Intermedin protects thapsigargin-induced endoplasmic reticulum stress in cardiomyocytes by modulating protein kinase A and sarco/endoplasmic reticulum Ca$^{2+}$-ATPase

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Abstract. Intermedin (IMD) is a calcitonin/calcitonin-related peptide that elicits cardioprotective effects in a variety of heart diseases, such as cardiac hypertrophy and heart failure. However, the molecular mechanism of IMD remains unclear. The present study investigated the effects of IMD on neonatal rat ventricular myocytes treated with thapsigargin. The results of the present study demonstrated that thapsigargin induced apoptosis in cardiomyocytes in a dose- and time-dependent manner. Thapsigargin induced endoplasmic reticulum stress, as determined by increased expression levels of 78-kDa glucose-regulated protein, C/EBP-homologous protein and caspase-12, which were dose-dependently attenuated by pretreatment with IMD. In addition, IMD treatment counteracted the thapsigargin-induced suppression of sarco/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA) activity and protein expression levels, and cytoplasmic Ca$^{2+}$ overload. IMD treatment also augmented the phosphorylation of phospholamban, which is a crucial regulator of SERCA. Additionally, treatment with the protein kinase A antagonist H-89 inhibited the IMD-mediated cardioprotective effects, including SERCA activity restoration, anti-Ca$^{2+}$ overload, endoplasmic reticulum stress inhibition and antiapoptosis effects. In conclusion, the results of the present study suggested that IMD may protect cardiomyocytes against thapsigargin-induced endoplasmic reticulum stress and the associated apoptosis at least partly by activating the protein kinase A/SERCA pathway.

Introduction

Intermedin (IMD), also termed adrenomedullin-2, is a member of the calcitonin/calcitonin gene-related peptide family and is widely expressed in the heart, blood vessels, brain, hypothalamus, kidney, lung, spleen, thymus, ovary and adipose tissue (1). The plasma levels of IMD are relatively low, between 100 and 200 pg/ml (2). Following proteolytic cleavage, pro-IMD generates IMD$_{1-53}$, IMD$_{1-40}$ and IMD$_{1-47}$, the major active fragments that act through the calcitonin-like receptor/receptor activity-modifying protein complexes to induce multiple biological effects, such as increased prolactin release, antidiuretic and natriuretic effects, and reduced food intake (3). Accumulating evidence has indicated extensive functions of IMD in maintaining cardiovascular homeostasis, such as accelerating angiogenesis, anti-apoptotic and fibroblast activity, and increasing cardiac contractility and perfusion (4-6). The plasma levels of IMD and the endogenous IMD in cardiomyocytes have been reported to be significantly increased in response to heart failure and acute cardiac infarction stimuli, and IMD supplementation inhibits cardiomyocyte injury induced by heart failure and cardiac infarction (4,7,8). These results also suggested that IMD may be a potential endogenous protector of the heart. Although these studies have confirmed the cardioprotective effects of IMD, the underlying protective mechanisms are still unclear. Teng et al (3) and Zhang et al (9) have reported that IMD attenuates tunicamycin and dithiothreitol-induced myocardial injury in rats by inhibiting endoplasmic reticulum (ER) stress. Tunicamycin and dithiothreitol induce ER stress via inhibition of protein glycosylation and disulfide bond formation, respectively. Similar to tunicamycin or dithiothreitol, thapsigargin is also an inducer of ER stress. Thapsigargin, originally isolated from the plant *Thapsia garganica*, is a specific and potent inhibitor for sarco/endoplasmic reticulum calcium ATPase (SERCA) (10). By inhibiting SERCAs, thapsigargin interferes with the ER lumen Ca$^{2+}$ flux that subsequently leads to ER stress (10,11).
SERCA has been reported to exert important effects in the heart, and a number of heart diseases are associated with SERCA dysfunction, such as cardiac hypertrophy and heart failure (12,13). Thapsigargin induces ER stress-related apoptosis by inhibiting SERCA activity to partially simulate the pathophysiological processes of various cardiovascular diseases (14).

The SERCA that is present in all organisms is a 110 kDa transmembrane protein encoded by three homologous genes (SERCA1, SERCA2 and SERCA3), with a dominant expression of SERCA2a in cardiomyocytes (15). SERCA2a is crucial for regulating 
Ca
2+ homoeostasis by transporting cytosolic 
Ca
2+ into the sarcoplastic reticulum (16,17). Phospholamban (PLB) located in the cardiac sarcoplasmic reticulum and, as an endogenous SERCA inhibitor, compromises SERCA affinity for 
Ca
2+ (18). PLB phosphorylation is the primary regulator of SERCA activity in cardiomyocytes (19,20).

