SUPPLEMENTAL MATERIAL

Supplemental Methods

Canine Tachypacing-Induced Heart Failure Model

Adult male mongrel dogs (n=14) underwent left bundle branch (LBB) radiofrequency ablation, and were followed for one week to ensure that there was no recovery and shortening of QRS duration. Then dogs were anesthetized and intubated and a left lateral thoractomy was performed to place bipolar leads (Medtronic) on the epicardial surfaces of the RA, right ventricular free wall and the postero-lateral LV wall. The atrial and both ventricular leads were connected to a pulse generator (Medtronic) and rapid RA pacing was initiated to produce DHF. After 3 weeks of RA tachy-pacing (190-200 bpm) to achieve LV dysfunction, dogs were divided into two groups; one group underwent continued RA tachy-pacing with progression to HF with LV dyssynchrony (DHF dogs; n=7), while another group underwent bi-ventricular pacing at the same pacing rate producing HF with synchronous ventricular contraction (CRT dogs; n=7).

Isolation of Ventricular Myocytes

Midmyocardial myocytes were isolated from the anterior and lateral wall by perfusion of the left anterior descending (LAD) artery and a branch of the left circumflex (LCx) artery, respectively with a nominally Ca^{2+} free solution containing collagenase and protease. Tissues were minced and cells were isolated by gentle trituration in a modified KB solution, and then myocytes were stored in Tyrode solution containing (in mmol/l) 140 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES and 10 glucose, adjusted to pH 7.4 with NaOH at room temperature (22-25˚C). Only Ca^{2+}-tolerant rod-shaped myocytes with cross striations and without spontaneous

contractions or significant granulation were selected for experiments.

Ionic current and calcium transient (CaT) measurements

Isolated ventricular myocytes were current and voltage clamped using the standard whole-cell patch-clamp technique with an Axopatch 200A patch-clamp amplifier (Axon Instruments) interfaced with a personal computer as previously described.[1,2] Voltage and current control, and data collection were performed using custom-written software. Borosilicate glass electrodes with tip resistances of ≈3.0 MΩ when filled with the pipette solution were used.

For the measurement of APs, standard Tyrode's was used as the external solution. The pipette solution contained (in mmol/l): 120 K^+-glutamate, 10 KCl, 10 HEPES, 5 EGTA and 5 Mg-ATP adjusted to pH 7.2 with KOH. The stimulation frequency was varied over the cycle lengths (CLs) of 4.0, 2.0, 1.0 and 0.5 sec, and the steady-state APs were recorded and analyzed at least 1 min after initiating pacing at each CL.

All currents were recorded in the whole-cell configuration of the patch clamp. Transient outward K^+ current (I_{to}) was recorded in Tyrode solution containing 5μmol/l nisoldipine to block I_{Ca} and 3 μmol/l E-4031 (Wako, Japan) and HMR1556 (Sanofi-Aventis Deutschland GmbH) to block the rapid (I_{Kr}) and slow (I_{Ks}) components of delayed rectifier K^+ currents (I_K). Inward rectifier K^+ current (I_{K1}) was quantified as the Ba^{2+}-sensitive (0.5 mmol/l) current in the normal Tyrode solution elicited by hyperpolarizing pulse from a holding potential of -40 mV. I_K was recorded as a composite current, and voltage dependence of I_K activation was evaluated by fitting the I-V relationship of tail currents to the Boltzmann equation: $I_{K,tail} = 1/\{1+\exp[(V_{1/2}-V_m)/k]\}$, where $I_{K,tail}$ was the tail current density. Ca^{2+} current (I_{Ca}) was recorded in Tyrode's solution with equimolar replacement of KCl by CsCl, and a pipette solution containing (in mmol/l): 80 Cs-glutamate, 40 CsCl, 10 HEPES, 5 EGTA and 5 Mg-ATP adjusted to pH 7.2 with CsOH. Cell

capacitance was estimated by integrating the area under an uncompensated depolarizing step of 10 mV from a holding potential of -80 mV.

