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PHYSIOLOGICAL CONSEQUENCES OF TRANSIENT OUTWARD K⁺ CURRENT ACTIVATION DURING HEART FAILURE IN THE CANINE LEFT VENTRICLE

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Abstract

Background—Remodeling of ion channel expression is well established in heart failure (HF). We determined the extent to which I_{to} is reduced in tachypacing-induced HF and assessed the ability of an I_{to} activator (NS5806) to recover this current.

Method and Results—Whole-cell patch clamp was used to record I_{to} in epicardial (Epi) ventricular myocytes. Epi- and endocardial action potentials were recorded from left ventricular wedge preparations. Right ventricular tachypacing-induced heart failure reduced I_{to} density in Epi myocytes (Control=22.13±1.9 pA/pF vs 16.12±1.4 after 2-weeks and 10.69±1.4 pA/pF after 5-weeks, +50 mV). Current decay as well as recovery of I_{to} from inactivation progressively slowed with development of heart failure. Reduction of I_{to} density was paralleled by a reduction in phase 1 magnitude, epicardial action potential notch and J wave amplitude recorded from coronary-perfused left ventricular wedge preparations. NS5806 increased I_{to} (at +50 mV) from 16.12±1.4 to 23.85±2.1 pA/pF (p<0.05) at 2 weeks and from 10.69±1.4 to 14.35±1.9 pA/pF (p<0.05) in 5 weeks tachypaced dogs. NS5806 increased both fast and slow phases of I_{to} recovery in 2 and 5-week HF cells and restored the action potential notch and J wave in wedge preparations from HF dogs.

Conclusions—The I_{to} agonist NS5806 increases the rate of recovery and density of I_{to}, thus reversing the HF-induced reduction in these parameters. In wedge preparations from HF dogs, NS5806 restored the spike-and-dome morphology of the Epi action potential providing proof of principal that some aspects of electrical remodelling during HF can be pharmacologically reversed.

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Keywords

heart failure; ventricle; electrophysiology; contractility; pharmacology

INTRODUCTION

Congestive heart failure (HF) is one of the most common causes of death and disability in United States affecting about 2.5 million in this country alone. Despite advances in pharmacological therapy, it is estimated that the mortality rate approaches 50% after 5 years. Associated with heart failure are a number of other problems such as cardiac arrhythmias occurring in both the atria and ventricle [1].

Causes of HF are numerous but include ischemic heart disease, myocardial infarction, hypertension and valvular disease. During the development of HF, the heart undergoes structural, functional and electrophysiological remodeling that ultimately results in a reduced cardiac output. Increased sympathetic tone initially compensates for the reduced cardiac output by maintaining blood pressure and perfusion. However, this added stress leads to increased metabolic demands. Current treatment involve administration of agents that reduce afterload (such as angiotensin converting enzyme inhibitors, diuretics or beta-blockers) thereby reducing the workload of the heart.

Altered intracellular Ca^{2+} handling appears to play a central role during the progression of heart failure as well as in the development of cardiac arrhythmias. In a process known as calcium induced calcium release (CICR), Ca^{2+} influx through L type calcium channels (I_{Ca}) initiates a much greater Ca^{2+} release from the sarcoplasmic reticulum resulting in cell contraction. Typically, studies have focused on defects in intracellular Ca^{2+} cycling and the subsequent remodeling of these processes during HF. Indeed, altered Ca^{2+} handling during heart failure has been linked to changes in Ca^{2+} handling proteins. The expression of several proteins involved in Ca^{2+} regulation is reduced, including a lower expression of the SR Calcium ATPase (SERCA). Interestingly, other proteins are upregulated such as the sodium-calcium exchanger (NCX) resulting in a greater proportion of Ca^{2+} ions being pumped out of the cell [2], whereby the intracellular and SR Ca^{2+} content is reduced. The decrease in SR Ca^{2+} loading results in reduced Ca^{2+} transients, a reduced synchronization in the release of Ca^{2+} from the SR and impaired contraction.

Down regulation of many repolarizing potassium currents during HF is well documented (for review see [3,4]) resulting in a prolongation of the action potential duration (APD) as well as a decrease in phase 1 repolarization, presumably due to a decrease in I_{to} . In canine failing hearts, this loss in I_{to} may be due to loss of either $\text{K}_{\text{v}}4.3$ and/or KChIP2 proteins [5,6]. Action potential waveform can profoundly affect size and kinetics of I_{Ca} . For example, the presence of a phase 1 repolarization can increase both peak and total charge of I_{Ca} during the course of an action potential [7–9]. Therefore in pathophysiological conditions where I_{to} is reduced, such as HF, loss of I_{to} may contribute to decreased calcium influx, CICR and contraction.