To date, there have been no published reports on the effects of IMD on cardiomyocyte injury induced by thapsigargin to the best of our knowledge. Therefore, the present study focused on the role of IMD in thapsigargin-induced cardiomyocyte apoptosis and examined SERCA activity during thapsigargin treatment to explore whether IMD restored ER stress in cardiomyocytes via a SERCA-dependent mechanism. Protein kinase A (PKA) has also been reported to increase SERCA activity (21); to further explore the underlying mechanisms through which IMD regulates SERCA activity and ER stress, the present study examined the involvement of the PKA pathway in the IMD-mediated protective effects in cardiomyocytes.

Materials and methods

Cell culture and treatment. The experimental protocols were approved by the Ethical Committee of Shanxi Medical University (Taiyuan, China) and complied with internationally accepted principles of laboratory animal care and use (22). Animals were housed at 23°C with 50% humidity, 12-h light/dark cycles, and free access to food and water. Briefly, 16 neonatal SD rats (male and female; age, 1-3 days; Laboratory Animal Center of Shanxi Medical University) were anesthetized by pentobarbital sodium (100 mg/kg) and decapitated for cardiac tissue harvesting. Left ventricular tissues were digested with collagenase II (Sigma-Aldrich; Merck KGaA) in Hank's balanced salt solution (Ca
2+- and Mg
2+-free) for 1 h at 37°C, as described previously (23). After centrifugation at 500 x g for 10 min at 4°C, the supernatants were discarded, and the cells were resuspended; ~90 min later, non-myocytes were attached to the dishes. The viable non-attached cells were collected and plated on 60-mm culture dishes at a density of 6x10^6 cells per dish and cultured in DMEM supplemented with 0.1 mM 1% penicillin-streptomycin and 20% FBS (all purchased from Sigma-Aldrich; Merck KGaA) for 24 h at 37°C. PLB phosphorylation was the primary regulator of SERCA activity in cardiomyocytes.

Measurement of intracellular 
Ca
2+ concentration. Intracellular 
Ca
2+ concentration ([Ca
2+]i) was measured using the 
Ca
2+-specific fluorescent probe fluo-3/AM (cat. no. 50013; Biotium, Inc.). Cardiomyocytes were loaded with 5 µM fluo-3/AM for 30 min at 37°C. The mean fluorescence intensity of the cells was monitored using an Olympus FV1000 laser scanning confocal microscope (Olympus Corporation). Fluorophores were excited at 488 nm, and the emission intensity was measured at 528 nm.

Flow cytometry analysis. Apoptotic cells were detected using an Annexin-V-FITC kit (BD Biosciences) with propidium iodide (PI) staining and flow cytometric analysis according to the manufacturer’s instructions. The double-negative
(Annexin V-negative/PI-negative) cells were defined as viable, the single-positive populations were considered to be early apoptotic (Annexin V-positive/PI-negative) or necrotic (Annexin V-negative/PI-positive) cells, and double-positive (Annexin V-positive/PI-positive) cells were considered to be in a late stage of apoptosis. Apoptotic cells were analyzed using
a FACScan flow cytometer (BD Biosciences) and CellQuest Pro software (version 5.1; BD Biosciences). Apoptosis was calculated as the total of early and late apoptotic cells.

**Statistical analysis.** Data are expressed as the mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, Inc.). All data on dose response and time series were analyzed with a one-way ANOVA followed by the Tukey test. Additional data were analyzed with student's t-test for two group comparisons. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Thapsigargin induces cardiomyocyte apoptosis in a dose- and time-dependent manner.** Neonatal rat cardiomyocytes were incubated with 1.5, 3 or 6 µM thapsigargin for 24 h to determine the working concentration of thapsigargin. Cell viability was determined using an MTT assay. As demonstrated in Fig. 1A, treatment with increasing concentrations of thapsigargin led to a dose-dependent loss of cardiomyocyte viability compared with the control group. Following exposure to 3 µM thapsigargin, 69.33±4.63% of the cardiomyocytes were viable relative to the control group (P<0.01). However, with 6 µM thapsigargin, the viability of cells was significantly reduced to 44.52±6.88% (Fig. 1A). Apoptosis was analyzed by flow cytometry using the Annexin V/PI double staining assay. The percentages of apoptotic cells were 9.8±7.55, 26.47.0±10.31 and 50.87±13.51% in the 1.5, 3 and 6 µM thapsigargin groups, respectively (Fig. 1B and C).

