for each competitive PCR between MIMIC and KCNQ1. The high intensity band in the DNA ladder (λ) corresponds to 1 kb. Size of fragments: MIMIC: 332 bp, KCNQ1: 176 bp. (B) Control experiment showing that the PCR product is not due to amplification of genomic DNA. When sterile water, total RNA or DNAse treated RNA (RNA(-DNA)) were used as template for amplification, no amplicons were observed. (C) Representative results from normal (Ctrl.) and CAVB dogs. The density of the KCNQ1 band was normalized to that of MIMIC (log KCNQ1/MIMIC) and plotted against the concentration of MIMIC template in each PCR tube (log [MIMIC]). A linear regression (solid line) was fitted to each set of data. (D,E) The concentration of target gene cDNA in attomoles was determined by the intersection of the linear regression with the abscissa and normalized to the amount of RT-cDNA template (mg) used in each PCR reaction. n=6 KCNE1 control dogs (Ctrl.), n=4 KCNQ1 Ctrl., n=4 CAVB dogs. Statistical significance (ANOVA): *P<0.05, **P<0.01 (Ctrl. vs. CAVB); †P<0.05 (RV vs. LV).

5B), no genomic DNA was amplified from the RNA templates.

To assess changes in protein expression, we performed immunoblots on proteins isolated from tissues. Fig. 6 shows that our KCNE1 antibody recognized a protein of 35 kDa and in some samples a lower molecular weight protein around 20 kDa (Fig. 6A). The density of the 35-kDa band was 23±10% higher in RV versus LV (P=0.048) of control dogs (Fig. 6B). CAVB reduced the expression of KCNE1 by 58±14 and 70±15% in LV and
Fig. 6. Expression of KCNE1 proteins is significantly reduced by CAVB. (A) Representative Western blot of tissue proteins (20 μg) extracted from the left (LV) and right ventricle (RV) of three normal and three CAVB dogs (D1–D3) and probed with affinity purified KCNE1 polyclonal antibody (pAb). The KCNE1 pAb recognized a band around ~35 kDa and a very faint band around ~20 kDa in control dogs. CAVB significantly reduced the amount of protein recognized by the KCNE1 pAb. (B) Intensity of each band, in arbitrary units (a.u.), was measured by densitometry to assess the total protein expression in LV and RV of normal (n=8) and CAVB dogs (n=9). Given the low intensity of the 20-kDa band, only the density of the 35-kDa band was measured in each experiment. (C) Experiment described in A was repeated on proteins isolated from cardiac myocytes acutely dissociated from the endocardium of the left ventricle. (D) Control Western blots using proteins extracted under low denaturing conditions and probed with anti-KCNE1 antibodies (Ctrl.) or with antibodies pre-absorbed against the KCNE1 antigen (Pre-Abs.). Statistical significance (ANOVA): ***P<0.001 (Ctrl. vs. CAVB); †P<0.05 (RV vs. LV).

RV, respectively, and abolished the small inter-ventricular difference (Fig. 6B).

Because CAVB-induced hypertrophy may induce changes in the cellular composition of the cardiac ventricles and reduce the relative contribution of KCNQ1 and KCNE1 to the tissue protein pool, we compared the banding patterns from tissue and acutely dissociated myocyte proteins. Fig. 6C shows that a similar reduction in band intensity was observed in myocyte protein. The same observations were made in three different batches of cells from the left ventricle with an average reduction of 47±25%. In control experiments (Fig. 6D), antibodies pre-absorbed against the KCNE1 antigen failed to detect the strong 35-kDa band observed in control.

In SR dogs, our KCNQ1 Ab recognized two bands of ~72 and ~120 kDa. Fig. 7A shows that the intensity of both bands was strongly reduced by CAVB. To quantitate the change in expression, we measured the density of each band from the RV and LV of CAVB dogs and normalized them to their respective values in SR dogs. Fig. 7B shows that CAVB similarly reduced the density of the 72- and 120-kDa bands in LV and RV, respectively. When the intensity of both bands was averaged, protein expression was reduced by 45±10% (RV) and 60±14% (LV).