The present study evaluates the effect of an I_{to} activator (NS5806) in tachypacing induced HF in canine heart. Results of our study show that there is a progressive loss in the magnitude of I_{to} as well as a change in kinetics of the current following prolonged ventricular tachypacing. Application of NS5806 increased I_{to} density toward normal levels, resulting in restoration of AP morphology in both single and multicellular preparations. Results have been previously presented in abstract form.

METHODS

Rapid-Pacing Induced Heart Failure

We used a well characterized rapid-pacing induced heart failure model which results in congestive HF model [10]. Adult mongrel dogs of either sex were used for the study. This investigation conforms to the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (The Eighth Edition of the *Guide for the Care and Use of Laboratory Animals* (NRC 2011)) and was approved by the Institutional Animal Care and Use Committee.

Dogs were premedicated with 0.1 mg/kg hydromorphone and 0.02 mg/kg acepromazine. Anesthesia was induced by intravenous administration of 12 mg/kg thiopental and maintained by isoflurane (1–1.5%) inhalation. Pacemaker generators (modified Medtronic) were implanted in a subcutaneous pocket in the left cervical region. An active fixation bipolar pacing lead was positioned in the interventricular septum of the right ventricle with the aid of fluoroscopy and transesophageal echocardiography. After recovery (1 day), the dogs were paced at 220 bpm for a period of either 2 or 5 weeks. Pulse rates were monitored daily and a 12 lead ECG was recorded weekly to ensure proper pacing. HF was verified by measurement of LV ejection fraction, fractional shortening, end-systolic volume, and end diastolic volume using echocardiography and by measurement of BNP before and at the end of the 2-week (9 dogs) or 5 week (8 dogs) pacing periods.

Ventricular Wedge Preparations

The animals were anticoagulated with heparin and anesthetized with pentobarbital (30–35 mg/kg, i.v.). The chest was open via a left thoracotomy, the heart excised, placed in a cardioplegic solution (4° C-Tyrode's solution with 12 mM $[K^+]_o$). Transmural wedges with dimensions of up to $3 \times 2 \times 1.5$ cm (left ventricular wedge) were dissected from the antero-apical aspects of the canine left ventricle as previously described [11]. During the cannulation procedure the preparations were initially arterially perfused with cardioplegic solution through a distal diagonal branch of the left anterior descending coronary artery. Subsequently, the wedges were placed in a tissue bath and perfused with Tyrode's solution of the following composition (mM): 129 NaCl, 4 KCl, 0.9 NaH_2PO_4 , 20 $NaHCO_3$, 1.8 $CaCl_2$, 0.5 $MgSO_4$, 5.5 glucose, buffered with 95% O_2 and 5% CO_2 ($37 \pm 0.5^\circ C$). The perfusate was delivered at a constant pressure (45–50 mmHg). A transmural ECG was recorded using two Ag/AgCl half cells placed at ~1 cm. from the Epi (+) and Endo (–) surfaces of the preparation and along the same axis as the transmembrane recordings. Action potentials were simultaneously recorded from the epicardial surface (Epi) and from subendocardial regions or endocardial surface (Endo) using floating microelectrodes. Pacing was applied to the endocardial surface (BCL= 2 s). All amplified signals were digitized and analyzed using Spike 2 for Windows (Cambridge Electronic Design [CED], Cambridge, UK).

Isolation of adult myocytes

Myocytes from Epi regions were prepared from canine hearts using techniques previously described [12–14]. A wedge consisting of the left ventricular free wall was cannulated and perfused with nominally Ca^{2+} -free Tyrode's solution containing 0.1% BSA for about 5 minutes. The wedge preparations were then subjected to enzyme digestion with the nominally Ca^{2+} -free solution supplemented with 0.5 mg/ml collagenase (Type II, Worthington), 0.1 mg/ml protease (Type XIV, Sigma) and 1 mg/ml BSA for 8–12 minutes. After perfusion, thin slices of tissue from the Epi (<2 mm from the epicardial surface) were shaved from the wedge using a dermatome. The tissue slices were then placed in a beaker, minced and incubated in fresh buffer containing 0.5 mg/ml collagenase, 1 mg/ml BSA and

agitated. The supernatant was filtered, centrifuged at 200 rpm for 2 minutes and the pellet containing the myocytes was stored in 0.5 mM Ca^{2+} HEPES buffer at room temperature.

