



Semaglutide inhibits ischemia/reperfusion-induced cardiomyocyte apoptosis through activating PKG/PKC ϵ /ERK1/2 pathway

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ABSTRACT

Apoptosis is a major pathophysiological change following myocardial ischemia/reperfusion (I/R) injury. Glucagon-like peptide 1 (GLP-1) and its receptor GLP-1R are widely expressed in the cardiovascular system and GLP-1/GLP-1R activates the protein kinase G (PKG)-related signaling pathway. Therefore, this study tested whether semaglutide, a new GLP-1 analog, inhibits I/R injury-induced cardiomyocyte apoptosis by activating the PKG/PKC ϵ /ERK1/2 pathway. We induced myocardial I/R injury in rats and hypoxia/reoxygenation (H/R) injury in H9C2 cells and detected the effects of semaglutide, a PKG analog (8-Br-cGMP), and a PKG inhibitor (KT-5823) on the PKG/PKC ϵ /ERK1/2 pathway and cardiomyocyte apoptosis. We found that semaglutide upregulated GLP-1R levels, and both semaglutide and 8-Br-cGMP activated the PKG/PKC ϵ /ERK1/2 pathway, inhibited myocardial infarction (MI), decreased hs-cTNT levels, increased NT-proBNP levels, and suppressed cardiomyocyte apoptosis in I/R rats and H/R H9C2 cells. However, KT-5823 exerted contrasting effects with semaglutide and 8-Br-cGMP, and KT-5823 weakened the cardioprotective effects of semaglutide. In conclusion, semaglutide inhibits I/R injury-induced cardiomyocyte apoptosis by activating the PKG/PKC ϵ /ERK1/2 pathway. The beneficial effect of GLP-1/GLP-1R, involved in the activation of the PKG/PKC ϵ /ERK1/2 pathway, may provide a novel treatment method for myocardial I/R injury.

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1. Introduction

According to a World Health Organization (WHO), approximately 1.8 million people (32% worldwide) have died from diseases related to cardiovascular diseases, which are the primary cause of death [1]. Myocardial infarction (MI) is a major health problem that leads to 75% of sudden cardiac deaths, mainly resulting from myocardial supply area ischemia and necrosis because of the reduction of coronary blood [2,3]. Persistent squeezing or discomfort in the posterior sternum is the most common symptom of MI. Intraarterial thrombolysis is required to achieve reperfusion.

However, some patients still develop cardiac failure, even with timely treatment such as anti-coagulation, anti-platelet, aggregation, or thrombolysis [4]. The main reason for the deterioration of heart function in patients who received reperfusion treatment may be that they underwent myocardial ischemia/reperfusion (I/R) injury [5].

The occurrence of such injury involves several pathological mechanisms, such as apoptosis and oxidative stress injury, among which apoptosis is considered an important factor associated with myocardial I/R injury [6,7]. Blocking cardiomyocyte apoptosis may exert a positive effect on I/R injury [8,9]. Previous studies have shown that protein kinase G (PKG) is one of the most important targets of cyclic guanosine monophosphate (cGMP), and the activation of cGMP-dependent PKG plays a vital role in maintaining cardiac homeostasis and has been used to treat heart failure [10,11]. PKG-induced phosphorylation of downstream proteins is important for regulating calcium signaling, cardiomyocyte and vascular

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smooth muscle cell activity, cardiomyocyte inflammation, cardiomyocyte oxidative stress, and cardiomyocyte apoptosis [12–14]. The extracellular signal-regulated kinase ERK includes two molecules, ERK1 and ERK2, which regulate the damage and repair process of cardiomyocytes and are involved in the regulation of cellular mitochondrial dynamics. PKC ϵ , a phospholipid-dependent filament/threonine kinase, is a downstream protein of PKG and an important endogenous cardiac protective protein [15]. Thus, activation of the PKG/PKC ϵ /ERK1/2 pathway may be an effective method to protect cells from myocardial ischemia/reperfusion injury.

