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Fluvoxamine rescues mitochondrial Ca^{2+} transport and ATP production through σ_1-receptor in hypertrophic cardiomyocytes

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ABSTRACT

Aims: We previously reported that fluvoxamine, a selective serotonin reuptake inhibitor with high affinity for the σ_1-receptor (σ_1R), ameliorates cardiac hypertrophy and dysfunction via σ_1R stimulation. Although σ_1R on non-cardiomyocytes interacts with the IP_3 receptor (IP_3R) to promote mitochondrial Ca^{2+} transport, little is known about its physiological and pathological relevance in cardiomyocytes.
Main methods: Here we performed Ca^{2+} imaging and measured ATP production to define the role of σ_1Rs in regulating sarcoplasmic reticulum (SR)-mitochondrial Ca^{2+} transport in neonatal rat ventricular cardiomyocytes treated with angiotensin II to promote hypertrophy.
Key finding: These cardiomyocytes exhibited imbalances in expression levels of σ_1R and IP_3R and impairments in both phenylephrine-induced mitochondrial Ca^{2+} mobilization from the SR and ATP production. Interestingly, σ_1R stimulation with fluvoxamine rescued impaired mitochondrial Ca^{2+} mobilization and ATP production, an effect abolished by treatment of cells with the σ_1R antagonist, NE-100. Under physiological conditions, fluvoxamine stimulation of σ_1Rs suppressed intracellular Ca^{2+} mobilization through IP_3Rs and ryanodine receptors (RyRs). In vivo, chronic administration of fluvoxamine to TAC mice also rescued impaired ATP production.
Significance: These results suggest that σ_1R stimulation with fluvoxamine promotes SR-mitochondrial Ca^{2+} transport and mitochondrial ATP production, whereas σ_1R stimulation suppresses intracellular Ca^{2+} overload through IP_3Rs and RyRs. These mechanisms likely underlie in part the anti-hypertrophic and cardioprotective action of the σ_1R agonists including fluvoxamine.

Introduction

Recently, we reported relatively high expression of σ_1Rs in rat heart compared to brain and aortic tissues (Bhuiyan et al., 2010a). Others report in vivo studies indicating that σ_1R expression in the heart is upregulated by stress stimuli such as immobilization and hypoxia (Novakova et al., 2007). Interestingly, we noted markedly reduced σ_1R levels in the left ventricle following progression of the left ventricular hypertrophy and heart failure in transverse aortic constriction (TAC) mice (Tagashira et al., 2010). More importantly, there is a significant positive correlation between reduced σ_1R expression in the heart and impaired cardiac function, as assessed by fractional shortening (Tagashira et al., 2010). Furthermore, treatment with a different σ_1R agonist, the neurosteroid dehydroepiandrosterone (DHEA), antagonized pressure overload (PO)-induced decreases in σ_1R expression in rat heart (Bhuiyan and Fukunaga, 2009) and thoracic arteries (Bhuiyan et al., 2010b, 2011). However, the functional consequences of reduced σ_1R expression seen in hypertrophic cardiomyocytes remain unclear.

In non-cardiomyocytes, σ_1R localizes to the mitochondria-associated ER membrane (MAM), and its interaction with the IP_3 receptor (IP_3R) promotes Ca^{2+} transport into mitochondria from the ER (Hayashi and Su, 2007). The type 2 IP_3R (IP_3R2) is the most prominent isoform in the heart (Bare et al., 2005; Li et al., 2005). We recently documented that selective σ_1R agonist SA4503 rescues the IP_3R-mediated Ca^{2+} transport into mitochondria and enhances mitochondrial ATP production in Ang II-induced hypertrophic cardiomyocytes (Tagashira et al., 2013). However, it is unclear whether fluvoxamine elicits the comparable effects with SA5403 on IP_3R-mediated Ca^{2+} transport into mitochondria in normal and hypertrophic cardiomyocyte.