Solutions

The nominally Ca^{2+} -free dissecting buffer contained (mM): NaCl 129, KCl 5.4, MgSO_4 2.0, NaH_2PO_4 0.9, glucose 5.5, NaHCO_3 20 and was bubbled with 95% O_2 /5% CO_2 . Ventricular cells were superfused with HEPES buffer (mM): NaCl 126, KCl 5.4, MgCl_2 1.0, CaCl_2 2.0, HEPES 10, glucose 11, pH=7.4 with NaOH. For I_{to} recordings 300 μM CdCl_2 was present in the extracellular solution to block I_{Ca} . The pipette solution consisted of (mM): K-aspartate 125, KCl 10, MgCl_2 1, EGTA 5, MgATP 5, HEPES 10, NaCl 10, pH=7.2 with KOH.

Electrophysiology

I_{to} recordings from myocytes were performed as previously described [15] and all myocyte experiments were performed at 36°C. Voltage-clamp and conventional recordings were made using a MultiClamp 700A amplifier and MultiClamp Commander (Axon Instruments). Patch pipettes were fabricated from borosilicate glass capillaries (1.5 mm O.D., Fisher Scientific, Pittsburg, PA). Pipettes were pulled using a gravity puller (Narishige Corp) and the resistance ranged from 0.9–3 M Ω when filled with the internal solution. Cell capacitance was measured by applying –5 mV voltage steps. Electronic compensation of series resistance to 60–70% was applied. All analog signals were acquired at 10–25 kHz, filtered at 4–6 kHz, digitized with a Digidata 1322 converter (Axon Instruments) and stored using pClamp9 software.

Statistics

Pooled data are presented as Mean $\pm$ SEM. Statistical analysis was performed using an ANOVA test followed by a Student-Newman-Keuls test or Student t-test, as appropriate, using SigmaStat software. Statistical analysis of RNA and protein expression was performed with a repeated measures ANOVA followed by a Tukey's post test. $p < 0.05$ was considered statistically significant.

RESULTS

Right ventricular tachypacing induced progressive development of HF as assessed by hemodynamic parameters measured using echocardiography following 2 and 5 weeks of tachypacing (Figure 1A). LV end systolic volume (LVESV) increased from 8.6 ± 1.4 to 19.2 ± 3.0 ml at 2 weeks and from 18.2 ± 4.0 to 48.6 ± 9.4 ml at 5 weeks and end diastolic volume (LVEDV) increased from 22.8 ± 3.1 to 29.8 ± 4.0 ml at 2 weeks and from 41.4 ± 7.6 to 70.5 ± 13.0 ml. Left ventricular ejection fraction (LVEF) decreased from 63.2 ± 2.0 to 35.9 ± 2.5 % at 2 weeks and from 59.0 ± 4.0 to 36.3 ± 4.5 % at 5 weeks.

As an initial basis of comparison, action potentials (AP) were recorded in Epi cells isolated from hearts subjected to either 2 or 5-weeks of rapid pacing. Representative APs from normal and 5 week paced Epi cells paced at a basic cycle length of 1 s are shown in Figure 1B. AP recordings from normal Epi cells show a prominent spike-and-dome configuration. In contrast, APs recorded from Epi cells isolated from a HF dog showed loss of the spike and dome configuration and smaller phase 1 repolarization, suggesting loss of I_{to} . This was accompanied by a prolongation of the AP duration suggesting down regulation of other repolarizing K^+ currents.

The density of I_{to} was examined in myocytes isolated from Epi in control animals and dogs tachypaced for 2 and 5 weeks. Cd^{2+} (300 μM) was present in the extracellular solution to

block the calcium current (I_{CaL}). Following a brief step to -50 mV to discharge sodium channels, voltage steps from -40 to $+50$ mV applied to Epi cells elicited fast activating and rapidly inactivating I_{to} currents. Representative currents recorded from normal and 5-week Epi are shown in Figure 2A–B. The mean current-voltage (I-V) relation of peak I_{to} showed a progressive reduction in I_{to} density in HF Epi cells compared to controls (Figure 2C). Analysis of the decay of I_{to} (τ) revealed a progressive slowing of current inactivation with 2 to 5 weeks of tachypacing (Figure 2D).

Changes in steady state gating parameters may contribute to the difference in current density between normal and HF cells. Steady state inactivation of I_{to} was evaluated using a prepulse-test pulse voltage clamp protocol (top of Figure 3) in presence of Cd^{2+} . Peak current following a 2 s prepulse was normalized to the maximum current and plotted as a function of the prepulse voltage to obtain the availability of the channels. A Boltzmann function was then fitted to the data. In normal Epi cells, the mid-inactivation voltage was -47.4 ± 0.31 mV (Figure 3C). In 2-week and 5-week HF Epi cells, mid-inactivation voltage was -49.9 ± 0.45 mV for 2-week and -46.8 ± 0.48 mV for 5-week Epi cells (Figure 3C).