The glucagon-like peptide 1 (GLP-1) analog can obviously reduce cardiac infarct size [16,17]. However, whether the GLP-1 analog exerts myocardial protection by activating the PKG/PKC ϵ /ERK1/2 pathway is unclear. In this study, we aimed to verify whether semaglutide, a novel GLP-1 analog in humans for 7 d [18], inhibits I/R-induced cardiomyocyte apoptosis by activating the PKG/PKC ϵ /ERK1/2 pathway.

2. Materials and methods

2.1. Experimental animals and myocardial I/R model

Male Sprague-Dawley (SD) rats (200 g) were purchased from the Animal Experiment Center of Southwest Medical University. When feeding the rats, the environmental conditions were set as follows: humidity, 60%; temperature, 23 °C; light and dark cycle alternated; and the diet was unrestricted. The relevant research contents and programs were approved by the Ethics Committee of the Southwest Medical University and were in line with the National Institutes of Health guide for the care and use of Laboratory animals.

The myocardial I/R model was created under anesthesia by injecting sodium pentobarbital (30 mg/kg), according to the method of a previous study [19]. The control group underwent the same operation, except that the suture placed under the left anterior descending artery was relaxed. I/R rats were treated with semaglutide (0.3 mg/kg; GL biochem, China) [20], the cGMP/PKG analog 8-Br-cGMP (Sigma-Aldrich, USA; 1 mg/kg ip) [21], and/or the PKG inhibitor KT-5823 (Aladdin, China; 0.5 mg/kg ip) [21] 30 min prior to I/R. Blood specimens and heart tissues were collected under anesthesia after 4 h of reperfusion.

2.2. Electrocardiograph (ECG)

The needle electrodes were inserted subcutaneously into the limbs of rats. Continuous ECG monitoring was performed by connecting the BL-420S Biological Function Experiment system to a multi-channel physiological recorder system. ECG waves were recorded before ligation and 60, 120, and 240 min after reperfusion.

2.3. Biochemical detection

Blood specimens were centrifuged at low speed for 10 min, and hs-cTNT and NT-proBNP were detected by the Shanghai Shenggong Bioengineering Factory (Shanghai, China).

2.4. Haematoxylin-eosin (HE) and immunohistochemical staining

Heart tissues were fixed in 10% buffered formalin, embedded in paraffin, and cut into slices (4 μ m) for HE and immunohistochemical staining. After deparaffinization, the slices were stained with HE according to the manufacturer's instructions. For immunohistochemical staining, after deparaffinization, sections were incubated with 3% H₂O₂ for 10 min and heated to repair antigen

temperature (95 °C). After blocking with 5% bovine serum albumin (BSA) for 1 h, slices were incubated with rabbit anti-GLP-1R antibody (1:100; Proteintech, China) at 4 °C overnight. Then, slices were incubated with the horseradish peroxidase (HRP) conjugated secondary antibody (1:200, Proteintech, China) at 25 °C for 1 h and colored using 3, 3'-diaminobenzidine (DAB). The Integrated optical density (IOD) was analyzed using Image-Pro Plus software.

2.5. Detection of infarct size

The heart was rapidly excised after 4-h reperfusion and frozen at a temperature of –20 °C for 30 min, then serially sectioned into 2-mm thick sections. The slices were placed in 1% triphenyltetrazolium chloride (TTC) solution (Coolaber, China) at 37 °C in term's instructions. Each slice was scanned and quantified using Image-Pro Plus software.

2.6. Cell culture and treatments

The H9C2 cell was obtained from the American Tissue Culture Collection (ATCC) of USA and cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) containing 10% fetal bovine serum (FBS; Gibco, USA). H9C2 cells were induced hypoxia/reoxygenation (H/R) injury to mimic myocardial I/R injury. Briefly, H9C2 cells were cultured under standard incubation conditions for 3 h. Then, H9C2 cells were maintained in an atmospheric environment with 5%–95% O₂ for 2 h. H9C2 cells were treated with semaglutide (5 mmol/L) [20], 8-Br-cGMP (100 μ M) [22], and/or KT-5823 (1 μ M) [22] 30 min prior to H/R. H9C2 cells were cultured in a humidified atmosphere of 5% CO₂ and 21% O₂ at 37 °C for 1 d, then the cells were harvested.

