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Original article

Role of late sodium current as a potential arrhythmogenic mechanism in the progression of pressure-induced heart disease

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ABSTRACT

The aim of the study was to determine the characteristics of the late Na current (I_{NaL}) and its arrhythmogenic potential in the progression of pressure-induced heart disease. Transverse aortic constriction (TAC) was used to induce pressure overload in mice. After one week the hearts developed isolated hypertrophy with preserved systolic contractility. In patch-clamp experiments both, I_{NaL} and the action potential duration (APD_{90}) were unchanged. In contrast, after five weeks animals developed heart failure with prolonged APDs and slowed I_{NaL} decay time which could be normalized by addition of the I_{NaL} inhibitor ranolazine (Ran) or by the Ca/calmodulin-dependent protein kinase II (CaMKII) inhibitor AIP. Accordingly the APD_{90} could be significantly abbreviated by Ran, tetrodotoxin and the CaMKII inhibitor AIP. Isoproterenol increased the number of delayed afterdepolarizations (DAD) in myocytes from failing but not sham hearts. Application of either Ran or AIP prevented the occurrence of DADs. Moreover, the incidence of triggered activity was significantly increased in TAC myocytes and was largely prevented by Ran and AIP. Western blot analyses indicate that increased CaMKII activity and a hyperphosphorylation of the Nav1.5 at the CaMKII phosphorylation site (Ser571) paralleled our functional observations five weeks after TAC surgery. In pressure overload-induced heart failure a CaMKII-dependent augmentation of I_{NaL} plays a crucial role in the AP prolongation and generation of cellular arrhythmogenic triggers, which cannot be found in early and still compensated hypertrophy. Inhibition of I_{NaL} and CaMKII exerts potent antiarrhythmic effects and might therefore be of potential therapeutic interest. This article is part of a Special Issue entitled "Na^+ Regulation in Cardiac Myocytes".

1. Introduction

Patients with heart failure either die from pump failure or life threatening arrhythmias. Major and well accepted determinants of electrical remodeling in heart failure include prolongation of the cardiac action potential (AP) and an increased sarcoplasmic reticulum (SR) Ca-leak occurring as spontaneous SR Ca-release events [1]. Both arrhythmic triggers are known to decrease the threshold of cardiac arrhythmias. A persistent Na current, also known as late Na current (I_{NaL}), has been discussed to be a potent contributor to the progression and complications of heart failure [2–10].

Under physiologic conditions Na channels open transiently and are quickly inactivated thereby generating the peak Na current (peak I_{Na}) which is responsible for the upstroke of the cardiac AP. However, some Na channels close and reopen or remain active, carrying I_{NaL} which persists throughout the whole AP. Although the amplitude of this current is very small compared to peak I_{Na}, the long persistence and its slow inactivation kinetics make this current substantial when elevated as it has been shown in pathological conditions like heart failure, ischemic metabolites, hypoxia, or pathological redox-signaling [6,9,11–13].

I_{NaL} is regarded as a relevant contributor to arrhythmias and its inhibition might be of antiarrhythmic potential (for review see [8,14]). As I_{NaL} generates Na influx and thereby an inward current throughout the AP, it is expected to contribute to the prolongation of the cardiac AP making early afterdepolarizations (EADs) more likely to occur [7,15]. Moreover, cellular Na cycling is tightly integrated with Ca homeostasis, as Na modulates the transport direction of the Na/Ca exchanger (NCX). Cellular Na overload as well as AP-prolongation stimulate the reverse mode NCX facilitating the efflux of Na in exchange for the influx of Ca

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[1]. The net result is an elevated diastolic Ca concentration. Additionally, the cardiac ryanodine receptor (RyR2) was shown to be "leaky" in heart failure. Spontaneous SR Ca-release events, the so called Ca-sparks, occur [1,16] and contribute to the elevation of diastolic Ca-levels. Ca, once released by spontaneously opening RyR2s, can be eliminated via the NCX, which possibly generates a depolarizing current (transient inward current, I_{TI}) that gives rise to delayed afterdepolarizations (DADs) [7,9,17]. Thus, an elevation of diastolic $[Ca]_i$ and changes of the cardiac RyR2 open propensity are arrhythmogenic mechanisms in failing ventricular cardiomyocytes.

There is strong evidence that I_{NaL} is increased in animal models of heart failure as well as in human heart failure [5,9,11,12,15]. However, very little is known about the role and impact of I_{NaL} in isolated cardiac hypertrophy with preserved contractility as it commonly occurs in patients suffering from hypertensive heart disease as well as from aortic stenosis. Therefore one objective of the present study was to investigate changes in I_{NaL} in the course from pressure-induced hypertrophy with preserved contractility to heart failure.

Moreover, there is controversy regarding the quantitative contribution of an increased I_{NaL} to cause AP prolongation in heart failure, and even more so in hypertrophy. As I_{NaL} induces Na-dependent Ca-overload, the impact of I_{NaL} on DADs remains to be elucidated in a pathophysiologically relevant animal model in the absence of pharmacologic I_{NaL} inducers. Therefore, the second objective of our study was to explore the role of I_{NaL} as a contributor to AP prolongation and its consequences for generation of EADs or DADs in isolated hypertrophy with preserved contractility and in heart failure.

Furthermore, we were also interested in the underlying mechanism and possible pharmacological treatment.