We next determined if I_{to} recovery from inactivation was altered following rapid pacing. Representative traces from a normal Epi (Figure 4A) and 5-week HF Epi (Figure 4B) are shown. Reactivation of I_{to} at -80 mV for normal and HF Epi cells showed a fast and a slow phase of recovery as follows: i) $\tau_1 = 28.4 \pm 2.54$ ms and $\tau_2 = 177.5 \pm 7.82$ ms for normal Epi cells, ii) $\tau_1 = 56.6 \pm 5.92$ ms and $\tau_2 = 337.8 \pm 28.9$ ms for 2-week and iii) $\tau_1 = 73.4 \pm 3.52$ ms and $\tau_2 = 546.3 \pm 38.6$ ms for 5-week Epi HF cells (Figure 4C). These results demonstrate that HF causes a significant time dependent slowing in both the fast and slow phase of recovery of I_{to} in Epi cells.

The results thus far demonstrate a reduction in the density of I_{to} as well as a slowing in the recovery of the current with progressive development of HF. In previous studies, we have identified a selective I_{to} agonist (NS5806). Although this compound also produces a minor inhibition of I_{Na} and I_{Ca} with no effect on other K^+ currents [11], we utilized its I_{to} agonist effect to restore this current in Epi cells isolated from HF hearts. In the first series of experiments the effect of NS5806 on I_{to} density in HF Epi cells was examined. Representative I_{to} recordings for Epi cells isolated from 5 week tachypaced dogs in the absence and presence of $10 \mu M$ NS5806 are shown in Figure 5A–B. An increase in I_{to} peak current density was observed as well as a slowing in the decay of the current. Analysis of the current-voltage (I-V) relation of peak I_{to} showed that NS5806 increased current density in both 2-week (Figure 5C) and 5-week HF Epi cells (Figure 5D). On a percentage basis, we found that NS5806 increased I_{to} (at $+50$ mV) by 180% in normal Epi cells, 148% in 2 weeks and 134% in 5 weeks tachypaced dogs. Analysis of the decay of I_{to} (τ) showed that NS5806 slowed current decay in HF Epi cells (Figure 5E–F), similar to previously described results in normal cells [12].

Steady state inactivation of I_{to} was next evaluated in HF cells. In the absence of drug, mid-inactivation voltage was -49.9 ± 0.45 mV in 2 week HF Epi cells and -46.8 ± 0.48 mV in 5 week HF Epi cells (Figure 6). Application of NS5806 ($10 \mu M$) caused a significant negative shift in the mid-inactivation potential in both HF Epi cells with mid-inactivation voltages of -54.3 ± 0.36 mV at 2 weeks (Figure 6C) and -52.1 ± 0.46 mV at 5 weeks (Figure 6D). This shift in mid-inactivation could not account for the increase in current density observed in the presence of NS5806.

We next examined the difference in time dependent recovery from inactivation of I_{to} in HF Epi cells in the absence and presence of $10 \mu M$ NS5806 (Figure 7A–B). Application of NS5806 significantly increased both the fast and the slow phase of recovery of I_{to} in both 2

and 5 weeks HF Epi cells. In 2 week HF Epi cells, the time constants of recovery were: $\tau_1=56.6\pm5.92$ ms and $\tau_2=337.8\pm28.9$ ms in the absence of drug and $\tau_1=31.1\pm4.28$ ms and $\tau_2=149.9\pm7.98$ ms after 10 μ M NS5806 (Figure 7C). In 5 week HF Epi cells recovered with $\tau_1=73.4\pm3.52$ ms and $\tau_2=546.3\pm38.6$ ms in the absence of drug and $\tau_1=51.2\pm4.22$ ms and $\tau_2=257.5\pm16.83$ ms after drug (Figure 7D).

The results demonstrate that NS5806 can restore I_{to} in Epi cells isolated from HF hearts. We next determined the effect of NS5806 in multicellular preparations. APs and corresponding pseudo-ECG were simultaneously recorded in Epi and Endo layers from a canine left ventricular wedge preparation (Figure 8). In a control wedge preparation, the Epi layer of the wedge exhibited a pronounced phase 1 repolarization with minimal phase 1 repolarization present in Endo layer (left panel). The resulting transmural voltage gradient inscribed a distinct J wave in the ECG. In wedge preparations isolated from dogs tachypaced for a period of 2-week, phase 1 and the epicardial action potential notch were dramatically reduced, as was the J wave in the ECG (middle panel). Application of 10 μ M NS5806 restored the spike-and-dome morphology in the Epi recording (phase 1 repolarization increased from 3% of the amplitude of phase 0 to 20.5%, $n=5$ wedges) with negligible effect in the Endo recording and no changes in APD₉₀. The dramatic increase in the Epi notch was accompanied by a comparable augmentation of the J wave amplitude of the ECG.