2.7. Cell viability assay

Cell Counting Kit-8 (CCK-8) testing was used to measure H9C2 cell viability. Harvested H9C2 cells were incubated with 10 μ L CCK-8 (Beyotime, China) for 2 h in the dark. Cell viability was tested using Multiskan Spectrum ThermoMultiskanGo (USA).

2.8. Western blot analysis

Total cellular protein was extracted from H9C2 cells and heart tissues using an extraction kit (Proteintech, China), according to the manufacturer's instructions. The protein was quantified using BCA technology and then subjected to polyacrylamide gel electrophoresis (PAGE; 10% separation). The proteins were transferred to Polyvinylidene fluoride (PVDF) membranes and incubated with rabbit anti-GLP-1R (1:1000, Proteintech, China), rabbit anti-PKG (1:1000, MedChemExpress, USA), rabbit anti-p-PKC ϵ (1:1000, APEXBio, USA), rabbit anti-PKC ϵ (1:1000, APEXBio, USA), rabbit anti-p-ERK (1:1000, MedChemExpress, USA), rabbit anti-ERK (1:1000, MedChemExpress, USA), rabbit anti-Bax (1:1000, Proteintech, China), rabbit anti-Bcl-2 (1:1000, Proteintech, China), rabbit anti-cleaved-caspase3 (1:1000, Proteintech, China), and rabbit anti- β -actin (1:4000, Proteintech, China) overnight at 4 °C. After the PVDF membrane was washed, it was incubated with a secondary antibody labeled with horseradish peroxidase for 30 min. The membrane was then washed and detected using the enhanced chemiluminescence (ECL) method.

2.9. Detection of cell apoptosis

The terminal transferase-mediated dUTP-biotin nick end labeling (TUNEL) method was used to test cell apoptosis. H9C2 cells were fixed with 4% paraformaldehyde at 23 °C, then washed with

phosphate-buffered saline (PBS) solution. After incubation with 0.5% Triton X-100, H9C2 cells and the samples were stained using the TUNEL Apoptosis Detection Kit (YEASEN, China). Finally, 4',6-diamidino-2-phenylindole (DAPI) was used to label the nuclei, and TUNEL-positive cells were detected using an Olympus confocal microscope (Olympus BX63, Japan).

2.10. Immunofluorescent staining

H9C2 and the slices were fixed at 23 °C for 15 min with 4% paraformaldehyde, then washed with PBS. The slices were placed in 0.5% Triton X-100 for incubation for 2 h, and then blocked at 37 °C for 1 h with 5% BSA. The sample was mixed with rabbit anti GLP1R antibody (1:100; Proteintech, China) and incubated at 4 °C for 12 h. The sample was then incubated with goat anti-rabbit IgG (1:100, Proteintech, China) combined with FITC and incubated at 37 °C in the dark for 2 h. Finally, DAPI was used to label the nuclei, and

images were obtained using an Olympus confocal microscope (Olympus BX63, Japan).

2.11. Statistical analysis

The data are expressed as standard error of the mean (SEM). Analysis of variance (ANOVA) was used to analyze between-group comparisons. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Semaglutide promoted expression of GLP1R and activated PKG/PKC ϵ /ERK1/2 pathway in I/R rats

The ECG results showed markedly elevated ST and T waves fused into a towering and broad composite wave after ischemia, whereas the ST segment decreased by > 50% with successful reperfusion

