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NHE-1 blockade reversed changes in calcium transient in myocardial slices from isoproterenol-induced hypertrophied rat left ventricle

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ABSTRACT

We previously reported that left ventricular (LV) slices from isoproterenol (ISO)-induced hypertrophied rat hearts showed an increase of energy expenditure due to remodeling of Ca^{2+} handling in excitation–contraction coupling, i.e., suppressed SERCA2a activity and enhanced Na^+/Ca^{2+}exchanger-1 (NCX-1) activity. Na^+/H^+ exchanger-1 (NHE-1) inhibitor (NHEI) has been demonstrated to exert beneficial effects in the development of cardiac remodeling. We hypothesized that a novel NHE-1 selective inhibitor, BIIB723 prevents remodeling of Ca^{2+} handling in LV slices of ISO-induced hypertrophied rat hearts mediated by inhibiting NCX-1 activity. The significant shortening in duration of multi-cellular Ca^{2+} transient in ISO group was normalized in ISO + BIIB723 group. The significant increase in amplitude of multi-cellular Ca^{2+} waves (CaW) generated at high $[Ca^{2+}]_o$ of LV slices in ISO group was also normalized in ISO + BIIB723 group. However, the enhanced NCX-1 activity was not antagonized by BIIB723. We recently reported that ISO-induced down-regulation of a Ca^{2+} handling protein, SERCA2a, was normalized by BIIB723. Therefore, it seems likely that BIIB723 normalized shortened multi-cellular Ca^{2+} transient duration and increased CaW amplitude in LV slices mediated via normalization of SERCA2a activity. Furthermore, the results presented here suggest the multi-cellular Ca^{2+} transient duration and CaW amplitude in LV slices might be better indices reflecting SERCA2a activity than SERCA2a protein expression level.

1. Introduction

Na^+/H^+ exchanger (NHE) is an integral membrane glycoprotein, plays a key role in maintaining intracellular pH and Na^+ concentration and cellular volume. Recently, the link between cardiac NHE-1 activity and myocardial hypertrophy has been clearly established in elevated sympathetic nerve activity models. Long-term isoproterenol (ISO)-induced cardiac hypertrophy was prevented and NHE-1 protein expression was normalized by the inhibition of NHE-1 in rats [1]. NHE-1 also contributes to ISO-induced abnormal Ca^{2+} handling associated with cardiac hypertrophy. Inhibition of NHE-1 ameliorates cardiac Ca^{2+} handling impairment by up-regulation of SERCA2a protein expression and prevents the development of cardiac dysfunction in ISO-infused rats [2]. Furthermore, the development of cardiac hypertrophy and fibrosis associated with increased NHE-1 protein expression in $ß_1$-adrenergic receptor transgenic mice was prevented by NHE-1 inhibition [3].

Myocardial oxygen consumption related with the total Ca^{2+} handling in excitation–contraction (E–C) coupling was increased in the left ventricular (LV) myocardial slice of ISO-induced hypertrophied rat heart [4]. Molecular and cellular physiological studies demonstrated that this increase was caused by functional increase in Na^+–Ca^{2+} exchange (NCX1) activity [4]. This functional increase seems to be induced by attenuation of the intrinsic inactivation mechanisms associated with functional depression of SERCA2 induced by lower protein expression of phospho-Ser^{16} PLB and SERCA2. On the other hand, LV myocardial oxygen consumption related with the total Ca^{2+} handling in E–C coupling was not increased in ISO-induced hypertrophied rat whole heart preparation perfused with blood at a lower rate, 240 bpm pacing [5]. Lowering the heart rate enabled ISO-induced hypertrophied rat hearts to exert normal myocardial oxygen consumption related with the total Ca^{2+} handling in E–C coupling.

However, whether changes in NCX1 activity and NHE-1 protein contribute to real Ca^{2+} handling in E–C coupling, i.e., Ca^{2+}-transient of myocardial slices of ISO-induced hypertrophied rat heart LV, are not analyzed yet. In the present study, to clarify this, a new

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approach for evaluating multi-cellular Ca^{2+}-transients and Ca^{2+} waves (CaW) measured at multiple points of each LV myocardial slice is performed to compare between ISO-induced hypertrophied and normal rat hearts.

2. Methods and materials

Experimental procedures followed the regulations of and were approved by the animal care and use committee of Nara Medical University.

2.1. Animals and drug infusion

Male Wistar rats weighing 250–370 g (8–10 weeks) were randomly divided into Vehicle (SA), NHEI, and ISO groups without or with pretreatment with NHEI. Delivery of drug was achieved by implanting an osmotic minipump (model 1003D, Alzet, Durect Corp, Cupertino, CA) subcutaneously in the neck under pentobarbital (50 mg/kg i.p.) anesthesia. Either ISO (2.4 mg kg^{-1} day^{-1} for 7 days) or vehicle (0.1% ascorbic acid in saline 2.4 μl/day for 7 days) was infused subcutaneously [5,6]. Rats were received an NHE-1 inhibitor, BIIB723 (Boehringer-Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA, 3.0 mg kg^{-1} day^{-1}) in drinking water 3 days before the start of ISO infusion [2].