DISCUSSION

The results of our study demonstrate that rapid-pacing induced heart failure causes a prolongation of the cardiac action potential and a marked reduction of phase 1 repolarization in both single and multicellular preparations. Patch clamp analysis of I_{to} showed a time-dependent reduction in current density as well as a slowing of current decay in failing hearts. A progressive slowing in recovery from inactivation was also observed. The I_{to} activator NS5806 increased I_{to} density toward normal in HF cells and restored early repolarization in ventricular wedge preparations from HF hearts.

In the normal canine ventricle, the majority of transient outward K^+ channels are comprised of $K_V4.3$ channels with lower levels of $K_V1.4$ [6,16]. The electrophysiological properties of $K_V4.3$ channels are modulated by several β -subunits including KChIP2, which increase peak $K_V4.3$ current density, accelerate recovery from inactivation, and slow the decay (τ) of the current [17]. The reduction in I_{to} coupled with the change in current kinetics during HF suggests that one or more of the molecular correlates of I_{to} is altered. Previous studies have found that both $K_V4.3$ and KChIP2 are reduced following rapid pacing induced HF in canine hearts [5]. In contrast, a reduction in $K_V4.3$ but no change in KChIP2 was observed in another study [6]. Interestingly, the expression of $K_V1.4$ proteins has been shown to be increased in failing canine hearts [6]. The fact that $K_V1.4$ typically displays very slow recovery from inactivation as well as a slow current decay [18], suggests the possibility that the slower τ values of I_{to} decay as well as the slowing of the time dependent recovery observed in the present study may be due to a relatively larger contribution of $K_V1.4$ to I_{to} in failing hearts. In addition, I_{to} may be affected by elevated levels of angiotensin II, a known modulator of I_{to} [19].

In rodent models of HF it has been shown that loss of I_{to} may be directly involved in the hypertrophic response [20]. Reducing I_{to} prolongs APDs, resulting in larger Ca^{2+} influx which activates the hypertrophic factor calcineurin [20] whereas increasing I_{to} slows the progression of HF [21–23]. In larger mammals I_{to} is not directly involved in the phase 3 repolarization and the increased APD is likely due to down-regulation of currents other than I_{to} , thus the mechanisms of the potential beneficial effect of increasing I_{to} is fundamentally different in smaller and larger mammals (see review by Sah et al. [24]).

Application of NS5806 resulted in a substantial increase in I_{to} density in isolated HF Epi cells. In 2 weeks HF Epi cells, I_{to} density in the presence of NS5806 was comparable to normal Epi cells (Figures 2 and 5). After 5 weeks, I_{to} density was further reduced in Epi HF cells and while NS5806 increased I_{to} density, the effect was smaller than for 2 week. The slower recovery and reduced ability of NS5806 to enhance I_{to} after 5 weeks may suggest a larger contribution of $K_{V1.4}$ to the remaining I_{to} . Some studies have demonstrated that a reduction in KChIP2 occurs during HF, suggesting that this is responsible for the reduced I_{to} density. However, in the absence of KChIP2, $I_{Kv4.3}$ has a faster decay [25] but in the present study we observed a slowing in tau. Furthermore, our previous work has demonstrated that NS5806 slows I_{to} current decay only in the presence KChIP2 [17]. This suggests that the reduction of I_{to} in HF is not merely due to a reduction in KChIP2 levels.

In LV wedge preparations from failing hearts, phase 1 repolarization of Epi APs was decreased, presumably due to a loss in I_{to} . Moreover, APD (and the corresponding ECG) was prolonged in Endo and Epi compared to normal canine hearts. Application of NS5806 restored the phase 1 repolarization in Epi, and normalized the pseudo-ECG by restoring the J-wave. AP morphology in Endo recordings was not affected by application of NS5806, consistent with very little expression of I_{to} in the endocardium [12]. In both Epi and Endo, the APD remained prolonged, which was reflected on the ECG as an increased QT interval compared to normal, suggesting that factors other than a decrease in I_{to} are responsible for the effects on APD. The early repolarization phase determines the electrochemical driving force for Ca^{2+} influx during the early plateau phase. Cell types with a large I_{to} exhibit a prominent early repolarization and have a significantly larger peak I_{CaL} as well as greater total Ca^{2+} influx compared to a cell type where I_{to} is absent [7–9]. Since the size of the Ca^{2+} transient is proportional to Ca^{2+} influx via I_{CaL} , a large influx translates into greater SR Ca^{2+} release as well as increased cell shortening. Furthermore, in the presence of a prominent phase 1 repolarization, the SR Ca^{2+} release exhibits more temporal synchronization and spatial synchronization on single cell level [7,8], which in turn increases contraction as well as accelerates time to peak contraction [7]. In the normal canine heart, Epi and M cells have a prominent phase 1 repolarization compared to Endo, reflecting the transmural gradient in I_{to} density. Since the normal activation pattern of the ventricle is from Endo to Epi, the differences in phase 1 repolarization may serve to synchronize contraction across the ventricle and may improve the contractile efficiency [26]. These results suggests that in HF cells, the reduction of I_{to} and loss of phase 1 repolarization will affect L-type Ca^{2+} currents and reduce the synchronization of the calcium transient as well as disrupt the transmural coordination of contraction.