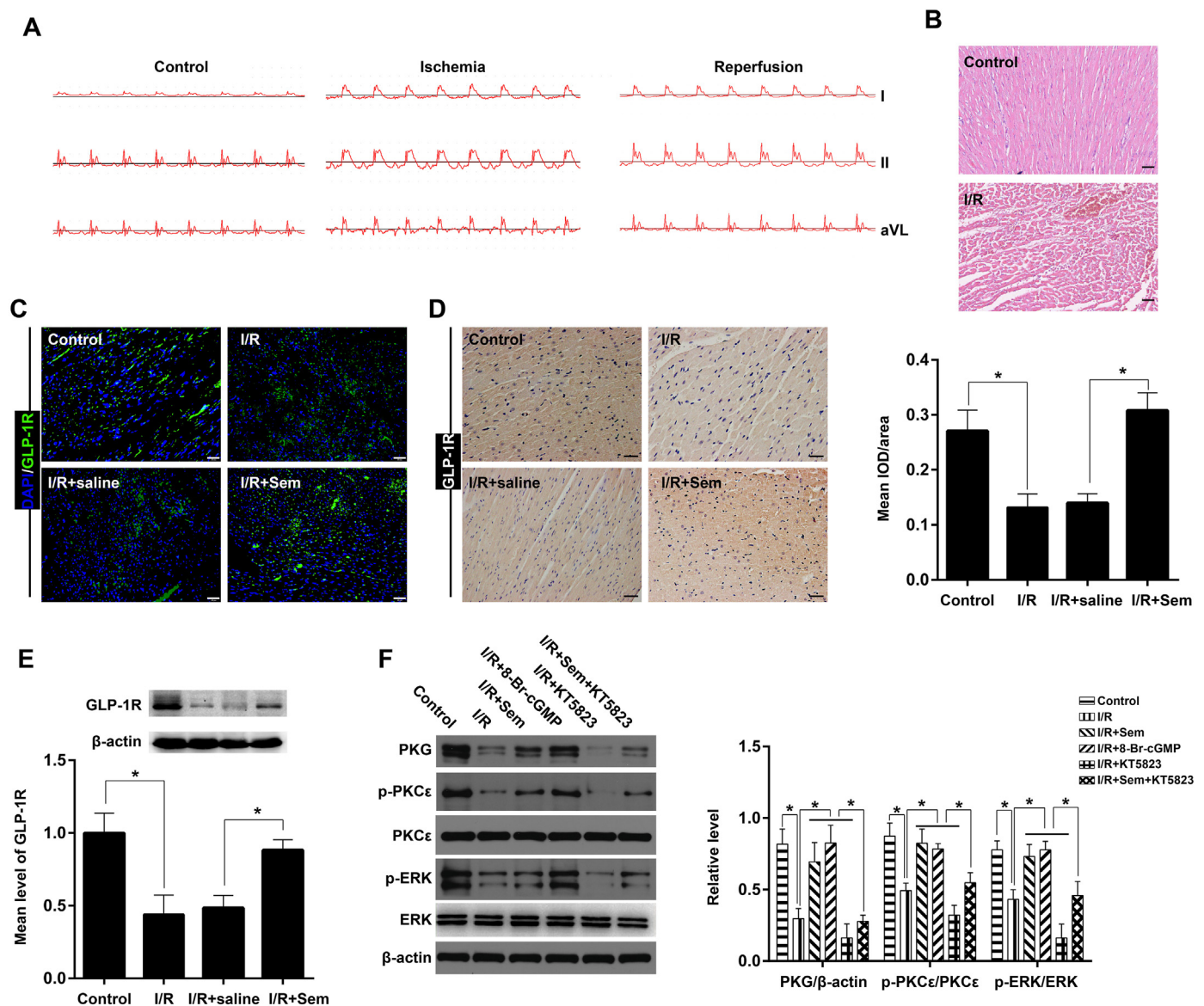


Fig. 1. Semaglutide promoted expression of GLP1R and the activated PKG/PKC ϵ /ERK1/2 signaling pathway in myocardial I/R rats. (A) The ECG in the control, ischemia, and reperfusion. (B) HE staining of heart tissues of control and myocardial I/R rats (Scale bar = 50 μ m). Immunofluorescent staining (C), immunohistochemical staining (D), and Western blot (E) of GLP-1R in the control, I/R, I/R + Saline, and I/R + Semaglutide groups (Scale bar = 50 μ m). (F) Western blot of PKG/PKC ϵ /ERK1/2 signaling pathway in the control, I/R, I/R + Semaglutide, I/R+8-Br-cGMP, I/R + KT-5823, and I/R + Semaglutide + KT-5823 groups. $n = 6$ per group; one-way ANOVA with Bonferroni post hoc analysis; * indicates $P < 0.05$.

(Fig. 1A). HE staining indicated that myocardial I/R injury caused the myocardial fibers to become loose and irregular (Fig. 1B). These data suggest that although reperfusion decreased the ST segment and improved myocardial ischemia, it also caused a new injury: myocardial I/R injury. Immunofluorescence staining (Fig. 1C), immunohistochemical staining (Fig. 1D), and western blotting (Fig. 1E) analyses revealed that GLP1R levels were decreased in myocardial I/R rats, whereas semaglutide stimulated GLP1R expression. The levels of PKG/ β -actin, p-PKC ϵ /PKC ϵ , and p-ERK/ERK in the I/R group were significantly decreased ($P < 