2.2. Animals and left ventricular myocardial slice preparation

Male Wistar rats (n = 98) were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and osmotic mini-pump was removed from the neck 7 days after the implantation. The heart and its slices were prepared as described before [7–10]. The whole heart was excised under perfusion with Tyrode solution oxygenated with 100% O_2 at 13 °C for 5 min. The composition of Tyrode solution (in mM) was 136.0 NaCl, 5.4 KCl, 1.0 $MgCl_2$, 0.3 NaH_2PO_4, 1.8 $CaCl_2$, 10.0 glucose, and 5.0 HEPES, with pH adjusted to 7.4 with NaOH at 30 °C. After the perfusion, both atria, the four valves including the connective tissue, the aorta and the pulmonary artery were removed from the heart. The heart was longitudinally cut into two or three pieces and each of the pieces was cut into 300 μm thick slices in parallel with the epicardium with a microslicer DTK-3000 (Dosaka EM, Kyoto, Japan). The slices with the thickness (300 μm) we chose seem to be sufficiently oxygenated by diffusion, according to the published data and calculation [10]. This cut was chosen so that most fibers were parallel-sectioned to fiber orientation according to the known fiber architecture of the heart wall. We obtained 12–20 slices (average single-side surface area: approximately 30 mm^2) from each LV.

2.3. Ca^{2+} imaging

The slices were stored in Tyrode solution oxygenated with 100% O_2 at 18 °C for 30 min. LV slices were incubated for 2–4 h at room temperature in Tyrode solution containing 10 μM fluo-3 acetoxymethyl ester (Dojindo, Kumamoto, Japan) and detergents (0.02% Pluronic F-127, Dojindo and 0.02% Cremophor EL, Sigma), after which changes in $[Ca^{2+}]_i$ were monitored using a digital imaging system (AQUACOSMOS, Hamamatsu Photonics, Shizuoka, Japan) mounted on an inverted microscope as previously reported [11]. Consequently, acute effects of isoproterenol were removed before starting experiments. The fluo-3-containing LV slices were illuminated at 488 nm, and the intensity of the fluorescent emission from the indicator at 515–565 nm was recorded. Digital Ca^{2+} images (527 × 511 pixels) were normally collected at 8-ms intervals, and the intensity of the fluorescence at a given time (F_t) was usually normalized to the fluorescence intensity at the start (F_0), yielding relative values representative of the integrated $[Ca^{2+}]_i$ recorded at 2–5 points in a single slice during 25–30 stimuli. Stimulation consisted of 1-Hz (low frequency) rectangular pulses, 10 ms in duration and current of 1 mA (voltage: 1 V). Tyrode solutions were oxygenated with 100% O_2 and preheated to 40 °C in a water bath. Slices without any mechanical load were placed into the dish superfused with the prepared Tyrode solution and fixed with a pair of the wire stimulation electrodes. The dish was warmed with a Microwarm Plate DC-MP10DM (Kitazato, Tokyo, Japan) at 36 °C.

2.4. Drugs

BIIB723, a gift from Boehringer-Ingelheim Pharmaceuticals, Inc. (Ridgefield, CT, USA) was used as an NHE-1 inhibitor, cyclopiazonic acid (CPA) (Sigma; St. Louis, MO) was used as a SERCA2 inhibitor [8,9], or SEA0400 (2-[4-[(2,5-difluorophenyl) methoxy] phenoxy]-5-ethoxyaniline), a gift from Taisho Pharmaceutical Co. Ltd. (Saitama, Japan), was used as a bidirectional-mode NCX 1 inhibitor [12–14].

2.5. Myocytes study

2.5.1. Cell isolation

The rats (n = 14) were deeply anesthetized by intraperitoneal injection of pentobarbital sodium (>0.1 mg/g body weight). LV myocytes were dissociated from SA (n = 10 myocytes from 4 rats) and ISO group hearts without (n = 18 myocyctes from 5 rats) or with pretreatment with NHEI (n = 13 myocytes from 5 rats) as described in previous studies [15]. The dissociated myocytes were kept in a HEPES-buffered DMEM solution (ICN Biomedicals) and used for the patch clamp and fluorescence studies within 8 h. All procedures were approved by the Animal Research Committee of the Graduate School of Medicine, Kyoto University.

2.5.2. Solutions

The standard pipette solution contained (in mM) 30 CsOH, 40 aspartate, 20 TEACl, 40 EGTA, 5 MgATP, 33.8 $CaCl_2$, 1.19 $MgCl_2$, 10 HEPES, 50 NaOH (pH = 7.2 with CsOH). Free Ca^{2+} concentration was calculated to be 0.8 mM. Composition of bath solution for recording NCX current (I_{NCX}) was 145 NaCl, 2 $BaCl_2$, 3 $MgCl_2$, 5 HEPES, 0.2 EGTA (or 0 when adding 2 $CaCl_2$), 0.05 mM ouabain and 0.002 nicardipine (pH = 7.4 with NaOH) according to a previous study [16].

2.5.3. Electrophysiology

The myocytes were voltage clamped using the whole cell method with an Axopatch 200B amplifier (Axon Instruments). Holding potential was −40 mV. Current–voltage (I–V) relationships were measured by applying ramp pulses and normalized by membrane capacitance. I_{NCX} was induced by applying 2 mM Ca^{2+} for 10 s at intervals of 30 s and determined as the difference current in the same manner as described in our previous studies [15,16].

2.6. Statistics

All data were presented as mean ± SD. Differences between two mean values were evaluated by paired or non-paired Student's t-test. Multiple comparisons were performed by one-way ANOVA and Bonferroni's t-test or Dunnett's t-test, or two-way ANOVA. In all statistical tests, P values less than 0.05 were considered statistically significant.