expression of Sarcomplasmic Reticulum Calcium-transporting ATPase (SERCA2a) decrease in diabetes, leading to diastolic and systolic dysfunction of myocardium. It was recently reported that SUMOylation could elevate the activity and stability of SERCA2a. We assume that diabetes might affect the intensity of SUMOylation of SERCA2a after MI.

METHODS Diet-induced type 2 diabetic rats and controls were divided into subcutaneous injected MI groups or sham groups. Primary cardiomyocytes were cultured in different concentrations of glucose and insulin, and underwent oxygen deprivation (OD) for 6 or 12 hours. Echocardiograph and left ventricular pressure were measured to determine cardiac function. The intensity of SUMOylation of SERCA2a, expressions of SERCA2a, SUMO1 and enzymes of SUMOylation were evaluated.

RESULTS Diabetes exacerbated diastolic and systolic dysfunction of myocardium after infarction. SUMOylation intensity of SERCA2a was enhanced in 1-week-post-MI non-diabetic rats and 6-hour-OD cardiomyocytes but not in 4-week-post-MI rats and 12-hour-OD cardiomyocytes. The expression of enzyme 2 of SUMOylation, namely Ubc9, was in accordance with the SUMOylation intensity, while SUMO1 and enzyme 1 were also not changed. Additionally, overexpression of Ubc9 with lentivirus neutralized the decreasing of SUMOylation intensity caused by glucose and insulin in vitro.

CONCLUSIONS SUMOylation intensity of SERCA2a was compensatory enhanced in post-MI non-diabetic rats, but not in diabetic rats. SUMOylation intensity of SERCA2a decreased in cardiomyocytes with addition of high glucose and insulin in vitro, which could be neutralized by overexpression of Ubc9. These observations provide evidence that Ubc9 and SUMOylation of SERCA2a is involved in diabetes-mediated exacerbation of left ventricular dysfunction after MI.

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DCPIB Attenuates Myocardial Ischemia/Reperfusion Injury Through Inhibiting Autophagy in Rat Model
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OBJECTIVES Autophagy plays a contradictory role in myocardial ischemia/reperfusion (I/R) process, which acts as both beneficial cardioprotective during ischemia stress and myocardial injury in response to subsequent reperfusion. DCPIB (4-[2-Butyl-6,7-dichloro-2-cyclopentyl-1,3-dihydro-1-oxo-1H-inden-5-yl] butanoic acid), a selective inhibitor of volume-sensitive outwardly rectifying (VSOR) chloride channel, has been determined to protect cardiomyocytes from reperfusion damage. However, the underlying mechanism remains unclear. The present study explored the possible mechanism of DCPIB in alleviating myocardial I/R injury.

METHODS Sprague-Dawley rats were randomly divided into sham operation group, I/R group, I/R+Rapamycin (RAPA) group, I/R+DCPIB group, I/R+3-methyladenine (3MA, an autophagic inhibitor) group and I/R+RAPA+DCPIB group, with 6 rats in each group. Rats were performed to ischemia 30 minutes and subsequent reperfusion 24 hours, DCPIB (10mg/kg), RAPA (4mg/kg) and 3MA (1mg/kg) were administrated as intraperitoneal injection 10 min before the onset of reperfusion, respectively. Serum myocardial enzymes were measured and light microscopic study was performed, the myocardial LC3 was detected by immunohistochemistry, nuclear factor-κB (NF-κB) and tumor necrosis factor α (TNF-α) were detected by enzyme-linked immunosorbent assay (ELISA).