Dysregulation of IP_3Rs, ryanodine receptors (RyRs) and transient potential channels (TRPCs) also functions in the development of hypertrophy through Ca^{2+} overload and/or leakage through both the plasma and SR membranes (Nakayama et al., 2010; Zou et al., 2011; Heinzel et al., 2011; Eder and Molkentin, 2011). For example, prolonged stimulation of α_1 adrenergic receptors or Ang II receptors activates TRPCs through diacyl glycerol (DG) and/or oxidative stresses (Onohara et al., 2006; Mohl et al., 2011). TRPC upregulation is

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essential for the development of hypertrophy (Niizeki et al., 2008). Cardiomyocytes from RyR-R176Q knock-in mice, which mutation increases catecholamine-induced SR Ca^{2+} release in ventricular myocytes (Kannankeril et al., 2006), show increased Ca^{2+} leakage from the SR after TAC, leading cardiac hypertrophy (van Oort et al., 2010). However, the interaction between σ_1R and RyRs remains unclear.

Therefore, we addressed the regulatory roles of fluvoxamine on IP_3R-mediated Ca^{2+} transport into mitochondria and ATP production in normal and Ang II-induced hypertrophic cardiomyocytes. We also defined a functional interaction of σ_1R with RyRs in intracellular Ca^{2+} mobilization in neonatal rat ventricular cardiomyocytes (NRVMs). We confirmed that σ_1R stimulation by fluvoxamine rescues Ang II-induced impairment of SR-mitochondrial Ca^{2+} transport and mitochondrial ATP production. σ_1R stimulation by fluvoxamine under physiological conditions in normal cardiomyocytes suppressed intracellular Ca^{2+} mobilization through IP_3Rs and RyRs. Furthermore, in cultured hypertrophic NRVMs and in vivo in TAC-induced hypertrophied mice, fluvoxamine stimulated σ_1R-mediated mitochondrial Ca^{2+} transport and ATP production through the IP_3R.

Materials and methods

Materials

Reagents and antibodies were obtained from the following sources: anti-σ_1 receptor antibody (a kind gift of Dr. Teruo Hayashi, National Institute on Drug Abuse, National Institutes of Health, Bethesda, Maryland, (Hayashi and Su, 2007)); anti-IP_3 receptor type 2 (American Research Products Inc. Waltham, MA); anti-ryanodine receptor (Abcam, Cambridge, UK); and anti-β-tubulin antibody (Sigma, St. Louis, MO). Fluvoxamine maleate and NE-100 (N, N-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride) were obtained from Sigma-Aldrich (Tokyo, Japan) and Santa Cruz (Santa Cruz, USA), respectively. Other reagents were of the highest quality available (Wako Pure Chemicals, Osaka, Japan).

Animals and operations

All procedures for handling animals complied with the *Guide for Care and Use of Laboratory Animals* and were approved by the Animal Experimentation Committee of Tohoku University Graduate School of Pharmaceutical Sciences. Adult male ICR mice weighing 35 to 40 g were obtained from Nippon SLC (Hamamatsu, Japan). Ten-week-old males were acclimated to the local environment for 1 week, which included housing in polypropylene cages at 23 ± 1 °C in a humidity-controlled environment maintained on a 12-h light/dark schedule (lights on 8:00 AM–8:00 PM). Mice were provided food and water ad libitum. Transverse aortic constriction (TAC) was performed as previously described (Tagashira et al., 2010) on male ICR mice under anesthesia using a mixture of ketamine (100 mg/kg, i.p.) (Daiichi Sankyo Pharmaceutical Co. Ltd, Tokyo, Japan) and xylazine (5 mg/kg, i.p.) (Sigma, St. Louis, MO). Adequate depth of anesthesia was confirmed by a negative toe-pinch reflex. If anesthesia was not sufficient, a top-up dose of 20% of the initial dose was given. Fluvoxamine (1.0 mg/kg) was administrated during the 4 weeks until sampling from the heart.