In order to further study the effects of thapsigargin, cardiomyocytes were treated with 3 µM thapsigargin for 12, 24 or 48 h. After exposure to 3 µM thapsigargin for the indicated times, cell viability was significantly decreased in the 24 and 48 h groups compared with that of the control group (Fig. 1D). The apoptotic rates in the 24 and 48 h groups, but not the 12 h group, were significantly higher compared with those in the control group (Fig. 1E and F). Thapsigargin induced apoptosis in cardiomyocytes in a dose- and time-dependent manner; thus, 3 µM thapsigargin treatment for 24 h was used in subsequent experiments.

**IMD inhibits thapsigargin-induced ER stress in cardiomyocytes.** Cardiomyocytes were incubated with 3 µM thapsigargin, 69.33±4.63% of the cardiomyocytes were viable relative to the control group (P<0.01). However, with 6 µM thapsigargin, the viability of cells was significantly reduced to 44.52±6.88% (Fig. 1A). Apoptosis was analyzed by flow cytometry using the Annexin V/PI double staining assay. The percentages of apoptotic cells were 9.8±7.55, 26.47.0±10.31 and 50.87±13.51% in the 1.5, 3 and 6 µM thapsigargin groups, respectively (Fig. 1B and C).
Thapsigargin in the presence or absence of IMD for 24 h. CHOP, GRP78 and caspase-12 are molecular markers specific for ER stress (24). Compared with the control group, the thapsigargin group exhibited significantly upregulated protein expression levels of GRP78, CHOP and caspase-12, indicating that thapsigargin treatment induced ER stress. IMD (10 and 100 nM) reduced the thapsigargin-mediated upregulation of ER stress in a dose-dependent manner. Pretreatment with 100 nM IMD resulted in a strong protective effect against thapsigargin-induced injury (Fig. 2A-D). Collectively, these results demonstrated that IMD pretreatment inhibited the thapsigargin-induced ER stress in a dose-dependent manner.

IMD attenuates SERCA suppression and [Ca²⁺]i overload induced by thapsigargin in cardiomyocytes. Thapsigargin is a highly selective inhibitor of SERCA. Therefore, the present study examined SERCA activity in cardiomyocytes treated with thapsigargin in the presence or absence of IMD (100 nM) to further investigate the possible relationship between IMD and SERCA. As demonstrated in Fig. 3A and B, thapsigargin treatment significantly reduced the activity and protein expression levels of SERCA2, which were reversed by IMD treatment.

Since SERCA serves a crucial role in Ca²⁺ homeostasis, subsequent experiments were performed to determine whether IMD decreased cytosolic Ca²⁺ overload in cardiomyocytes using the fluorescent indicator fluo-3AM. As presented in Fig. 3C and D, the intensity of [Ca²⁺]i fluorescence was significantly elevated in the thapsigargin group compared with that in the vehicle group. This increase in [Ca²⁺]i fluorescence was significantly reduced by IMD pretreatment (Fig. 3C and D).

IMD improves SERCA function by regulating PLB phosphorylation. PLB is a key regulatory protein of SERCA (25). Unphosphorylated PLB binds SERCA and inhibits its activity, which is abolished upon PLB phosphorylation (25).

**Figure 3.** IMD inhibits TG-induced endoplasmic reticulum stress by regulating SERCA activity and [Ca²⁺]i homeostasis. Cardiomyocytes were treated with 3 µM TG in the presence or absence of 100 nM IMD for 24 h. (A) SERCA activity. (B) Representative western blots and semi-quantification of SERCA2 protein levels. (C and D) [Ca²⁺]i, intracellular Ca²⁺ concentration; TG, thapsigargin; nmol Pi/mg prot/min, nanomoles of phosphorus ions per milligram of protein per minute.

**Figure 4.** IMD improves SERCA activity by regulating PLB phosphorylation. Cardiomyocytes were treated with 3 µM TG in the presence or absence of 100 nM IMD for 24 h. p-PLB and t-PLB protein expression levels were assayed by western blot analysis. Representative western blots and semi-quantification of p-PLB and t-PLB. Data were analyzed with a student’s t-test for two group comparison. Data are presented as the mean ± SD. n=3. **P<0.01. IMD, intermedin; SERCA, sarco/endoplasmic reticulum calcium ATPase; PLB, phospholamban; p-, phosphorylated; t-, total; TG, thapsigargin.
Therefore, elucidating the changes in the SERCA regulatory protein PLB may help understand the protective mechanisms of IMD. Thus, t-PLB and p-PLB protein levels were analyzed in the present study by western blotting. As demonstrated in Fig. 4, the levels of p-PLB, which is the active form of the SERCA regulatory protein, were reduced in the thapsigargin group compared with those in the vehicle group. This reduction was reversed by IMD pretreatment (Fig. 4).

