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Sarcolemmal α2-adrenoceptors control protective cardiomyocyte-delimited sympathoadrenal response

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ABSTRACT

Sustained cardiac adrenergic stimulation has been implicated in the development of heart failure and ventricular dysrhythmia. Conventionally, α2 adrenoceptors (α2-AR) have been assigned to a sympathetic short-loop feedback aimed at attenuating catecholamine release. We have recently revealed the expression of α2-AR in the sarcolemma of cardiomyocytes and identified the ability of α2-AR signaling to suppress spontaneous Ca^{2+} transients through nitric oxide (NO) dependent pathways. Herein, patch-clamp measurements and serine/threonine phosphatase assay revealed that, in isolated rat cardiomyocytes, activation of α2-AR suppressed L-type Ca^{2+} current (I_{CaL}) via stimulation of NO synthesis and protein kinase G- (PKG) dependent activation of phosphatase reactions, counteracting isoproterenol-induced β-adrenergic activation. Under stimulation with norepinephrine (NE), an agonist of β- and α-adrenoceptors, the α2-AR antagonist yohimbine substantially elevated I_{CaL} at NE levels >10 nM. Concomitantly, yohimbine potentiated triggered intracellular Ca^{2+} dynamics and contractility of cardiac papillary muscles. Therefore, in addition to the α2-AR-mediated feedback suppression of sympathetic and adrenal catecholamine release, α2-AR in cardiomyocytes can govern a previously unrecognized local cardiomyocyte-delimited stress-reactive signaling pathway. We suggest that such aberrant α2-AR signaling may contribute to the development of cardiomyopathy under sustained sympathetic drive. Indeed, in cardiomyocytes of spontaneously hypertensive rats (SHR), an established model of cardiac hypertrophy, α2-AR signaling was dramatically reduced despite increased α2-AR mRNA levels compared to normal cardiomyocytes. Thus, targeting α2-AR signaling mechanisms in cardiomyocytes may find implications in medical strategies against maladaptive cardiac remodeling associated with chronic sympathoadrenal stimulation.

1. Introduction

The pilot of cardiac excitation-contraction coupling, the voltage-gated sarcolemmal Ca^{2+} influx, is tightly regulated via activation of the sympathoadrenal system [1,2], and can thereby govern intracellular Ca^{2+} release and overall myocardial performance under various physiological stimuli [3,4]. In particular, under the fight-or-flight response, G-protein-coupled α- and β-adrenoceptors are the main targets for endogenous catecholamines (epinephrine, norepinephrine) that in cardiac myocytes promote the mobilization of diverse cellular processes, including potentiation of L-type Ca^{2+} current (I_{CaL}), aimed at augmenting cardiac output to support the body's requirement for enhanced performance [1,3–5]. However, under chronically elevated physical workload, emotional stress or hypertension hearts undergo sustained sympathetic drive [1,6–8], which is considered a major risk-factor

Abbreviations: 7NI, 7-nitroindazole, NOS inhibitor; 8Br-cGMP, 8-bromoguanosine 3′,5′-cyclic monophosphate, membrane permeable cGMP analog; Akt/PKB, Akt or protein kinase B, serine/threonine-specific protein kinase; DAF-FM, 4-amino-5-methylamino-2′,7′-Difluorofluorescein; eNOS, endothelial NO synthase; I_{CaL}, voltage-gated L-type Ca^{2+} current; NE, norepinephrine; NO, nitric oxide; PDE, phosphodiesterase; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; PP1 and PP2A, protein phosphatases 1 and 2A, respectively; SD, Sprague-Dawley rats; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase; sGC, soluble guanylate cyclase; SHR, spontaneously hypertensive rats; SNAP, S-nitroso-N-acetyl-D,L-penicillamine, NO donor; SNP, Sodium nitroprusside, NO donor; SR, sarcoplasmic reticulum.

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contributing to cardiomyopathy and leading to the development of heart failure [9–12].