2. Methods

An expanded Methods section is available in the Online Data Supplement.

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996) and was approved by a local ethics review board and by the Veterinary Institute of the Lower Saxony State Office for Consumer Protection and Food Safety (G10/220).

2.1. Transverse aortic constriction (TAC) and echocardiography

8 weeks old female C57/BL6J mice were anesthetized using intraperitoneal injections of ketamine and xylazine (100 mg/kg + 5 mg/kg) and pressure overload was induced by transversal aortic contstriction (27G needle). For analgesia (metamizole 1.33 mg/mL) was added to the drinking water 2 days before surgery and continued for 7 days after operation.

Transthoracic echocardiography was performed blinded using a Vevo2100 (VisualSonics, Toronto, Canada) system with a 30 MHz center frequency transducer. The animals were anesthetized with 3% isoflurane, and temperature-, respiration-, and ECG-controlled anesthesia was maintained with 1.5% isoflurane. Maximal left ventricular length (L), thicknesses of the septum, the posterior myocardial wall, the inner diameter of the left ventricle (LVEDD) and the area of the left ventricular cavity (Area) were measured according to standard procedures. The ejection fraction (EF) was calculated using the area-length method.

After completion of the experiments mice were killed in isofluran anesthesia (5%) by cervical dislocation.

2.2. Cell isolation

The excised hearts were mounted on a Langendorff perfusion apparatus and were retrogradely perfused. Cardiomyocytes were isolated with liberase 1 (Roche Diagnostics GmbH, Mannheim, Germany) and trypsin 0.6% digestion and were plated onto superfusion chambers. The glass inlays had been pretreated with laminin to allow cell adhesion and were then used for immediate measurements.

2.3. Patch-clamp experiments

Ruptured-patch whole-cell voltage- and current-clamp was used to measure action potentials and I_{NaL} as described previously [18,19]. Measurements were performed at increasing stimulation frequencies to elicit Na currents or action potentials (APs).

For Na current measurements myocytes were held at −120 mV and I_{NaL} was elicited using 250 ms depolarizing pulses to −20 mV. Each pulse was preceded by a 5 ms pre-pulse to +50 mV in order to optimize voltage control. The measured currents were normalized to the membrane capacitance. I_{Na} decay (first 200 ms) was fitted using a double exponential function $y(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + y_0$ as it was done previously [5,18,19].

For action potential recordings, low-resistance pipettes were used. Resting cell membrane potentials were similar in WT (−65 ± 0.94 mV), TAC (compensated hypertrophy) (−64.86 ± 0.63 mV) and in TAC (heart failure) (−64.94 ± 0.77 mV) ventricular myocytes. All patch-clamp experiments were conducted at room temperature.

2.4. Confocal microscopy

Cardiomyocytes were incubated with a Fluo-3 AM loading buffer. Experimental solution contained (mmol/L): NaCl 136, KCl 4, NaH_2PO_4 0.33, $NaHCO_3$ 4, $CaCl_2$ 2, $MgCl_2$ 1.6, HEPES 10, glucose 10 (pH 7.4, NaOH, room temperature) as well as 10^{-8} mol/L isoproterenol and the respective drugs. Cardiomyocytes were continuously superfused during experiments after washing out the loading buffer and any extracellular dye. Ca-spark measurements were performed with a laser scanning confocal microscope (LSM 5 Pascal, Zeiss, Jena, Germany) using a 40× oil-immersion objective. Fluo-3 was excited by an argon ion laser (488 nm) and emitted fluorescence was collected through a 505 nm long-pass emission filter. Fluorescence images were recorded in the line-scan mode with 512 pixels per line (width of each scanline: 38.4 μm) and a pixel time of 0.64 μs. One image consists of 10,000 unidirectional line scans, equating to a measurement period of 7.68 s. Experiments were conducted at resting conditions after loading the SR with Ca by repetitive field stimulation at 4 Hz. Ca-sparks were analyzed with the program SparkMaster for Image. The mean spark frequency of the respective cell (CaSpF) resulted from the number of sparks normalized to cell width and scan rate ($100\ \mu m^{-1} * s^{-1}$). Spark size (CaSpS) was calculated as product of spark amplitude (F/F0), duration and width. From this, we inferred the average leak per cell by multiplication of CaSpS with CaSpF.

2.5. Drugs

10 μmol/L Ran ([+]N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)-propyl]-1-piperazine acetamide dihydrochloride]) was used because it is within the range of therapeutic plasma levels and inhibitory concentrations of 50% for inhibition of I_{NaL} (6 to 15 μmol/L), which does not significantly inhibit I_{Ca}, $I_{Na/Ca}$, or I_{Ks} [10]. Tetrodotoxin (TTX) selectively blocks I_{Na} and at low concentrations relatively selective I_{NaL} [15]. Thus, we used TTX concentrations of 2 μmol/L. Autocamide-2-related inhibitory peptide (AIP, 1 μmol/L) was used to selectively inhibit Ca/calmodulin-dependent protein kinase II (CaMKII). Isoproterenol was used at a concentration of 10^{-8} mol/L.

2.6. Western blots

Myocardium was homogenized and protein concentration was determined by BCA assay (Pierce Biotechnology, Rockford, USA). Denatured tissue homogenates (on ice in 2% beta-mercaptoethanol) were