**PKA contributes to SERCA function and ER stress regulated by IMD.** As PKA regulates PLB phosphorylation and SERCA activity (21,26), the present study further examined whether
the PKA signaling pathway was involved in IMD-mediated cardioprotection, PLB phosphorylation and SERCA function. As presented in Fig. 5A-D, co-treatment with the PKA inhibitor H-89 (10 µM) inhibited the effects of IMD on PLB phosphorylation and SERCA activity in cardiomyocytes treated with thapsigargin. These results suggested that IMD-induced PLB phosphorylation and restoration of SERCA activity were mediated in part by the PKA pathway.

PKA inhibitor H-89 treatment also abolished the protective effects of IMD on ER stress and ER stress-related cardiomyocyte apoptosis induced by thapsigargin, as demonstrated by western blotting and Annexin V/PI double staining assay (Figs. 5B, 5E-G, 6A and 6B). These results suggested that IMD may ameliorate ER stress and ER stress-related cardiomyocyte apoptosis induced by thapsigargin at least in part via the PKA/SERCA pathway.

Discussion

The results of the present study demonstrated that IMD decreased the thapsigargin-induced upregulation of GRP78, CHOP and caspase-12, the specific markers of ER stress. IMD alleviated thapsigargin-induced ER stress by restoring SERCA activity and Ca\(^{2+}\) overload in cultured cardiomyocytes. PLB phosphorylation may also contribute to IMD-enhanced SERCA activity. Co-treatment with the PKA inhibitor H89 appeared to counteract the protective effects of IMD on PLB phosphorylation and restoration of SERCA activity, suggesting the involvement of the PKA signaling pathway in the effects of IMD.

IMD belongs to a multifunctional calcitonin/calcitonin gene-related peptide superfamily and shares common receptors with calcitonin gene-related peptide, adrenomedullin and amylin, with unique and important cardioprotective functions, including improving cardiac function, pro-angiogenesis, anti-oxidation and anti-ER stress (5). ER stress is increasingly recognized as an important contributor to myocardial injury (27). ER, as the primary site of proper folding and sorting of proteins, is vulnerable to ischemia, hypoxia and oxidative stress. Various pathophysiological conditions disturb the ER function and initiate the unfolded protein response to promote cell survival (24). However, persistent and excessive ER stress triggers apoptosis and aggravates cardiovascular diseases, including heart failure, cardiac hypertrophy and ischemic heart disease. ER stress and its induced apoptosis have been highlighted as important mechanisms underlying myocardial injury (28,29). Alleviating ER stress is accepted as a promising therapeutic approach for the treatment of cardiovascular diseases (30).

In previous studies, IMD has been demonstrated to exert a cardioprotective effect against ER stress induced by tunicamycin and dithiothreitol (6,7). The pathways by which IMD inhibits ER stress and protects myocardial injury are not well understood. Thapsigargin is also a classic ER stress inducer. Tunicamycin and dithiothreitol induce ER stress via inhibition of protein glycosylation or disulfide bond formation, respectively (31), whereas thapsigargin possesses a unique mechanism to induce ER stress. To date, there have been no published reports on the effects of IMD on thapsigargin-induced cardiomyocyte injury. The present study assessed the protein expression levels of ER stress-related markers GRP78, CHOP and caspase-12 in cardiomyocytes by western blotting; thapsigargin treatment significantly upregulated the expression levels of GRP78, CHOP and
caspase-12, whereas IMD pretreatment significantly ameliorated these changes in a dose-dependent manner, suggesting that IMD inhibited thapsigargin-induced ER stress. To the best of our knowledge, the present study demonstrated for the first time that IMD may rescue cardiomyocytes from thapsigargin-triggered ER stress.