0.05$, Fig. 1F), and this inactivation of the PKG/PKC ϵ /ERK1/2 signaling pathway in I/R rats was inhibited by semaglutide ($P < 0.05$, Fig. 1F). To further test whether semaglutide can activate the PKG/PKC ϵ /ERK1/2 signaling pathway, we used 8-Br-cGMP and KT-5823 to activate and inactivate this pathway, respectively. As shown in Fig. 1F, 8-Br-cGMP was activated, whereas KT-5823 inhibited the PKG/PKC ϵ /ERK1/2 pathway in I/R rats ($P < 0.05$). Moreover, semaglutide weakened the

inactivation effects of KT-5823 on the PKG/PKC ϵ /ERK1/2 pathway in experimental animals ($P < 0.05$, Fig. 1F).

3.2. Semaglutide reduced the MI and apoptosis in myocardial I/R rats

TTC staining was used to measure the effect of semaglutide on MI. As shown in Fig. 2A, we detected visible infarction in the I/R group, whereas semaglutide markedly decreased infarct size. 8-Br-cGMP decreased the myocardial, but KT-5823 aggravated MI in the I/R group ($P < 0.05$, Fig. 2A). During the study, observation of the samples in the I/R group showed that the corresponding infarct was highly visible, and the corresponding infarct area was significantly reduced after treatment with semaglutide, which indicates that it has a certain inhibitory effect on MI. In addition, the experimental comparison results also showed that after 8-Br-cGMP intervention, the MI area of the I/R group decreased, but KT-5823 had the

