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Original article

Ranolazine decreases diastolic calcium accumulation caused by ATX-II or ischemia in rat hearts

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Abstract

Cardiac pathologies are associated with increased late I_{Na} that contributes to the dysregulation of ion homeostasis and causes electrical and contractile dysfunction. This study was designed to test the hypothesis that an increased late sodium channel current (I_{Na}) leads to Ca^{2+} overload and left ventricular (LV) dysfunction, and thereby inhibition of late I_{Na} (e.g., by ranolazine) improves Ca^{2+} homeostasis and reduces LV dysfunction. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) and LV function were measured simultaneously in rat isolated perfused hearts. Augmentation of late I_{Na} with sea anemone toxin-II (ATX-II, 12 nM) increased diastolic $[Ca^{2+}]_i$ ($d[Ca^{2+}]_i$), and impaired LV mechanical function, but had no effect on $[Ca^{2+}]_i$ transient amplitude. Although ranolazine (4 and 9 μM), an inhibitor of late I_{Na}, had no direct effects on $d[Ca^{2+}]_i$ or LV function, it significantly reduced the deleterious effects of ATX-II. Global ischemia increased $d[Ca^{2+}]_i$ and inhibited Ca^{2+} transient amplitude. During reperfusion, Ca^{2+} transient amplitude recovered fully, but $d[Ca^{2+}]_i$ remained elevated and LV function was depressed, indicative of Ca^{2+} overload. Ranolazine (9 μM) reduced $d[Ca^{2+}]_i$ accumulation during ischemia as well as reperfusion and improved recovery of LV function. These results show that augmentation of late I_{Na} with ATX-II or by ischemia is associated with diastolic Ca^{2+} overload and LV dysfunction. The beneficial effects of ranolazine in reducing Ca^{2+} overload and LV mechanical dysfunction during ischemia/reperfusion is consistent with the inhibition of late I_{Na} mechanism of action.

Keywords: LV function; Ca^{2+} overload; Voltage-gated sodium channels; Ischemia; Ranolazine

1. Introduction

Voltage-gated sodium channels (VGSCs) play a fundamental role in the propagation of action potentials in the myocardium. VGSC activation is triggered by membrane depolarization and results in a rapid influx of Na^+ leading to further depolarization, Ca^{2+} entry and the initiation of excitation-contraction coupling. Under normal conditions, once activated, VGSCs rapidly inactivate (within a few ms); hence, influx of Na^+ is transient [1]. The resulting increase in the intracellular concentration of Na^+ ($[Na^+]_i$) is small and Na^+ homeostasis can be restored by activity of the Na^+-K^+-ATPase [1].

The VGSC inactivation process is slowed and/or is incomplete under certain conditions, causing a sustained/persistent influx of Na^+, herein referred to as late I_{Na} [2–4]. Variant 3 of the long QT syndrome (LQT3) is caused by mutations in the cardiac VGSC gene *SCN5A* [3] that destabilize the I_{Na} inactivation process and cause an increase in late I_{Na}. Likewise, phosphorylation of cardiac VGSCs by stress-activated kinases is also associated with increased late I_{Na} [5]. An increase in late I_{Na} may increase action potential duration (APD), APD dispersion and induce early after depolarizations (EADs) that may lead to the development of life-threatening polymorphic ventricular tachycardias such as torsade de pointes [6]. Moreover, the increase in $[Na^+]_i$ and resultant extrusion of the excess Na^+ ions via reverse-mode Na^+-Ca^{2+} exchange (NCX) may cause simultaneous Ca^{2+} entry and may cause Ca^{2+} overload and subsequent mechanical dysfunction [4,7–10].

VGSCs may contribute to hypoxia-induced Na^+-dependent Ca^{2+} loading after cells become unexcitable [8], and augmentation of late I_{Na} is associated with pathological conditions such

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as heart failure [11,12] and hypoxia [13,14], and with exposure of the myocardium to reactive oxygen species [15,16], ischemic metabolites [17,18] or glycolytic intermediates [19].

Despite accumulating evidence for a beneficial effect of inhibition of late I_{Na} and Na^+-dependent Ca^{2+} overload in the treatment of myocardial ischemia [20], evidence for the involvement of late I_{Na} in intracellular Ca^{2+} accumulation and LV mechanical dysfunction at the whole heart level is lacking. Therefore, an aim of the present study was to investigate the role of late I_{Na} on Ca^{2+} accumulation and LV function in intact heart.

The potential role of late I_{Na} in ischemia-related cardiac pathologies is supported by studies with ranolazine, a compound recently approved for the chronic treatment of angina pectoris. Ranolazine reduces the severity of angina pectoris at plasma concentrations that cause no or minimal changes in cardiac function or systemic hemodynamics [21,22]. It is approximately 38-fold more potent at inhibiting late I_{Na} than inhibiting peak I_{Na} in ventricular myocytes from canine failing hearts [23]. Consistent with these findings, ranolazine has been shown to suppress EADs and cardiac arrhythmias in guinea pig and rabbit models of long-QT syndrome [24,25], but it has yet to be demonstrated that ranolazine will limit Ca^{2+} accumulation in the heart and thereby reduce Ca^{2+} overload and LV dysfunction when late I_{Na} is enhanced.