In addition to the stimulation by β-adrenoceptor-activated cAMP-dependent protein kinase A (PKA), L-type Ca^{2+} channels can be modulated via targeting of alternative phosphorylation sites by cGMP-dependent protein kinase (PKG) [13]. Although PKG-dependent phosphorylation may activate or inhibit L-type Ca^{2+} channels, in most cases such stimulation of channel activity has been only indirectly linked to PKG-mediated mechanisms [14–16]. Nonetheless, the overall amplitude of Ca^{2+} entry through L-type Ca^{2+} channels depends on a dynamic balance between phosphorylation and dephosphorylation of the channel protein [13,17–19]. In fact, intracellular dialysis of ventricular myocytes with protein phosphatases 1 (PP1) or 2A (PP2A) decreases cytosolic intracellular Ca^{2+} levels indicating a specific role of these serine-threonine phosphatases in regulating L-type Ca^{2+} channels [19–21]. The spatial anchoring of L-type Ca^{2+} channels with the ryanodine receptor, PKA, PP2A and PP1 within a compartmentalized functional unit by scaffolding membrane proteins underscores the orchestration of kinase and phosphatase activities aimed at effectively tuning Ca^{2+} influx, adequate excitation-contraction coupling and cardiac output [22–27]. Discoordination between functional components of such units may eventually lead to fatal arrhythmia, maladaptive cardiac remodeling and heart failure [28].

We have previously identified the expression of all three isoforms of α2-AR ($\alpha2_A$, $\alpha2_B$ and $\alpha2_C$) in the sarcolemma of cardiac myocytes [29]. In these cells, endothelial NO synthase (eNOS) was a central effector of the α2-AR signaling outcome. Specifically, α2-AR through PI_3-kinase stimulated Akt protein kinase (PKB) and NO production that collectively inhibited spontaneous intracellular Ca^{2+} spikes presumably via potentiation of Ca^{2+} re-uptake into the sarcoplasmic reticulum (SR) by Ca^{2+}-ATPase (SERCA) [29]. This indicates that local α2-AR signaling in cardiac myocytes can improve the utilization of intracellular Ca^{2+} and prevent detrimental catecholamine-induced Ca^{2+} overload [30–32]. So far, α2-AR have been assigned a sympathetic short-loop feedback function aimed at attenuating sarcolemmal β-adrenoceptor stimulation resulted from an inhibition of catecholamine release from presynaptic adrenergic nerve terminals and the adrenal medulla [33–36]. A loss of normal synaptic autoinhibitory feedback caused by dysfunction of these adrenoceptors resulted in enhanced presynaptic release of norepinephrine increasing the risk for heart failure [37–39]. However, in transgenic mice lacking $\alpha2_A$ and $\alpha2_C$ adrenoceptors the targeted rescue of α2-AR in sympathetic neurons provided only a weak restoration of left ventricular response to the intravenous infusion of α2-AR agonists [40]. Although this may point towards an alternative localization of these adrenergic receptors in the myocardium, where they can participate in regulation of the local adrenergic response, such scenario has not been reported to date. Therefore, the comprehensive characterization of adrenergic signaling outcome on myocardial functions requires assessment of α2-AR-dependent regulation of I_{CaL} and induced intracellular Ca^{2+} dynamics in isolated cardiac myocytes.

In this study, we investigated the functional role of α2-AR in the sarcolemma of cardiomyocytes by assessing their regulation of I_{CaL}, intracellular Ca^{2+} transients and contractility in response to catecholamine stimulation, and tested whether aberrant α2-AR signaling may be associated with cardiac hypertrophy in spontaneously hypertensive rats (SHR) characterized by persistently elevated catecholamine levels [41].