CaTs were measured by indo-1 fluorescence exited at 365 nm with a Xenon arc lamp, emitted light at 405 and 495 nm was collected with a two-channel photomultiplier tube assembly. Fluorescence signals were digitized and stored with electrophysiological recordings for off-line analysis with custom-written software.[3] The ratio of indo-1 fluorescence ($R = F_{405\ nm}/F_{495nm}$) was determined after subtraction of cellular autofluorescence. The rate of Ca^{2+} removal (τ_{Ca}) was determined by fitting a single exponential to the Ca^{2+} time course. All the electrophysiological and intracellular Ca^{2+} measurements were performed at the physiological temperature (37ºC)

Real Time-PCR

Real-time quantitative PCR (RT-PCR) was performed using an ABI Prism 7900 Sequence-Detection System (Applied Biosystems). One step RT-PCR for the desired amplicon was conducted with total RNA isolated from cardiac tissue using the Qiagen RNeasy Midi Kit with an on column DNase digestion according to the manufacturer's instructions. Primers and probes were designed using the Primer Express software (Supplement Table 1). A trio of oligonucleotides with appropriate melting temperatures and structural features were selected and synthesized. At the 5' end, the probe was covalently linked to a fluorescent reporter dye 6-carboxyfluorescein (FAM or VIC) while the 3' end was linked to the quenching dye 6-carboxy-N,N,N',N'- tetramethylrhodamine (TAMRA). During PCR, the 5' nuclease activity of the AmpliTaq Gold DNA polymerase cleaves the reporter dye from the quenching dye; hence, releases a fluorescent signal that is proportional to the amount of starting target template. Reverse transcription and amplification reactions were conducted in one step under the following conditions: 48ºC for 38 min (reverse transcription), 95ºC for 10 min (initial denaturation), and

the next two steps (amplification) were repeated 40 times: 95ºC for 15 sec and 60ºC for 1 min. Optimization of both the primer (between 50-900 nM) and probe (between 50-250 nM) concentrations giving the lowest threshold cycle (C_T) and maximum change in the normalized reporter signal (ΔR_n) were determined. ROX served as the internal passive reference dye for normalization. No amplification (i.e. no reverse transcriptase) and no template samples served as negative controls. The efficiency of the RT-PCR reaction per sample was evaluated based on the same sample RT-PCR results with 18S rRNA control reagents. Each amplicon C_T was normalized against its 18S rRNA C_T.

Western Immunoblotting

LV tissue sections were carefully dissected from epicardium, endocardium and mid-myocardial from anterior and lateral regions in normal, DHF and CRT dogs, which were rapidly frozen in liquid nitrogen after organ harvest. Samples were run in duplicate or triplicate on 10%, 12.5%, or 15% Tris HCl precast gels (Bio-Rad, Hercules, CA) in 25 mM Tris, 192 mM glycine, and 0.1% (wt/vol) SDS running buffer. A standard control sample was also run on all gels to allow for comparisons across gels. Primary antibody incubations were performed overnight at 4°C with the appropriate antibodies. Antibodies to Cav1.2 (AB5412), Kir2.1 (AB5374,), Kv4.3 (AB5194), and ERG (AB5908) were purchased from Millipore. Antibodies to KChIP2 (PA1927), RyR2 (MA3-916), PLN (MA3-922), SERCA2 (MA3-910), and NCX (MA3-926) were purchased from Affinity Bioreagents. The antibody to KvLQT1 was made for our laboratory. Secondary horseradish peroxidase-conjugated antibodies were obtained from Jackson Immuno Research (West Grove, PA). Membranes were exposed and developed with enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions.