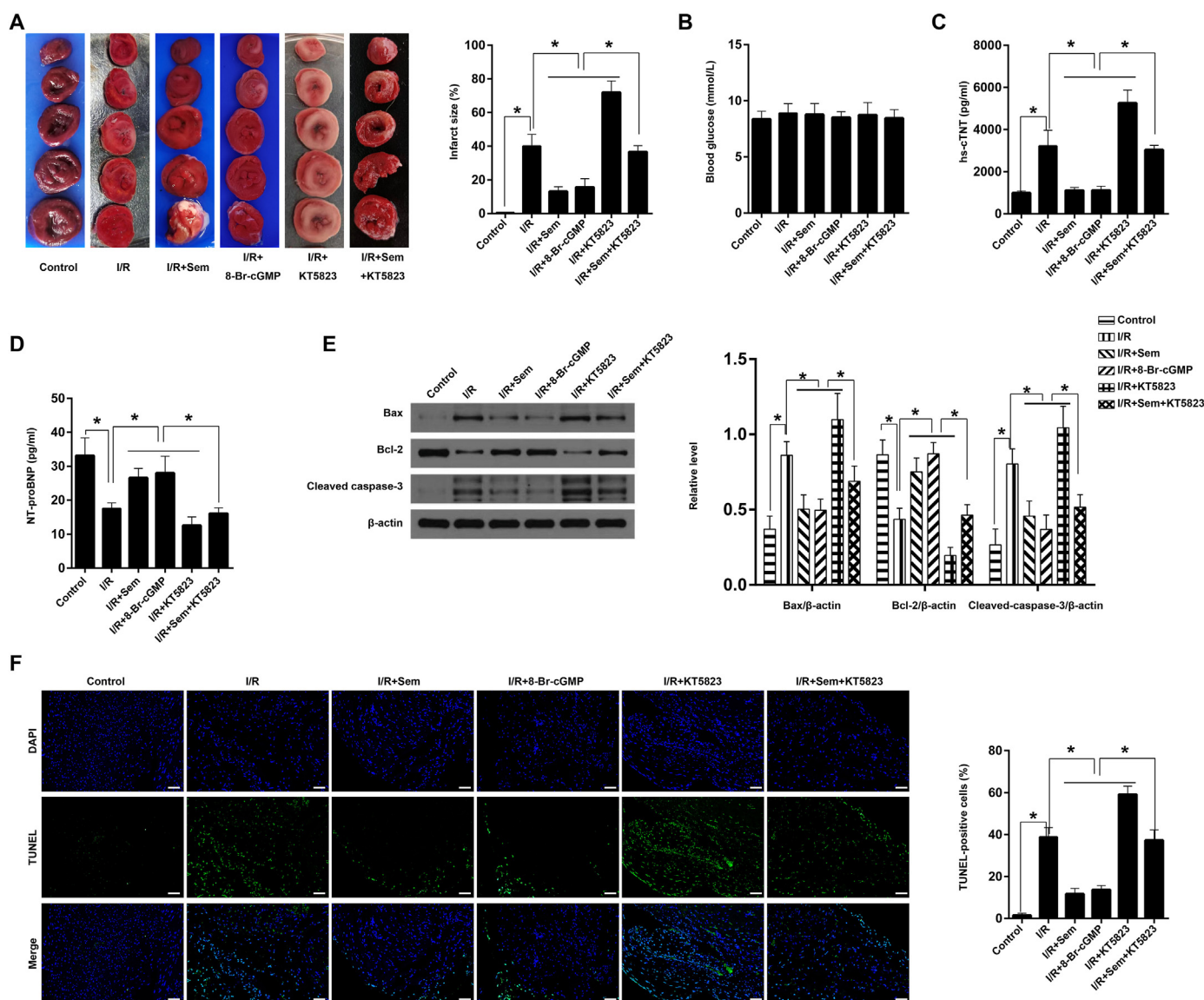


Fig. 2. Semaglutide reduced the MI and apoptosis in myocardial I/R rats. (A) TTC staining of heart tissues in the control, I/R, I/R + Semaglutide, I/R+8-Br-cGMP, I/R + KT-5823, and I/R + Semaglutide + KT-5823 groups. The levels of blood glucose (B), hs-cTNT (C) and NT-proBNP (D) in the control, I/R, I/R + Semaglutide, I/R+8-Br-cGMP, I/R + KT-5823, and I/R + Semaglutide + KT-5823 groups. (E) Western blot of Bax, Bcl-2, and cleaved-caspase3 in the control, I/R, I/R + Semaglutide, I/R+8-Br-cGMP, I/R + KT-5823, and I/R + Semaglutide + KT-5823 groups. (F) TUNEL staining of heart tissues in the control, I/R, I/R + Semaglutide, I/R+8-Br-cGMP, I/R + KT-5823, and I/R + Semaglutide + KT-5823 groups (Scale bar = 50 μ m). n = 6 per group; one-way ANOVA with Bonferroni post hoc analysis; * indicates $P < 0.05$.

opposite effect. Moreover, I/R rats that underwent semaglutide plus KT-5823 treatment had a smaller infarct sizes than that of I/R rats that underwent KT-5823 treatment ($P < 0.05$, Fig. 2A), and the larger infarct size than I/R rats that underwent semaglutide treatment ($P < 0.05$, Fig. 2A), indicating KT-5823 could weaken the effects of semaglutide on MI. Therefore, attention should be given to this aspect during the treatment process.