To assess whether the RNA distribution observed with RT-PCR translated into inter-ventricular differences in protein expression, we normalized the density of each immunoblot band from RV to its respective intensity in LV. Fig. 7C shows that significantly more KCNQ1 protein was present in the right ventricle. The RV/LV density ratio of both 72- and 120-kDa bands was greater than 1 in sinus rhythm dogs. In CAVB dogs, the RV/LV ratio of the 72-kDa band was slightly smaller but when the density of the 120- and the 72-kDa were summed the difference was not significant. Thus, CAVB significantly reduced the inter-ventricular difference in protein and RNA distribution. A 72-kDa band is predicted based on the KCNQ1 protein sequence. Fig. 7D shows that both the 72- and 120-kDa bands were recognized in the total protein preparation but only the 120-kDa band was predominant in proteins isolated from the membrane fraction. Thus, the 120-kDa band is linked to the cytoplasmic (mature) form of the protein while the 72-kDa band is likely to be related to cytosolic proteins. To verify that both bands were linked to KCNQ1, we performed immunoblots using pre-absorbed KCNQ1 antibodies. Fig. 7D (right panel) shows that the intensity of both bands was strongly reduced when the KCNQ1 antibody was pre-absorbed against the control antigen. A search for homologies in the NIH GenBank and SWISS-PROT databases revealed that the epitope recog-
Fig. 7. Expression of KCNQ1 proteins is significantly reduced by CAVB. (A) Representative Western blot of tissue proteins (20 μg) extracted from the left (LV) and right ventricle (RV) of a normal sinus rhythm dog (SR) and a CAVB dog and probed with affinity purified KCNQ1 polyclonal antibody (pAb). The pAb recognized two bands with molecular weights (MW) of ~72 and ~120 kDa, respectively, in normal dogs. Intensity of each band was measured by densitometry. (B) To quantitate the CAVB-induced reduction in KCNQ1 expression, the average density of the bands from the left and right ventricles of CAVB dogs were normalized to the intensities obtained in SR dogs. All measurements showed a significant reduction in intensity during CAVB (P<0.001). (C) To measure inter-ventricular differences in the expression of KCNQ1, a density ratio of right versus left ventricle was calculated for the 72- and 120-kDa bands obtained from the normal (SR, n=8) and CAVB dogs (n=9). Statistical significance: *P<0.05, **P<0.01 (ratio against 1). (D) Left panel: experiments as described in A were repeated on total proteins (Total) and membrane proteins (Membr.) isolated from cardiac myocytes acutely dissociated from the endocardium of the left ventricle of SR. Right panel: Control immunoblot of proteins extracted under low denaturing conditions and probed with anti-KCNQ1 antibodies (Ctrl.) or with antibodies pre-absorbed against the KCNQ1 antigen (Pre-Ab.).

nized by the polyclonal antibody (pAb) is specific to KCNQ1. Our results show that the 72- and 120-kDa antigens contained the KCNQ1 specific epitope.

4. Discussion

We show that chronic AV block induces a significant reduction in the expression of KCNE1 and KCNQ1, the putative genes responsible for I_Ks. The reduction in KCNE1 and KCNQ1 proteins that we report is in close agreement with the previous [13] demonstration of a 50 and 55% reduction in I_Ks density in the LV and RV of CAVB dogs.

KCNQ1 mRNA was 300% more abundant in RV versus LV. That inter-ventricular mRNA distribution was abolished during CAVB. Thus, our data suggest that a common transcriptional factor, possibly stabilizing mRNA, may be responsible for the inter-ventricular difference in the expression of KCNQ1 and KCNE1 and that CAVB reduces the activity of this factor.

We observed a disproportionate CAVB-induced down-regulation in KCNQ1 mRNA (82–93%) when compared to protein levels (45–60%) and I_Ks current (50–55%). In view of its proposed role as a chaperone protein [15,16], KCNE1 may play a determining role in the expression of KCNQ1 at the level of the plasma membrane. This hypothesis is supported by our observation of a proportional reduction of KCNQ1 protein, mRNA and I_Ks, and may explain the lack of correlation with the reduction of KCNQ1 mRNA in the CAVB dog.

Our finding of a proportional reduction in the density of KCNQ1 membrane protein and cytosolic proteins, 120- and 72-kDa bands, respectively (Fig. 7B,D), also raise the interesting possibility that KCNE1 regulates the expression of KCNQ1 through a feedback mechanism coordinating
the translation of KCNQ1 with the formation/recycling of the KCNQ1/KCNE1 protein complex. Although the composition of the 120-kDa band remains unclear, it is likely to be associated with a glycosylated protein complex formed by KNCQ1 and KCNE1 or another ancillary subunit. Our experiments with different glycosidase gave mixed results and remaining unsppecific IgGs in our affinity purified polyclonal antibodies prevented efficient immunoprecipitation of KCNQ1. Commercially available KCNQ1 antibodies failed to recognize the dog epitope. Further experiments are therefore needed to fully identify the components of the 120-kDa band and validate the hypothesis of a feedback mechanism.

Previous studies have shown an up-regulation of Na\(^{+}\)-Ca\(^{++}\) exchange (NCX1) current during CAVB [25]. An increase in NCX1 mRNA in models of pressure overload hypertrophy [26] was also reported. But no significant changes in the density of the transient outward current I\(_{\text{to}}\), the inward rectifier current I\(_{\text{Ks}}\) and the slow inward calcium current were observed in our model [13,25]. Down-regulation of KCNE1 and KCNQ1 expression therefore contributes importantly to the electrical remodeling of the ventricular myocardium following volume overload hypertrophy.

Our results also provide insight into the molecular basis for the inter-ventricular difference in I\(_{\text{Ks}}\) previously shown to exist in normal hearts. We found interventricular ratios of 1.32 and 1.84 for KCNQ1 low and high molecular weight antigens, respectively. These results are are in good agreement with the data of Volders et al. [12] who reported I\(_{\text{Ks}}\) densities of 0.72±0.12 and 0.38±0.13 pA/pF for the right and left ventricle, respectively (RV/LV ratio: 1.89). This concordance between the electrophysiological and the biochemical data strengthens the notion that a KCNE1/KCNQ1 protein complex is responsible for I\(_{\text{Ks}}\).