Experimental and clinical studies have indicated that IMD suppresses cardiac hypertrophy and heart failure, a major health issue that is a leading cause of death worldwide (32-34). During this process, cytosolic Ca\(^{2+}\) overload serves a crucial role in the development of pathological cardiac hypertrophy and heart failure. Cytosolic Ca\(^{2+}\) homeostasis is tightly controlled by Ca\(^{2+}\)-handling enzymes, proteins, channels and transporters in the plasma membrane and Ca\(^{2+}\) storage organelles (35). The ER is the major Ca\(^{2+}\) storage organelle that releases Ca\(^{2+}\) predominately via the Inositol 1,4,5-trisphosphate and the ryanodine receptor, and uptakes Ca\(^{2+}\) via SERCA, which is the only active Ca\(^{2+}\) transporter from the cytosol to the ER in the heart (36). Suppression of SERCA activity and subsequent alteration of cytosolic Ca\(^{2+}\) signaling can severely impair the systolic and diastolic function of the heart, which are major etiologies of cardiac hypertrophy and heart failure (37-39). As the primary function of SERCA is to replenish the sarcoplasmic reticulum Ca\(^{2+}\) load during the contraction-relaxation cycle of the heart, resulting in cytosolic Ca\(^{2+}\) overload, which is associated with heart failure and cardiac hypertrophy, it was hypothesized in the present study that restoration of SERCA activity may mediate the cardio-protective effects of IMD on ER stress and ER stress-related apoptosis. Thapsigargin is a highly selective inhibitor of SERCAs (40). By inhibiting SERCAs, thapsigargin disrupts Ca\(^{2+}\) transport into the ER lumen, leading to an increase in cytoplasmic Ca\(^{2+}\) concentration, and subsequently activates ER stress (41). To further investigate this signaling pathway, thapsigargin was used in the present study, as it is a well-established model to study ER stress and SERCA activity (42). The present results demonstrated that thapsigargin attenuated the protein expression and activity of SERCA2a in cardiomyocytes, and IMD pretreatment reversed this change. Furthermore, [Ca\(^{2+}\)]\(_{\text{ER}}\) in thapsigargin-treated cardiomyocytes was significantly increased, whereas IMD pretreatment inhibited this increase, suggesting that modulating SERCA function and Ca\(^{2+}\) homeostasis may contribute to IMD-mediated cardioprotection. Thus, the results of the present study indicated that the regulation of SERCA activity may serve an important role in the IMD-mediated protection of cardiomyocytes against ER stress, which in part explains the underlying mechanism of IMD improving cardiac hypertrophy and heart failure.

PLB inhibits SERCA activity by reducing its affinity for Ca\(^{2+}\) (42). Unphosphorylated PLB binds SERCA to inhibit SERCA activity; this inhibition is abolished upon PLB phosphorylation (25). PLB is a key regulator of SERCA (42). Considering the relationship between PLB and SERCA, the present study assessed the protein levels of p-PLB and t-PLB in cardiomyocytes treated with thapsigargin in the presence or absence of IMD to further investigate the underlying mechanisms of IMD. The results demonstrated that the expression of p-PLB was decreased in the thapsigargin group compared with those in the vehicle group, whereas IMD reversed this change, suggesting that PLB phosphorylation may be associated with the protective effects of IMD on SERCA activity and cytosolic Ca\(^{2+}\) influx.

IMD stimulates cardiomyocyte PKA activity (43), and SERCA activity can be increased by PKA (20), which provides a potential mechanism by which IMD stimulates SERCA activity and subsequently attenuates ER stress-related apoptosis in thapsigargin-treated cardiomyocytes. To investigate this in the present study, cardiomyocytes were co-pretreated with the PKA inhibitor H-89 and/or IMD, and incubated with thapsigargin. The results demonstrated that the PKA inhibitor H-89 inhibited the protective effect of IMD on ER stress and SERCA function, as indicated by the changes in the expression levels of GRP78, CHOP and caspase-12, and SERCA activity. H-89 pretreatment also abrogated the antiapoptotic effects of IMD. These results suggested that IMD may exert antiapoptotic effects at least partly via the regulation of the PKA signaling pathway in cardiomyocytes treated with thapsigargin.

H-89 is a highly selective, but not exclusive inhibitor of PKA, and reportedly also binds to other protein kinases, including ribosomal protein S6 kinase β1, ribosomal protein S6 kinase α5, rho-associated protein kinase 2, RAC-α serine/threonine-protein kinase and ribosomal protein S6 kinase alpha-1, with a low binding affinity (44,45). Thus, the possibility that these protein kinases may in part contribute to the thapsigargin-induced cardiomyocyte apoptosis cannot be eliminated. In addition, the present study did not investigate whether other Ca\(^{2+}\)-handling enzymes, proteins, channels and transporters, such as inositol 1,4,5-trisphosphate receptor type, ryanodine receptor 1 and stromal interaction molecule 1, are involved in the protection of IMD on thapsigargin-induced cardiomyocyte apoptosis. Accumulating evidence has suggested that thapsigargin-induced apoptosis is mediated by autophagy in cardiomyocytes (46,47). The present results indicated that IMD attenuated thapsigargin-induced cardiomyocyte apoptosis by inhibiting ER stress with the possible involvement of the PKA/SERCA signaling pathway. The present study focused on the mechanisms upstream of ER stress, but did not explore the involvement of autophagy, which is downstream of ER stress. This is a limitation of this study, and future studies should evaluate whether autophagy contributes to the effects of IMD on ER stress-related apoptosis. In addition, PLB has been demonstrated to be a major regulator of SERCA activity. It is the only SERCA-associated protein directly involved in the development of cardiac disease, including heart failure (18). Thus, the present study focused on the PLB/SERCA signaling pathway. Accumulating evidence has indicated that sarcoplin also inhibits the affinity of SERCA for Ca\(^{2+}\) (48,49). In future studies, we will explore whether the effect of IMD on cardiomyocyte apoptosis is associated with sarcoplin and other SERCA regulators.