Semaglutide 8-Br-cGMP did not influence blood glucose levels ($P < 0.05$, Fig. 2B). I/R rats had higher levels of hs-cTNT than control rats, and both semaglutide and 8-Br-cGMP decreased hs-cTNT levels, while KT-5823 increased hs-cTNT levels in I/R rats ($P < 0.05$, Fig. 2C). Furthermore, the inhibitory effect of semaglutide on hs-cTNT levels in I/R rats was weakened by KT-5823 ($P < 0.05$, Fig. 2C). However, the effects of semaglutide, 8-Br-cGMP, and KT-5823 on NT-proBNP in I/R rats were the opposite of the effects of semaglutide, the two on hs-cTNT in I/R rats ($P < 0.05$, Fig. 2D).

Myocardial apoptosis was more obvious in I/R rats than in the control group ($P < 0.05$, Fig. 2E and F). Both semaglutide and 8-Br-cGMP inhibited the level of Bax, up-regulate Bcl-2, and decreased myocardial apoptosis ($P < 0.05$, Fig. 2E and F), indicating that semaglutide and 8-Br-cGMP suppresses myocardial apoptosis. However, KT-5823 up-regulated Bax and cleaved-caspase3, down-regulated Bcl-2, and increased myocardial apoptosis ($P < 0.05$, Fig. 2E and F), indicating that KT-5823 promotes myocardial

apoptosis. Moreover, the inhibitory effects of semaglutide on myocardial apoptosis were weakened by KT-5823 ($P < 0.05$, Fig. 2E and F).

3.3. Semaglutide promoted expression of GLP1R and activated PKG/PKCe/ERK1/2 signaling pathway in H/R H9C2 cells

H9C2 cells were subjected to H/R injury to mimic I/R injury. Immunofluorescence staining (Fig. 3A) and WB (Fig. 3B) indicated that GLP1R expression was lower in H/R H9C2 cells, whereas semaglutide up-regulated expression GLP1R. To further test whether semaglutide can activate the PKG/PKCe/ERK1/2 pathway in vitro, H/R H9C2 cells were treated with 8-Br-cGMP and KT-5823 to activate and inactivate this signaling pathway, respectively. As shown in Fig. 3B, both semaglutide and Br-cGMP activated the PKG/PKCe/ERK1/2 signaling pathway, whereas KT-5823 inactivated the PKG/PKCe/ERK1/2 signaling pathway. Moreover, the activating effects of semaglutide on the PKG/PKCe/ERK1/2 signaling pathway were weakened by KT-5823.

3.4. Semaglutide inhibited apoptosis of H/R H9C2 cells

The viability of H9C2 cells was lower than that of control cells, whereas that of H/R H9C2 cells was higher in the semaglutide and