Recent evidence suggests that other ancillary sub-units from the KCNE family may modulate KCNQ1 expression [27,28]. However the kinetics of currents described for KCN2/KCNQ1 or KCN3/KCNQ1 (in heterologous systems) differ from those normally present in dog cardiomyocytes. Moreover, our data strongly suggest that the association of KCNQ1 with KCNE1 is primarily responsible for I\(_{\text{Ks}}\), and that CAVB-induced reduction of KCN1 and KCNQ1 expression is a sufficient, but not necessarily exclusive, mechanism to account for the reduction in I\(_{\text{Ks}}\) density. Recent evidence also indicated that KCN1 may interact with KCNH2 (HERG), the gene coding for the alpha sub-unit generating I\(_{\text{Kr}}\) [29]. Co-expression studies however demonstrated a competitive interaction between KCN1 and KCN2 for co-assembly with HERG largely favoring KCN2 [29]. This observation notwithstanding, it is possible that the reduction of KCN1 demonstrated in this study may also contribute to the marked reduction in I\(_{\text{Ks}}\) known to be associated with CAVB.

We found a molecular weight between 28 and 35 kDa for KCNE1, slightly higher than what was previously reported (25–30 kDa). We attributed the discrepancy to technical differences. The MW of KCNE1 was strongly dependent on the gel temperature and density and the protein standard used. The Bio-Rad Kaleidoscope marker, used for most of our experiments, indicated an MW around 30–35 kDa. Other markers (Promega) indicated a band closer to 29 kDa on the same gel (not shown).

4.1. Clinical implications

Prolongation of the QT interval has been a consistent finding in our model and in several other models of hypertension as well as humans. Our observations support the hypothesis that the electrical remodeling attending cardiac hypertrophy is akin to acquired long QT syndrome. The reduction of KCNQ1 and KCNE1 expression results in electrophysiology similar to the most severe form of LQTS in which I\(_{\text{Ks}}\) is drastically reduced due to dominant negative mutations in KCNQ1 or KCNE1 genes [30–33].

Torsade de pointes (TdP) commonly develops in the setting of LQTS due to an increased dispersion of repolarization across the ventricular wall and septum of the heart, which creates the substrate for reentry [2,14,34–36]. In the canine and human heart, M cells normally display low levels of I\(_{\text{Ks}}\). M cells have been identified as the main culprits in LQTS-related arrhythmias because their action potentials prolong out of proportion in the presence of agents with Class III actions, thus augmenting transmural dispersion of repolarization. M cells are also prone to develop EADs as well as delayed afterdepolarizations under these same conditions [35]. The reduction in KCN1 and KCNQ1 expression may confer to all ventricular cells arrhythmogenic properties similar to those normally found in the M region. By drastically reducing I\(_{\text{Ks}}\), CAVB-induced hypertrophy may increase the dependence of late repolarization on I\(_{\text{Ks}}\) and, as a consequence, its sensitivity to Class III antiarrhythmic agents.

4.2. Limitations

The degree to which CAVB caused a reduction in KCNQ1 and KCNE1 RNA was smaller when assessed using Northern blot versus quantitative PCR assay. Moreover, the differences in message between RV and LV in SR dogs were less apparent using the Northern blot assay. We attribute this largely to the superior sensitivity and accuracy of the quantitative PCR versus Northern blot analysis, which relies on ratiometric measurements against an endogenous ‘housekeeping’ gene RNA. These discrepancies may also be due in part to differences in the origin of the tissues along the apico-basal axis of the ventricle.

A dominant negative N-terminal truncated KVLQT1 isoform 2 lacking 129 amino acids is expressed in the human heart [37]. In immunoblots, Demolombe et al. [37] observed a small difference in the migration of the two isoforms and associated part of the weaker I\(_{\text{Ks}}\) current...
recorded in the left mid-myocardium to enhanced expression of the truncated isoform [38]. Our Ab against KCNQ1 could not detect bands other than those previously attributed to the long isoform of KCNQ1 [37]. The epitope recognized by our polyclonal antibody is found in human and dog KCNQ1 and is located in that part of the COOH terminal region common to both isoforms. Our immunoassays suggest that lower molecular weight isoform 2 is not present in the ventricles of the dog. In other studies (not shown), we were not able to detect RNA corresponding to the truncated isoform 2 by RT-PCR or by RACE (rapid amplification of cohesive ends) PCR on our dog cDNA library. Since isoform 2 expression is cardiogenic-specific through genomic imprinting [39], chromosomal variations between species may limit its expression in dog ventricles. Comparisons between species should always be made cautiously; nonetheless, our results show that isoform 1 expression is reduced during CAVB. In human, the dominant negative effect of KCNQ1 isoform 2 may add to the downregulation of KCNQ1 (isoform 1) and potentiate the reduction in $I_{Ks}$. Thus, our model may underestimate the reduction in $I_{Ks}$ linked to reduced expression of KCNQ1 and KCNE1 in human.

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