In theory, the augmentation of I_{to} in the setting of heart failure should have a beneficial effect on cardiac output for several reasons: Application of an I_{to} activator will result in restoration of the spike-and-dome morphology resulting in an immediate increase in Ca^{2+} influx into the cell during the course of an action potential. Since an AP with spike and dome morphology is a more efficient releaser of Ca^{2+} from the SR, this may result in a better synchronization of the calcium transients in single cells [8,27]. Furthermore, restoration of transmural gradient of phase 1 repolarization may improve synchronization of contraction across the ventricular wall. On the long term basis, enhancement of I_{to} will maintain SR load since a greater amount of Ca^{2+} will enter the cell during each action potential. The improved contractile efficiency may outweigh the risk of increasing metabolic workload of the failing heart.

In summary, we demonstrate a time dependent reduction in the magnitude of I_{to} as well as a slowing in the recovery from inactivation in response to rapid pacing-induced HF. Application of NS5806 increased I_{to} density in Epi ventricular myocytes as well as phase 1 repolarization in multicellular preparations. Our results suggest that restoration of I_{to} may be

a novel approach to the treatment of HF or other pathophysiological conditions (such as diabetic cardiomyopathy) where I_{to} is known to be reduced.

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References

1. Tomaselli GF, Zipes DP. What causes sudden death in heart failure? *Circ Res.* 2004; 95:754–763. [PubMed: 15486322]
2. Xiong W, Tian Y, DiSilvestre D, Tomaselli GF. Transmural Heterogeneity of Na^+-Ca^{2+} Exchange: Evidence for Differential Expression in Normal and Failing Hearts. *Circ Res.* 2005; 97:207–209. [PubMed: 16002750]
3. Akar FG, Tomaselli GF. Ion channels as novel therapeutic targets in heart failure. *Ann Med.* 2005; 37:44–54. [PubMed: 15902846]
4. Aiba T, Tomaselli GF. Electrical remodeling in the failing heart. *Curr Opin Cardiol.* 2010; 25:29–36. [PubMed: 19907317]
5. Zicha S, Xiao L, Stafford S, Cha TJ, Han W, Varro A, et al. Transmural expression of transient outward potassium current subunits in normal and failing canine and human hearts. *J Physiol.* 2004; 561:735–748. [PubMed: 15498806]
6. Akar FG, Wu RC, Juang GJ, Tian Y, Burysek M, DiSilvestre D, et al. Molecular mechanisms underlying K^+ current downregulation in canine tachycardia-induced heart failure. *Am J Physiol Heart Circ Physiol.* 2005; 288:H2887–H2896. [PubMed: 15681701]
7. Cordeiro JM, Greene L, Heilmann C, Antzelevitch D, Antzelevitch C. Transmural heterogeneity of calcium activity and mechanical function in the canine left ventricle. *Am J Physiol Heart Circ Physiol.* 2004; 286:H1471–H1479. [PubMed: 14670817]
8. Sah R, Ramirez RJ, Backx PH. Modulation of Ca^{2+} release in cardiac myocytes by changes in repolarization rate: role of phase-1 action potential repolarization in excitation-contraction coupling. *Circ Res.* 2002; 90:165–173. [PubMed: 11834709]
9. Hund TJ, Rudy Y. Rate dependence and regulation of action potential and calcium transient in a canine cardiac ventricular cell model. *Circulation.* 2004; 110:3168–3174. [PubMed: 15505083]
10. Li D, Fareh S, Leung TK, Nattel S. Promotion of atrial fibrillation by heart failure in dogs: atrial remodeling of a different sort. *Circulation.* 1999; 100:87–95. [PubMed: 10393686]
11. Calloe K, Cordeiro JM, Di Diego JM, Hansen RS, Grunnet M, Olesen SP, et al. A transient outward potassium current activator recapitulates the electrocardiographic manifestations of Brugada syndrome. *Cardiovasc Res.* 2009; 81:686–694. [PubMed: 19073629]
12. Calloe K, Soltysinska E, Jespersen T, Lundby A, Antzelevitch C, Olesen SP, et al. Differential effects of the transient outward K^+ current activator NS5806 in the canine left ventricle. *J Mol Cell Cardiol.* 2010; 48:191–200. [PubMed: 19632239]
13. Cordeiro JM, Malone JE, Di Diego JM, Scornik FS, Aistrup GL, Antzelevitch C, et al. Cellular and subcellular alternans in the canine left ventricle. *Am J Physiol Heart Circ Physiol.* 2007; 293:H3506–H3516. [PubMed: 17906109]
14. Murphy L, Renodin DM, Antzelevitch C, Di Diego JM, Cordeiro JM. Extracellular proton depression of peak and late sodium current in the canine left ventricle. *Am J Physiol Heart Circ Physiol.* 2011; 301:H936–H944. [PubMed: 21685271]
15. Dumaine R, Cordeiro JM. Comparison of K^+ currents in cardiac Purkinje cells isolated from rabbit and dog. *J Mol Cell Cardiol.* 2007; 42:378–389. [PubMed: 17184792]