RESULTS The expressions of myocardial LC3, TNFα and NF-κB were significantly increased (P < 0.05), and cardiac function was declined in I/R group compared with that in sham-operation group (P < 0.05). myocardial LC3 were furthermore increased in RAPA group, whereas these could be reversed through 3MA, a autophagic inhibitor. Of note, DCPIB administration caused significant reduction of myocardial LC3, TNF-α and NF-κB (P < 0.05), and significantly improved the cardiac functional recovery and reduced myocardial enzymes activity compared with that in I/R group and I/R+RAPA+DCPIB group (P < 0.05). Specific details for hemodynamics, compared to sham-operated group, left ventricular systolic pressure (LVSP), maximal rate of increase of ventricular pressure (+dp/dt max) and maximal rate of decrease of ventricular pressure (-dp/dt max) decreased while left ventricular end diastolic pressure (LVEDP) significantly increased markedly in I/R group and I/R+RAPA (P < 0.05), however, DCPIB indeed reversed the disorder.

CONCLUSIONS Our results demonstrated that DCPIB attenuated excessive autophagy to protect rat heart from I/R injury.
Cellular Electrophysiological Mechanisms and Drug Intervention Study of the CACNA1C Gene Mutation Related to Early Repolarization

 OBJECTIVES To investigate the electrophysiological mechanisms of mutated cardiac L-type calcium channel (LTCC) caused by a CACNA1C gene mutation in ER from cellular level, and screening of effective drug mutation in a 34-year-old male patient. The mutant L-type calcium current (ICa,L) in HEK293 cells declined when compared to the wild calcium channel, (P < 0.05, P < 0.01), there is no difference of the voltage dependent SSA between the 2 groups. The tetrodotoxin (10 μM) significantly reduces the current density (P < 0.05). The therapeutic drug for ERS of isoproterenol (10 μM) increases the wild calcium current density, while the increased mutant calcium current density without statistical significance. The quinidine (5 μM) has no effect on the calcium current density and voltage dependent SSA in the both groups.

 RESULTS A missense mutation in CACNA1C gene (c.5747A > G, p.Q1916R) was identified in a 34-year-old male patient. The mutant L-type calcium current (ICa,L) in HEK293 cells declined when compared to the wild calcium channel, (P < 0.05, P < 0.01), there is no difference of the voltage dependent SSA between the 2 groups. The tetrodotoxin (10 μM) significantly reduces the current density (P < 0.05). The therapeutic drug for ERS of isoproterenol (10 μM) increases the wild calcium current density, while the increased mutant calcium current density without statistical significance. The quinidine (5 μM) has no effect on the calcium current density and voltage dependent SSA in the both groups.

 CONCLUSIONS In conclusion, a novel CACNA1C mutation might be associated with the ERS. The CACNA1C-Q1916R is a loss-of-function mutation with decreased calcium current. The testosterone exacerbates the decreased CACNA1C-Q1916R mutant calcium current density, which suggests that the male gender is an important risk factor ERS. There might be no therapeutic effect of isoproterenol in the CACNA1C-Q1916R mutant carrier related to the early repolarization we studied here.

 Up-Regulation of Neuronal Nitric Oxide Synthase Modulates Myofilament Ca2+ Sensitivity and Controls Left Ventricular Myocyte Contractility in Hypertensive Rats

 OBJECTIVES Hypertension is one of the major risk factors for developing cardiac hypertrophy and heart failure. Although cellular signaling pathways, intracellular Ca2+ handling and myofilament Ca2+ sensitivity are known to be altered in hypertensive myocardium, mechanisms mediating left ventricular (LV) contractile function remain to be defined. It is known that neuronal nitric oxide synthase (nNOS) is up-regulated in hypertensive myocardium, however, it’s role in myocyte Ca2+ handling and myofilament Ca2+ sensitivity and their interplays in regulating contractile function is not clear. Therefore, we aim to test the functional regulation by nNOS of LV myocyte contractin in angiotensin II (Ang II)-induced hypertensive rats.

 METHODS Sprague-Dawley rats (8 weeks old, male) were subjected to Ang II infusion subcutaneously using osmotic minipump for 4 weeks (12.5ng/min/kg). These animals were paired with sham-operated groups. LV myocytes were isolated using a standard enzymatic dispersion technique. Contraction and relaxation were measured in LV myocytes (field-stimulation at 2 Hz, 36±1°C) by using a video-sarcomere detection system (IonOptix Corp).

