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Original article

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 = 22.1 ± 1.9 pA/pF vs 16.1 ± 1.4 after 2 weeks and 10.7 ± 1.4 pA/pF after 5 weeks, +50 mV). Current decay as well as recovery of I_{to} from inactivation progressively slowed with the 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 16.1 ± 1.4 to 23.9 ± 2.1 pA/pF ($p<0.05$) at 2 weeks and from 10.7 ± 1.4 to 14.4 ± 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.

1. 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 involves 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.

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Downregulation of many repolarizing potassium currents during HF is well documented (for review see Refs. [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 the loss of either K_v4.3 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.

2. Methods

2.1. 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.

2.2. 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 $3\times2\times1.5$ 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 ± 0.5 °C). The perfusate was delivered at a constant pressure (45–50 mm Hg). 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).

2.3. 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 min. 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 min. 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 min and the pellet containing the myocytes was stored in 0.5 mM Ca^{2+} HEPES buffer at room temperature.

2.3.1. 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.

2.3.2. 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, Pittsburgh, PA). Pipettes were pulled using a gravity puller (Narishige Corp) and the resistance ranged from 0.9 to 3 MΩ 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.

2.3.3. Statistics

Pooled data are presented as Mean ± 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. $p<0.05$ was considered statistically significant.

3. Results

Right ventricular tachypacing induced progressive development of HF as assessed by hemodynamic parameters measured using echocardiography following 2 and 5 weeks of tachypacing (Fig. 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