16. Dixon EJ, Shi W, Wang H-S, McDonald C, Yu H, Wymore RS, et al. Role of the Kv4.3 K⁺ channel in ventricular muscle. A molecular correlate for the transient outward current. *Circ Res*. 1996; 79:659–668. [PubMed: 8831489]
17. Lundby A, Jespersen T, Schmitt N, Grunnet M, Olesen SP, Cordeiro JM, et al. Effect of the I(to) activator NS5806 on cloned K(V)4 channels depends on the accessory protein KChIP2. *Br J Pharmacol*. 2010; 160:2028–2044. [PubMed: 20649599]
18. Wickenden AD, Tsushima RG, Losito VA, Kaprielian R, Backx PH. Effect of Cd²⁺ on K_v4.2 and K_v1.4 expressed in *Xenopus* oocytes and on the transient outward currents in rat and rabbit ventricular myocytes. *Cell Physiol Biochem*. 1999; 9:11–28. [PubMed: 10352341]
19. Yu H, Gao J, Wang H, Wymore R, Steinberg S, McKinnon D, et al. Effects of the renin-angiotensin system on the current I(to) in epicardial and endocardial ventricular myocytes from the canine heart. *Circ Res*. 2000; 86:1062–1068. [PubMed: 10827136]
20. Kassiri Z, Zobel C, Nguyen TT, Molkentin JD, Backx PH. Reduction of I(to) causes hypertrophy in neonatal rat ventricular myocytes. *Circ Res*. 2002; 90:578–585. [PubMed: 11909822]
21. Zobel C, Kassiri Z, Nguyen TT, Meng Y, Backx PH. Prevention of hypertrophy by overexpression of Kv4.2 in cultured neonatal cardiomyocytes. *Circulation*. 2002; 106:2385–2391. [PubMed: 12403671]
22. Jin H, Hadri L, Palomeque J, Morel C, Karakikes I, Kaprielian R, et al. KChIP2 attenuates cardiac hypertrophy through regulation of Ito and intracellular calcium signaling. *J Mol Cell Cardiol*. 2010; 48:1169–1179. [PubMed: 20051248]
23. Lebeche D, Kaprielian R, Del MF, Tomaselli G, Gwathmey JK, Schwartz A, et al. In vivo cardiac gene transfer of Kv4.3 abrogates the hypertrophic response in rats after aortic stenosis. *Circulation*. 2004; 110:3435–3443. [PubMed: 15557376]
24. Sah R, Ramirez RJ, Oudit GY, Gidrewicz D, Trivieri MG, Zobel C, et al. Regulation of cardiac excitation-contraction coupling by action potential repolarization: role of the transient outward potassium current (I(to)). *J Physiol*. 2003; 546:5–18. [PubMed: 12509475]
25. Deschenes I, DiSilvestre D, Juang GJ, Wu RC, An WF, Tomaselli GF. Regulation of Kv4.3 current by KChIP2 splice variants: a component of native cardiac I_{to}? *Circulation*. 2002; 106:423–429. [PubMed: 12135940]
26. Kerckhoffs RC, Bovendeerd PH, Kotte JC, Prinzen FW, Smits K, Arts T. Homogeneity of cardiac contraction despite physiological asynchrony of depolarization: a model study. *Ann Biomed Eng*. 2003; 31:536–547. [PubMed: 12757198]
27. Cooper PJ, Soeller C, Cannell MB. Excitation-contraction coupling in human heart failure examined by action potential clamp in rat cardiac myocytes. *J Mol Cell Cardiol*. 2010; 49:911–917. [PubMed: 20430038]

Highlights

- Heart failure (HF) reduced phase 1 repolarization in both single and multicellular preparations.
- I_{to} current density was reduced in HF Epi cells compared to normal Epi cells.
- The I_{to} activator NS5806 increased I_{to} density toward normal in HF cells
- NS5806 restored phase 1 repolarization in ventricular wedge preparations from failing hearts.
- Restoration of I_{to} may be a novel approach to the treatment of HF.