2. Methods

2.1. Experimental animals and cell isolation

All animal procedures performed in this study with male Sprague-Dawley (SD) rats and SHR (laboratory of experimental animals, Pushchino, Russia) were approved by the Biological Safety and Ethics Committee (Institute of Theoretical and Experimental Biophysics) in accord with Directive 2010/63/EU of the European Parliament and by the Institutional Animal Care and Use Committee (Mayo Clinic) in accord with United States National Institutes of Health guidelines. Experiments were performed with weight-matched 2–2.5-month-old SD rats, and with SD animals matched to corresponding 5–7-month-old SHR. Blood pressure was measured noninvasively using the IN125 NIBP Controller in conjunction with an R-type transducer/tail cuff and the PowerLab data acquisition system (ADInstruments, New Zealand). Hearts were dissected from anesthetized animals (pentobarbital, 50 mg/kg i.p.), and solutions for retrograde perfusion and ventricular myocytes isolation were prepared based on a "low-Ca^{2+} medium" containing (in mM): NaCl, 80; KCl, 10; KH_2PO_4, 1.2; $MgSO_4$, 5; glucose, 20; taurine, 50; L-arginine, 1; HEPES, 10 (pH 7.2), as described previously [29]. Isolated cardiomyocytes were stored in low-Ca^{2+} medium supplemented with 200 μM $CaCl_2$. Only rod-shaped cardiomyocytes with clear striations were used.

2.2. Electrophysiology

Membrane currents in isolated cardiac myocytes were measured using the perforated mode of the whole-cell patch clamp technique, which allows preserving the intracellular milieu essential to maintain signaling pathways and minimize run-down of recorded currents [42,43]. Whole-cell membrane potential was controlled through the electrical access obtained by membrane patch perforation induced by amphotericin B (200–250 μg/mL) added to the pipette (3–5 MΩ) containing (in mM): CsCl, 130; $MgSO_4$, 5; HEPES, 10 (pH 7.25). The bath solution contained (in mM): NaCl, 80; $CaCl_2$, 2; $MgSO_4$, 5; KH_2PO_4, 1.2; CsCl, 10; tetraethylammonium chloride (TEA-Cl), 20; glucose, 20; L-arginine, 1; HEPES, 10 (pH 7.25). In pipette and bath solutions, cesium and TEA ions were used to diminish K^+ currents. L-type Ca^{2+} currents were elicited by depolarizing 200 ms-long rectangular pulses from a holding potential of −50 mV, chosen to inactivate low threshold voltage-gated channels, at a pacing rate of 0.03–0.04 Hz (see figures). Currents were measured using an Axopatch 200B amplifier (Molecular Devices, USA). Protocol of stimulation, determination of cell parameters and data acquisition were performed using the custom BioQuest software [43] and a L-154 AD/DA converter (L-card, Moscow, Russia). Perforated patch and cellular membrane resistance along with cell capacitance were defined online based on analysis of capacitive transient currents. Only cells providing perforated patches with series resistance less than 30MΩ were used. Series resistance, compensated by 50–60%, and uncompensated cell capacitances were continuously monitored for the quality of the whole-cell recording configuration throughout experiments. In control 10–15 min long recordings ($n = 4$), the rate of Ca^{2+} current run-down, estimated using linear regression of peak current values, did not exceed 2.3 pA/min. Measurements were performed at 20–22 °C.

2.3. Phosphatase assay

Serine/threonine phosphatase activity was determined by using a nonradioactive molybdate dye-based phosphatase assay kit (Promega) according to the manufacturer's recommendations. Following 15 min retrograde perfusion of isolated hearts with DMEM supplemented with 10 mM HEPES (pH 7.2), in the absence or in the presence of drugs tested, left ventricles were harvested and homogenized at 1 mg of tissue per 3 mL of buffer containing (in mM): sucrose, 250; β-mercaptoethanol, 15; EDTA, 0.1; phenylmethylsulfonyl fluoride, 0.1; TRIS-HCl, 50 (pH 7.4). Free phosphate was removed from the lysate supernatants using a Sephadex G-25 resin spin column. Phosphatase reactions were assessed in 50 μL samples at 37 °C, in buffer containing 50 mM imidazole, 0.2 mM EGTA, 0.02% β-mercaptoethanol, and 0.1 mg/mL bovine serum albumin (pH 7.2) based on the dephosphorylating rate of the synthetic-754 Da phosphopeptide RRA[pT]VA, a preferential substrate for PP2A. Specificity of the phosphatase reaction was tested by calyculin A (0.5 μM), a serine/threonine phosphatase