The studies reported here used isolated ejecting/working hearts from rats to test the following hypotheses: (1) the sea anemone toxin ATX-II, known to increase, selectively, late I_{Na}, causes Ca^{2+} accumulation and LV dysfunction; (2) ranolazine, known to inhibit late I_{Na}, reduces Ca^{2+} accumulation and limits LV dysfunction caused by ATX-II; and (3) ischemia-induced Ca^{2+} accumulation and LV dysfunction, which may in part be due to an increase in late I_{Na}, is reduced by ranolazine.

2. Materials and methods

2.1. Heart perfusions

Male Sprague–Dawley rats (300–400 g) were anesthetized with pentobarbital (150 mg/kg, i.p.) according to the University of Alberta Animal Policy and Welfare Committee and the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996). Each heart was rapidly removed, the aorta was cannulated and a non-working (Langendorff) perfusion was promptly (within 30 s) initiated with Krebs–Henseleit (KH) solution. After 10 min of Langendorff perfusion, working mode perfusion was initiated as described previously [26] and hearts were paced at 5 Hz. The perfusate (recirculating volume of 100 mL, 37 °C, pH 7.4, gassed with a 95% O_2–5% CO_2 mixture) consisted of a modified KH solution containing the following (in mM): KCl (4.7), NaCl (118), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $CaCl_2$ (2.5), $NaHCO_3$ (25), glucose (11), palmitate (1.2) and insulin 100 mU/L. Palmitate was pre-bound to bovine serum albumin (3%). Perfusions were performed at a constant workload (preload, 11.5 mm Hg; afterload, 80 mm Hg).

2.2. Measurement of LV mechanical function

Heart rate, systolic and diastolic aortic pressures (mm Hg), cardiac output (mL/min) and aortic flow (mL/min) were acquired digitally using Chart V5.0 software (AD Instruments, Colorado Springs, CO). LV minute work (L/min/mm Hg), calculated as cardiac output × LV developed pressure served as a continuous index of LV function. Coronary flow (mL/min) was calculated as the difference between cardiac output and aortic flow. CVC (mL/min/mm Hg) was calculated as coronary flow/aortic diastolic pressure.

2.3. Measurement of intracellular Ca^{2+}

Hearts were loaded with the fluorescent Ca^{2+} indicator, indo-1AM (5 μM) and indo-1 fluorescence was measured from the epicardial surface of a ~0.3 cm^2 area of the LV-free wall using a spectrofluorometer (Photon Technology International, London, Ontario, Canada) fitted with a bifurcated fiber optic cable containing both excitation (354 nm) and emission bundles [27]. Signals were acquired at 500 Hz and the ratio of indo-1 fluorescence emitted at 405 nm and 485 nm was calculated to provide an index of intracellular $d[Ca^{2+}]_i$ and $s[Ca^{2+}]_i$. Ca^{2+} transient amplitude was calculated as $s[Ca^{2+}]_i$ minus $d[Ca^{2+}]_i$.

2.4. Activation and inhibition of late I_{Na}

To increase late I_{Na}, the sea anemone toxin II (ATX-II) was added to the recirculating perfusate of aerobic hearts to inhibit selectively Na^+ channel inactivation [28,29]. After 10 min of ATX-II exposure, hearts were treated with either vehicle (DMSO final concentration ≤0.25%, $n=11$), 4 μM ($n=9$) or 9 μM ranolazine ($n=9$). In other experiments, hearts ($n=4$) were treated with 9 μM ranolazine to determine the direct effects of ranolazine on $[Ca^{2+}]_i$ and LV function. Thereafter, the effects of ATX-II in ranolazine-pretreated hearts ($n=4$) were measured and compared to the effects of ATX-II in untreated hearts ($n=11$).

2.5. Ischemia-reperfusion (IR) protocol

This comprised a 15-min period of baseline aerobic perfusion (hearts paced at 5 Hz), 20 min of global, no-flow ischemia (no pacing), followed by 30 min of aerobic reperfusion (pacing at 5 Hz re-attempted within 2 min of reperfusion). One group of hearts was exposed to vehicle alone (DMSO final concentration ≤0.25%, $n=9$) whereas a second group was pretreated (5 min prior to the onset of ischemia) with ranolazine (9 μM, $n=6$).

2.6. Sources of drugs

Ranolazine 1-piperazineacetamide, *N*-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-, (±)-, Lot # E4-NE-002, from CV Therapeutics Inc. was dissolved in DMSO. Concentrations of ranolazine in perfusate samples were analyzed by CV Therapeutics using LC/MS/MS. ATX-II (Lot # AT-05) was purchased from Alomone Labs (Jerusalem, Israel) and was