In conclusion, the results of the present study identified an additional signaling pathway through which IMD responds to ER stress. The protective role of IMD in attenuating thapsigargin-induced cardiomyocyte apoptosis may be mediated by inhibiting ER stress with the possible involvement of the PKA/SERCA signaling pathway. These findings provided a novel insight into the mechanisms underlying the cardioprotective effects of IMD.
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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MZ and ZL designed the experiments. ZL and JG performed the experiments. YB and MZ analyzed the data. ZL and JG wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The experimental protocols were approved by the Ethical Committee of Shanxi Medical University (approval no. SYXk:2015-0507) and complied with the internationally accepted principles of laboratory animal care and use.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Takei Y, Inoue K, Ogoshi M, Kawahara T, Bannai H and Miyano S: Identification of novel adrenomedullin in mammals: A potent cardiovascular and renal regulator. FEBS Lett 556: 53-58, 2004.
2. Taylor MM and Samson WK: Stress hormone secretion is altered by central administration of intermedin/adrenomedullin-2. Brain Res 1045: 199-205, 2005.
3. Teng X, Song J, Zhang G, Cai Y, Yuan F, Du J, Tang C and Qi YF: Inhibition of endoplasmic reticulum stress by intermedin-(53) protects against myocardial injury through a PI3 kinase-Akt signaling pathway. J Mol Med (Berl) 89: 1195-1205, 2011.
4. Tang B, Zhong Z, Shen HW, Wu HP, Xiang Pand Hu B: Intermedin as a prognostic factor for major adverse cardiovascular events in patients with ST-segment elevation acute myocardial infarction. Peptides 58: 98-102, 2014.
5. Yang SM, Liu J and Li CX: Intermedin protects against myocardial ischemia-reperfusion injury in hyperlipidemia rats. Genet Mol Res 13: 8309-8319, 2014.
6. Ni X, Zhang J, Tang CX and Qi YF: Intermedin/adrenomedullin2: An autocrine/paracrine factor in vascular homeostasis and disease. Sci China Life Sci 57: 781-789, 2014.
7. Bell D, Gordon BJ, Lawrey A, Megaw K, Kinney MO and Harbison MT: Plasma levels of intermedin (adrenomedullin-2) in healthy human volunteers and patients with heart failure. Peptides 76: 19-29, 2016.
8. Lv Z, Wu K, Chen X, Zhang X and Hong B: Plasma intermedin levels in patients with acute myocardial infarction. Peptides 43: 121-125, 2013.
9. Zhang JS, Hua YL, Lu WW, Ni XQ, Lin F, Yu YR, Tang CS and Qi YF: Intermedin-(53) protects against myocardial fibrosis by inhibiting endoplasmic reticulum stress and inflammation induced by homocysteine in apolipoprotein E-deficient mice. J Atheroscler Thromb 23: 1294-1306, 2016.
10. Canova NK, Kmonickova E, Martinek J, Zidek Z and Farghali H: Thapsigargin, a selective inhibitor of sarco/endoplasmic reticulum Ca2+-ATPases, modulates nitric oxide production and cell death of primary rat hepatocytes in culture. Cell Biol Toxicol 23: 337-354, 2007.
11. Chen G, Shen Y, Li X, Jiang Q, Cheng S, Gu Y, Liu L and Cao Y: The endoplasmic reticulum stress induced thapsigargin enhances the toxicity of ZnO nanoparticles to macrophages and macrophage-endothelial co-culture. Environ Toxicol Pharmacol 50: 103-110, 2017.
12. Chen X, Zhang X, Gross S, Houser SR and Soboloff J: Acetylation of SERCA2a, another target for heart failure treatment? Circ Res 124: 1285-1287, 2019.