Preparation of primary cultures and treatments

Neonatal rat ventricular cardiomyocytes were isolated from hearts of 1- to 3-day-old Wistar rats that had been sacrificed by decapitation, and cardiomyocytes were cultured as described (Tagashira et al., 2010). Briefly, rat pups were decapitated and their hearts were removed immediately. Ventricles were separated from the heart and washed in Hank's balanced salt solution (137.0 mM NaCl, 5.4 mM KCl, 0.4 mM KH_2PO_4, 0.3 mM $Na_2HPO_4 \cdot 12H_2O$, 6.12 mM glucose, 4.2 mM $NaHCO_3$), and myocytes were dissociated from the ventricles by serial digestion with 0.1% trypsin and 0.05% DNase I in the same solution. After each digestion, dissociated cardiomyocytes were collected and suspended in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS) and 0.02% trypsin. Cells were collected by centrifugation (4 °C, 1000 ×*g* for 10 min). After the supernatant was removed, DMEM containing 10% FBS was added. Cells were gently agitated and then plated on uncoated 90-mm culture dishes. Plates were allowed to stand for 90 min at 37 °C in a CO_2 incubator to remove non-myocytes, which is attached to the culture plates. Unattached myocytes were collected and plated at a density of 1–2 × 10^6 cells per 5-mm dish in 10% FBS in DMEM. Cells were cultured in serum-free DMEM before treatment with angiotensin II (Ang II) (100 nM). Fluvoxamine (5 μM) was treated during the last 24 h.

σ_1 receptor knockdown

To silence σ_1 receptor expression, we transfected NRVMs with σ_1 receptor siRNA (sense, 5′-ACACGTGGATGGTGGAGTA-3′ and anti-sense, 5′-TACTCCACCATCCACGTGT-3′) (Exgen Ltd., Tokyo, Japan) using methods described previously (Tagashira et al., 2010). Cultured myocytes were transfected with 100 nM σ_1 receptor siRNA using Lipofectamine 2000 (Invitrogen) in opti-MEM (Invitrogen) media. Cultured cells were washed with cold PBS and stored at −80 °C until western blot analysis was performed.

Morphological analysis and immunocytochemistry

Cultured NRVMs were plated on collagen-coated glass slides at a density of 1–2 × 10^6 cells per coverslip of 12 mm diameter. After incubation in the presence or absence of angiotensin II for 72 h, cultured cells were washed 3 times in phosphate-buffered saline (PBS; pH 7.4) and fixed with 4% formaldehyde. For mitochondrial staining, cardiomyocytes were stained for 20 min with 0.02 μM MitoTracker Red CMXRos (Molecular Probes) before fixation with 4% formaldehyde. After permeabilization with 0.1% Triton X-100 in PBS, fixed cells were incubated with 1% bovine serum albumin in PBS for 30 min. For cell size measurement, cardiomyocytes were incubated for 3 h at room temperature with rhodamine-conjugated phalloidin (1:300; Molecular Probes, Eugene, OR) in PBS containing 1% BSA. After cell images were acquired using an Olympus fluorescence microscope, the surface area of cells was quantitated using ImageJ program. All cells from randomly selected fields were examined in each condition (at least 100 myocytes per group). The surface area of control cells was set at 100% and compared with that of treated cells. For immunocytochemistry, cells were incubated 24 h at 4 °C with anti-σ_1 receptor antibody (1:500) and anti-IP3R2 antibody (1:200) in PBS containing 1% BSA. After washing, cells were incubated for 24 h with a species-specific secondary antibody that were detected with either Alexa 594 or 488 in PBS containing 1% BSA. Immunofluorescent images were obtained with a confocal laser scanning microscope (TCS SP, Leica Microsystems).

Western blot analysis

Western blotting of NRVMs was performed as previously described (Lu et al., 2007). After experimental treatment, cultured NRVMs were washed with PBS at 4 °C and stored at −80 °C until immunoblotting analyses were performed (Lu et al., 2007). For assays, each frozen sample was homogenized by methods described (Tagashira et al., 2010; Bhuiyan et al., 2009). An equal amount of protein for each sample was separated on 7.5–15% SDS-polyacrylamide gels and transferred onto polyvinylidene difluoride membranes (Millipore Corporation, Billerica, MA). Membranes were blocked for 1 h in 5% non-fat dried milk in