Figure 1.

Panel A: Summary of left ventricular hemodynamic changes in response to rapid pacing.

Hemodynamic parameters were collected before pacing and at the end of the 2-week (9 dogs) or 5 week (8 dogs) pacing periods. EF=ejection fraction, IDD=internal diameter in diastole, IDS=internal diameter in systole, EDV=end diastolic volume, ESV=end systolic volume, FS=fractional shortening, FWD=front wheel drive, FWS=wall thickness in systole

Panel B: Representative recordings of action potentials recorded from a Normal and 5-week HF Epi myocyte. Each panel shows 5 consecutive APs paced at a cycle length of 1 second. HF Epi cells show a drastically reduced phase 1 repolarization and longer AP duration.

Figure 2.

Representative I_{to} traces recorded from a normal Epi (**Panel A**) and 5-week HF Epi cell (**Panel B**). The voltage clamp protocol is shown at the top of the figure and Cd^{2+} was present to block I_{CaL} . Mean I-V relation for peak I_{to} from normal, 2-week HF and 5-week HF Epi cells (**Panel C**). The time constants of decay (Tau) were obtained by fitting a single exponential equation to the decaying phase of the currents and plotted as a function of voltage (**Panel D**). A time-dependent reduction in the density of I_{to} as well as a slowing of current decay was observed with prolonged pacing.

Figure 3. Representative traces recorded from a normal Epi (**Panel A**) and 5-week HF Epi (**Panel B**) showing voltage dependence of inactivation of I_{to} . Boltzmann curves showing mid-inactivation voltages for normal, 2-week HF and 5-week HF Epi (**Panel C**).

Figure 4. Representative traces recorded from a normal (**Panel A**) and 5-week HF Epi cell (**Panel B**) showing recovery of I_{to} . Cd^{2+} was present to block I_{CaL} . Mean data showing the recovery time-course of I_{to} recorded from normal, 2-week HF and 5-week HF Epi cells (**Panel C**).

Figure 5.

Representative I_{to} traces recorded from a 5-week HF Epi cell under control conditions (**Panel A**) and after 10 μM NS5806 (**Panel B**). Cd^{2+} was present to block I_{CaL} . Mean I-V relation for peak I_{to} from 2-week (**Panel C**) and 5-week (**Panel D**) HF Epi cells in absence and presence of 10 μM NS5806. Mean I-V relation for peak I_{to} from normal Epi cells are also illustrated for comparison. The time constants of decay (Tau) were obtained by fitting a single exponential equation to the decaying phase of the currents and plotted as a function of voltage for 2-week (**Panel E**) and 5-week (**Panel F**) HF Epi cells. Tau values from normal Epi cells are illustrated for comparison. NS5805 increased I_{to} peak current density and

slowed current decay in HF Epi cells paced for 2 or 5 weeks. *Statistically significant from normal Epi cells, $p<0.05$; #Statistically significant from HF Epi cells, $p<0.05$.

Figure 6. Representative traces recorded from a 5-week HF Epi cell under control conditions (**Panel A**) and after 10 μ M NS5806 (**Panel B**) showing voltage dependence of inactivation of I_{to} . Boltzmann curves showing mid-inactivation voltages for 2-week (**Panel C**) and 5-week (**Panel D**) HF Epi in absence and presence of NS5806.

Figure 7.

Representative traces recorded from a 5-week HF Epi cell under control conditions (**Panel A**) and after 10 μ M NS5806 (**Panel B**) showing recovery of I_{to} . Cd^{2+} was present to block I_{CaL} . The recovery time-course of I_{to} recorded from 2-week HF Epi (**Panel C**) and 5-week Epi cells (**Panel D**) in the absence and presence of drug.

Figure 8. Ventricular tachypacing-induced heart failure-induced reduction in epicardial action potential notch and ECG J wave is reversed by NS5806. **Left Panel:** Representative LV wedge isolated from a normal heart showing Epi and Endo AP as well as corresponding pseudo-ECG. A pronounced spike-and-dome AP is apparent in the Epi recording. **Middle Panel:** Representative LV wedge isolated from a 2-week HF heart showing a decrease in phase 1 repolarization, loss of spike-and-dome morphology and J wave. **Right panel:** 10 μ M NS5806 restores action potential notch and J wave toward control. Basic cycle length = 2000 ms.