13. Prasad AM, Ma H, Sumbilla C, Lee DI, Klein MG and Inesi G: Phenylephrine hypertrophy, Ca2+-ATPase (SERCA2A), and Ca2+ signaling in neonatal rat cardiac myocytes. Am J Physiol Cell Physiol 292: C2269-C2275, 2007.
14. Liu M, Xue M, Wang XR, Tao TQ, Xu FF, Liu XH and Shi DZ: Panax quinquefolium saponin attenuates cardiomyocyte apoptosis induced by thapsigargin through inhibition of endoplasmic reticulum stress. J Geriatr Cardiol 12: 540-546, 2015.
15. Adachi T: Modulation of vascular sarco/endoplasmic reticulum calcium ATPase in cardiovascular pathophysiology. Adv Pharmaco 59: 165-195, 2010.
16. Cook NL, Viola HM, Sharov VS, Hool LC, Schoneich C and Davies MJ: Myloperoxidase-derived oxidants inhibit sarco/endoplasmic reticulum Ca2+-ATPase activity and perturb Ca2+ homeostasis in human coronary artery endothelial cells. Free Radic Biol Med 52: 951-961, 2012.
17. Zhang C, Bose DD and Thomas DW: Paradoxical effects of sarco/endoplasmic reticulum Ca2+-ATPase (SERCA2A) activator ginginer on NG115-401L neuronal cells: Failure to augment ER Ca2+ uptake and protect against ER stress-induced cell death. Eur J Pharmacol 762: 165-173, 2015.
18. Kranias EG and Hajjar RJ: Modulation of cardiac contractility by the phospholamban/SERCA2a regulon. Circ Res 110: 1646-1660, 2012.
19. Gorski PA, Ceholski DK and Young HS: Structure-function relationship of the SERCA pump and its regulation by phospholamban and sarcoplacin. Adv Exp Med Biol 981: 77-119, 2017.
20. Cerra MC and Imbrogno S: Phospholamban and cardiac function: A comparative perspective in vertebrates. Acta Physiol (Oxf) 205: 9-25, 2012.
21. Xu J, Han Q, Shi H, Liu W, Chu T and Li H: Role of PKA in the process of neonatal cardiomyocyte hypertrophy induced by trotenisin II. Int J Mol Med 40: 499-504, 2017.
22. Ogden BE, Pang William W, Agui T and Lee BH: Laboratory Animal Laws, Regulations, Guidelines and Standards in China Mainland, Japan, and Korea. ILAR J 57: 301-311, 2016.
23. Bian YF, Hao XY, Gao F, Yang HY, Zhang N and Xiao CS: Adiponectin attenuates hypoxia/reoxygenation-induced cardiomyocyte injury through inhibition of endoplasmic reticulum stress. J Investig Med 59: 921-925, 2011.
24. Sozen E, Karademir B and Ozer NK: Basic mechanisms in endoplasmic reticulum stress and reperfusion to cardiovascular diseases. Free Radic Biol Med 78: 30-41, 2015.
25. Gustavsson M, Traaseth NJ, Karim CB, Lockamy EL, Thomas DD and Veglia G: Lipid-mediated folding/unfolding of phospholamban as a regulatory mechanism for the sarcoplasmic reticulum Ca2+-ATPase. J Mol Biol 408: 755-765, 2011.
26. Periasamy M, Bhupathy P and Babu GJ: Regulation of sarcoplasmic reticulum Ca2+ and Ca2⁺-ATPase pump expression and its relevance to cardiac muscle physiology and pathology. Cardiovasc Res 77: 265-273, 2008.
27. Dickhout JG, Carlisle RE and Austin RC: Interaction between cardiac hypertrophy, heart failure, and chronic kidney disease: Endoplasmic reticulum stress as a mediator of pathogenesis. Circ Res 108: 629-642, 2011.
28. Wang J, Hu X and Jiang H: ER stress-induced apoptosis: A novel therapeutic target in myocardial ischemia and reperfusion injury. Int J Cardiol 214: 233-234, 2016.
29. Wang M, Meng XB, YuYL, Sun GB, Xu XD, Zhang XP, Dong X, Ye JX, Xu HB, Sun YF and Sun XB: Elatoside C protects against hypoxia/reoxygenation-induced apoptosis in H9c2 cardiomyocytes through the reduction of endoplasmic reticulum stress partially depending on STAT3 activation. Apoptosis 19: 1727-1737, 2014.