 RESULTS Systemic blood pressure was elevated in Ang II-treated rats (osmotic minipump, 4 weeks) compared to that in shams. Functional analysis showed that LV myocyte sarcomere shortening was unchanged and relaxation was faster in Ang II-rats compared to those from shams. L-type Ca2+ channel activity (Ica) was not different between two groups, however peak [Ca2+]i was increased (Fura 2, F360/F380) and decay of [Ca2+]i (tau) was faster in Ang II rats. These results were accompanied by reduced myofilament Ca2+ sensitivity (EC50). Immunoblotting results revealed that nNOS protein expression was significantly increased (optical density of nNOS/β-actin in LV myocytes) in Ang II-rats compared to those from shams. Up-regulation of nNOS with specific inhibitors, 5-methyl-L-thiocitulline (SMTc, 100 nM) or N5-(1-imino-3-butenyl)-L-ornithine (L-VNIO, 100 μM) restored myofilament Ca2+ sensitivity and reduced [Ca2+]i in Ang II-rats. As a result, LV myocyte contraction was maintained unaltered. Interestingly, Ica was increased by nNOS inhibition (similar to that in sham) but tau of [Ca2+]i was not affected, despite that relaxation was significantly prolonged by SMTc or L-VNIO in Ang II-rats. A specific inhibitor of protein kinase G (KT 5823, 1 μM) mimicked the effect of nNOS and increased myofilament Ca2+ sensitivity, [Ca2+]i and LV myocyte contractility.

 Inhibition of VDAC1 Prevents Ca2+-Mediated Oxidative Stress and Apoptosis Induced by 5-Aminolevulinic Acid Mediated Sonodynamic Therapy in THP-1 Macrophages

 OBJECTIVES Ultrasound combined with endogenous protoporphyrin IX derived from 5-aminolevulinic acid (ALA-SDT) is known to induce apoptosis in multiple cancer cells and macrophages. Persistent retention of macropahages in the plaque has been implicated in the pathophysiology and progression of atherosclerosis. Here we investigated the effects of inhibition of voltage-dependent anion channel 1 (VDAC1) on ALA-SDT-induced THP-1 macrophages apoptosis.

 METHODS Cells were pre-treated with VDAC1 inhibitor 4, 4’-diisothiocyanostilbene-2, 2’-disulfonic acid (DIDS) disodium salt for 1 h or downregulated VDAC1 expression by small interfering RNA and exposed to ultrasound. Cell viability was assessed by MTT assay, and cell apoptosis along with necrosis was evaluated by Hoechst 33342/ propidium iodide staining and flow cytometry. Levels of cytochrome c release was assessed by confocal microscope and Western blots. The levels of full length caspases, caspase activation, and VDAC1 isomorphs were analyzed by Western blot. Intracellular reactive oxygen species generation, mitochondrial membrane permeability, and intracellular Ca2+ ([Ca2+]i) levels were measured with fluorescent probes.

 RESULTS The pharmacological inhibition of VDAC1 by DIDS notably prevented ALA-SDT-induced cell apoptosis in THP-1 macrophages. DIDS significantly inhibited intracellular ROS generation and apoptotic biochemical changes such as inner mitochondrial membrane permeabilization, loss of mitochondrial membrane potential, cytochrome c release and activation of caspase-3 and caspase-9. Moreover, ALA-SDT elevated the [Ca2+]i levels and it was also notably reduced by DIDS. Furthermore, both of intracellular ROS generation and cell apoptosis were predominately inhibited by Ca2+-chelating reagent BAPTA-AM. Intriguingly, ALA-treatment markedly augmented VDAC1 protein levels exclusively, and the down-regulation of VDAC1 expression by specific siRNA also significantly abolished cell apoptosis.

 CONCLUSIONS VDAC1 plays a crucial role in ALA-SDT-induced THP-1 macrophages apoptosis and targeting VDAC1 is a potential way regulating macropahages apoptosis. This finding may be relevant to therapeutic strategies against atherosclerosis.