Supplemental Table S1. Primers for RT-PCR

Current	Clone	Primer Pair	Accession
I_{K1}	cKir2.1	F: GTCACAGACGAATGCCCAGTT	AF277647
		R: TTCTTTGGCTTGGCCATCTT	
		T: CCTTCATCATCGGCGCGGTCA T	
I_{to}	cKv4.3	F: AAGTCCTTCGCTGTCCAGTCA	AF049887
		R: TGGAGTTGGGCA GGTGTGT	
		T:ACGACCACCTGCTGCTCCCGTC	
	cKChIP	F: GCTGGTTTGTCGGTGATTCTTC	AF458385
		R: AGCCATCCTTGTTGAGGTCATAC	
		T:AACCATAGATGACAGACTAAACTGGGCCTTCAA	
I_{Kr}	cKv11.1(ERG)	F: CAGTGTGGGCTTTGGCAA T	AF017429
		R: CCGAAGAT CTGGCGTAC AT	
		T: TCCATCTGTGTCATGCTCATCGGC TC	
I_{Ks}	cKv7.1(KVLQT1)	F: CCAGCAGAAGCAGAGACAGAAG	XM_540790
		R: CTCCACGCCGTCTGAATGA	
		T: CAGATCCCGGCGGCAGCCT	
	cminK	F: ACCCGTTCAACGTATACATCGA	XP_544868
		R: AACACGGGCCTGGCAATA	
		T: CTTGTCCTTCTCCTGCCAGGTGTCAGA	
$I_{Ca.L}$	cCav1.2 (α1c)	F: CGATCTTCACCA ACCTGA TCC T	AF458389
		R: GGTGTGCTGGACGGGATCT	
		T: CTCAGCAGCATTTCCCTGGCA GCT	
	cCACNB1	F:GGCAGAGCGCCAAGCAT	ENSCAFT00000026162*
		R:GGTTGTAGCCGACATTTGTC	
		T:AGCCCAGCTCGAGAAAGCTAAGACCAAG	
	cCACNB2	F:CAGTTGGAAAAGGCAAAGACAA	ENSCAFT00000007219*
		R:CCTGGCACTGGGACATCATC	
		T:CCCGTTGCATTTGCTGTTCGGAC	
NCX		F:TCCTCATGATTGGCATACTGA	M57523
		R:TTTCAAGCCGATGGTGCAT	
		T:CATTGGAGACCTGGCTTCCCACTTTG	
SR	cRyR	F: CTG AGTCTGGCATTCGCT ACA C	AF440217
		R: CCA ACG AGACATGTAGCTGCA A	
		T:CAAATGCCACATGTAATGGAAGTCATA	
	cPLN	F: GCA TCA TTGTGATGCTTCTCTGA	M35393
		R: TTC ATGGGATGGCAGATA TTA AGTT	
		T: TTCTGCTGCAATCTCCAGTGA TGC	
	cSERCa2	F: GATTGA AGTGGCCTCCTCTGT AA	U94345
		R: GCCGTGCCCTTGTTGTCT	
		T: CAAGCAGGCATCCGAGTCATTAT	

Primers labeled T represent the Taqman fluorescent probes. They are labeled on the 5' end with FAM and the 3' end with Tamra. Mink (*KCNE1*) and KvLQT1 (*KCNQ1*) sequences are from genomic DNA sequence which is available at http://www.ensembl.org/index.html.

Primer set shown for KChIP = cKChIPE6dd which recognizes KChIP26, KChIP24, KChIP2, KChIP2T, and KChIP2S but not KChIP25 splice variants.

*Ensemble transcript ID

Supplemental Table S2. Cell Capacitance

	ANT		LTR	
	Capacitance (pF)	Cell#	Capacitance (pF)	Cell#
Control	184 ±39	27	198 ±35	36
DHF	220 ±56 *	23	246 ±58 *	39
CRT	236 ±61 *	20	266 ±101 *	28

Mean ±SD. * $p<0.05$ vs. control

Supplemental Table S3. Action Potential Duration and Early After Depolarizations

		APD_{20} (ms)		APD_{90} (ms)		APD_{20}/APD_{90}	
	EAD	-	+	-	+	-	+
Control	A	387±115		675±140		0.57±0.08	
	L	384±90	316±45	681±117	637±52	0.57±0.06	0.50±0.11
DHF	A	578±100	408±83 ‡	963±69	1187±286 †	0.60±0.09	0.38±0.11 ‡
	L	759±225	549±199 †	1255±324	1402±262	0.60±0.08	0.39±0.11 ‡
CRT	A	580±294	386±140	899±306	1006±341	0.62±0.14	0.38±0.08 ‡
	L	615±206	491±118	986±282	1050±215	0.62±0.04	0.47±0.07 ‡

Mean ± SD

A: anterior, L : lateral, APD_{20} = action potential duration at 20% recovery APD_{90} = action potential duration at 90% recovery, EAD = early after depolarization

† $p<0.05$ vs. EAD(-), ‡ $p<0.01$ vs. EAD(-)

Supplemental References:

1. Kaab S, Dixon J, Duc J, Ashen D, Nabauer M, Beuckelmann DJ, Steinbeck G, McKinnon D, Tomaselli GF. Molecular basis of transient outward potassium current downregulation in human heart failure: a decrease in Kv4.3 mRNA correlates with a reduction in current density. *Circulation*. 1998;98:1383-93.
2. Kaab S, Nuss HB, Chiamvimonvat N, O'Rourke B, Pak PH, Kass DA, Marban E, Tomaselli GF. Ionic mechanism of action potential prolongation in ventricular myocytes from dogs with pacing-induced heart failure. *Circ Res*. 1996;78:262-73.
3. O'Rourke B, Kass DA, Tomaselli GF, Kaab S, Tunin R, Marban E. Mechanisms of altered excitation-contraction coupling in canine tachycardia-induced heart failure, I: experimental studies. *Circ Res*. 1999;84:562-70.