30. Hong J, Kim K, Kim JH and Park Y: The role of endoplasmic reticulum stress in cardiovascular disease and exercise. Int J Vasc Med 2017: 2049217, 2017.

31. Li B, Yi P, Zhang B, Xu C, Liu Q, Pi Z, Xu X, Chevet E and Liu J: Differences in endoplasmic reticulum stress signalling kinetics determine cell survival outcome through activation of MKP-1. Cell Signal 23: 35-45, 2011.

32. Chen H, Wang X, Tong M, Wu D, Wu S, Chen J, Wang X, Wang X, Kang Y, Tang H, Tang C and Jiang W: Intermedin suppresses pressure overload cardiac hypertrophy through activation of autophagy. PLoS One 8: e64757, 2013.

33. Liu K, Deng X, Gong L, Chen X, Wang S, Chen H, Chen X, Amir B and He S: The effect of intermedin on angiotensin II and endothelin-1 induced ventricular myocyte hypertrophy in neonatal rat. Clin Lab 59: 589-596, 2013.

34. Hirose T, Totsune K, Mori N, Morimoto R, Hashimoto M, Nakashige Y, Metoki H, Asayama K, Kikuya M, Ohkubo T, et al.: Increased expression of adrenomedullin 2/intermedin in rat hearts with congestive heart failure. Eur J Heart Fail 10: 840-849, 2008.

35. Reddish FN, Miller CL, Gorkhali R and Yang JJ: Calcium dynamics mediated by the endoplasmic/sarcoplasmic reticulum and related diseases. Int J Mol Sci 18: 1024, 2017.

36. Chemaly ER, Troncone L and Lebeche D: SERCA control of cell death and survival. Cell Calcium 69: 46-61, 2018.

37. Li L, Louch WE, Niederer SA, Aronsen JM, Christensen G, Sejersted OM and Smith NP: Sodium accumulation in SERCA knockout-induced heart failure. Biophys J 102: 2039-2048, 2012.

38. Roe AT, Ruud M, Espe EK, Manfra O, Longobardi S, Aronsen JM, Norden ES, Husebye T, Kolstad TRS, Cataliotti A, et al.: Regional diastolic dysfunction in post-infarction heart failure: Role of local mechanical load and SERCA expression. Cardiovasc Res 115: 752-764, 2019.

39. Shi H, Han Q, Xu J, Liu W, Chu T and Zhao L: Urotensin II induction of neonatal cardiomyocyte hypertrophy involves the CaMKII/PLN/SERCA 2a signaling pathway. Gene 583: 8-14, 2016.

40. Kamiya T, Hara H and Adachi T: Effect of endoplasmic reticulum (ER) stress inducer thapsigargin on the expression of extracellular-superoxide dismutase in mouse 3T3-L1 adipocytes. J Clin Biochem Nutr 52: 101-105, 2013.

41. Lytton J, Westlin M and Hanley MR: Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca2+-ATPase family of calcium pumps. J Biol Chem 266: 17067-17071, 1991.

42. Schmitt JP, Ahmad F, Lorenz K, Hein L, Schulz S, Asahi M, Maclennan DH, Seidman CE, Seidman JG and Lohse MJ: Alterations of phospholamban function can exhibit cardiotoxic effects independent of excessive sarcoplasmic reticulum Ca2+-ATPase inhibition. Circulation 119: 436-444, 2009.

43. Bell D and McDermott BJ: Intermedin (adrenomedullin-2): A novel counter-regulatory peptide in the cardiovascular and renal systems. Br J Pharmacol 153 (Suppl 1): S247-S262, 2008.

44. Lochner A and Moolman JA: The many faces of H89: A review. Cardiovasc Drug Rev 24: 261-274, 2006.

45. Saad NS, Elnakish MT, Ahmed AAE and Janssen PML: Protein kinase a as a promising target for heart failure drug development. Arch Med Res 49: 530-537, 2018.

46. Zhang X, Yuan Y, Jiang L, Wang J, Gao J, Shen Z, Zheng Y, Deng T, Yan H, Li W, et al.: Endoplasmic reticulum stress induced by tunicamycin and thapsigargin protects against transient ischemic brain injury: Involvement of PARK2-dependent mitophagy. Autophagy 10: 1801-1013, 2014.

47. Lindner P, Christensen B, Nissen P, Møller JV and Engedal N: Cell death induced by the ER stressor thapsigargin involves death receptor 5, a non-autophagic function of MAP1LC3B, and distinct contributions from unfolded protein response components. Cell Commun Signal 18: 12, 2020.

48. Bhat N, Babu GJ and Periasamy M: Sarcolipin and phospholamban as regulators of cardiac sarcoplasmic reticulum Ca2+ ATPase. J Mol Cell Cardiol 42: 903-911, 2007.

49. Asahi M, Nakayama H, Tada M and Otaka K: Regulation of sarco(endoplasmic reticulum Ca2+-adenosine triphosphatase by phospholamban and sarcolipin: Implication for cardiac hypertrophy and failure. Trends Cardiovasc Med 13: 152-157, 2003.

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