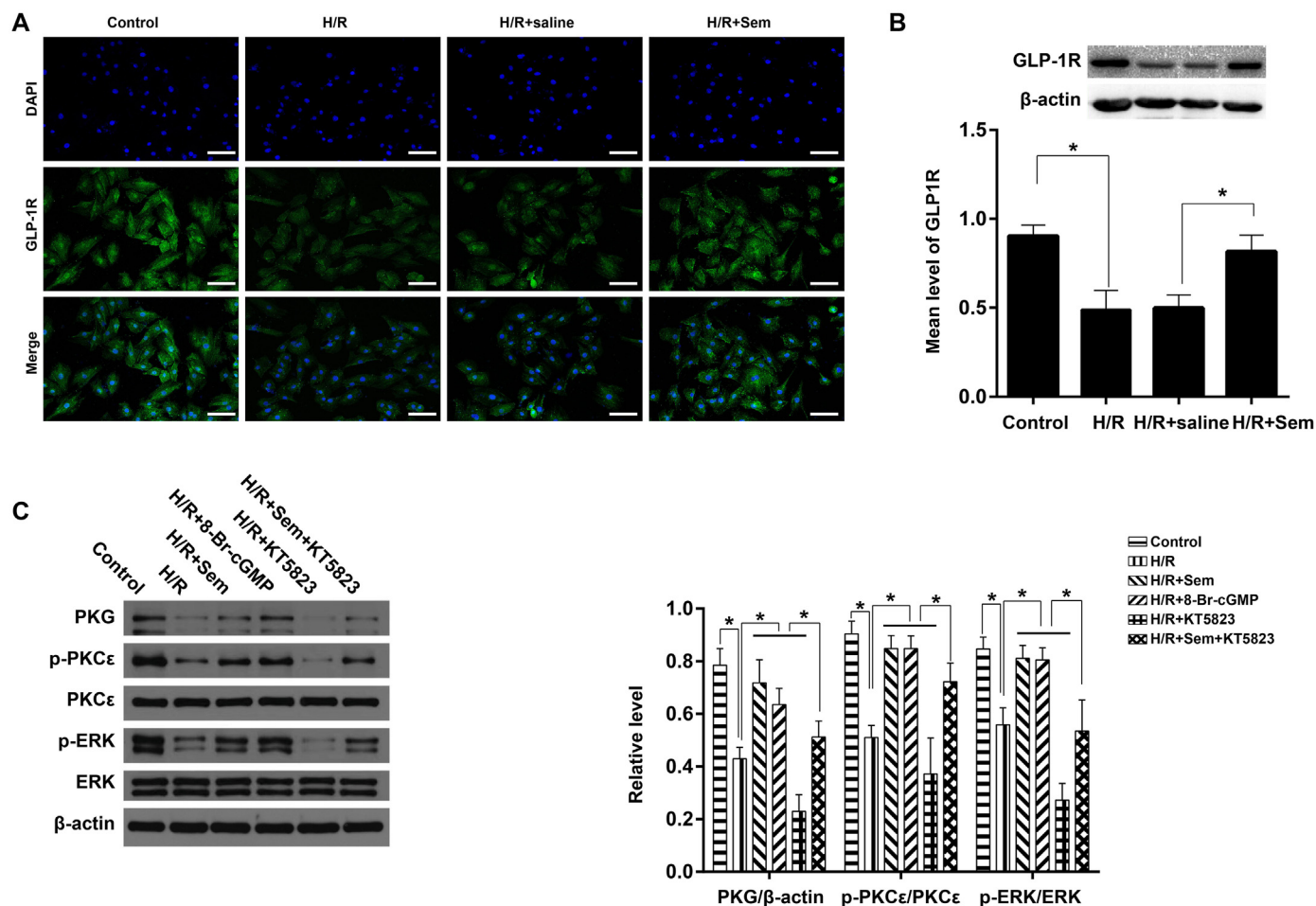


Fig. 3. Semaglutide promoted expression of GLP1R and activated the PKG/PKCe/ERK1/2 signaling pathway in H/R H9C2 cells. Immunofluorescent staining (A) and Western blot (B) of GLP-1R in the control, H/R, H/R + Saline and H/R + Semaglutide groups (Scale bar = 50 μ m). (C) Western blot of PKG/PKCe/ERK1/2 signaling pathway in the control, H/R, H/R + Semaglutide, H/R+8-Br-cGMP, H/R + KT-5823, and H/R + Semaglutide + KT-5823 groups. $n = 6$ per group; one-way ANOVA with Bonferroni post hoc analysis; * indicated $P < 0.05$.

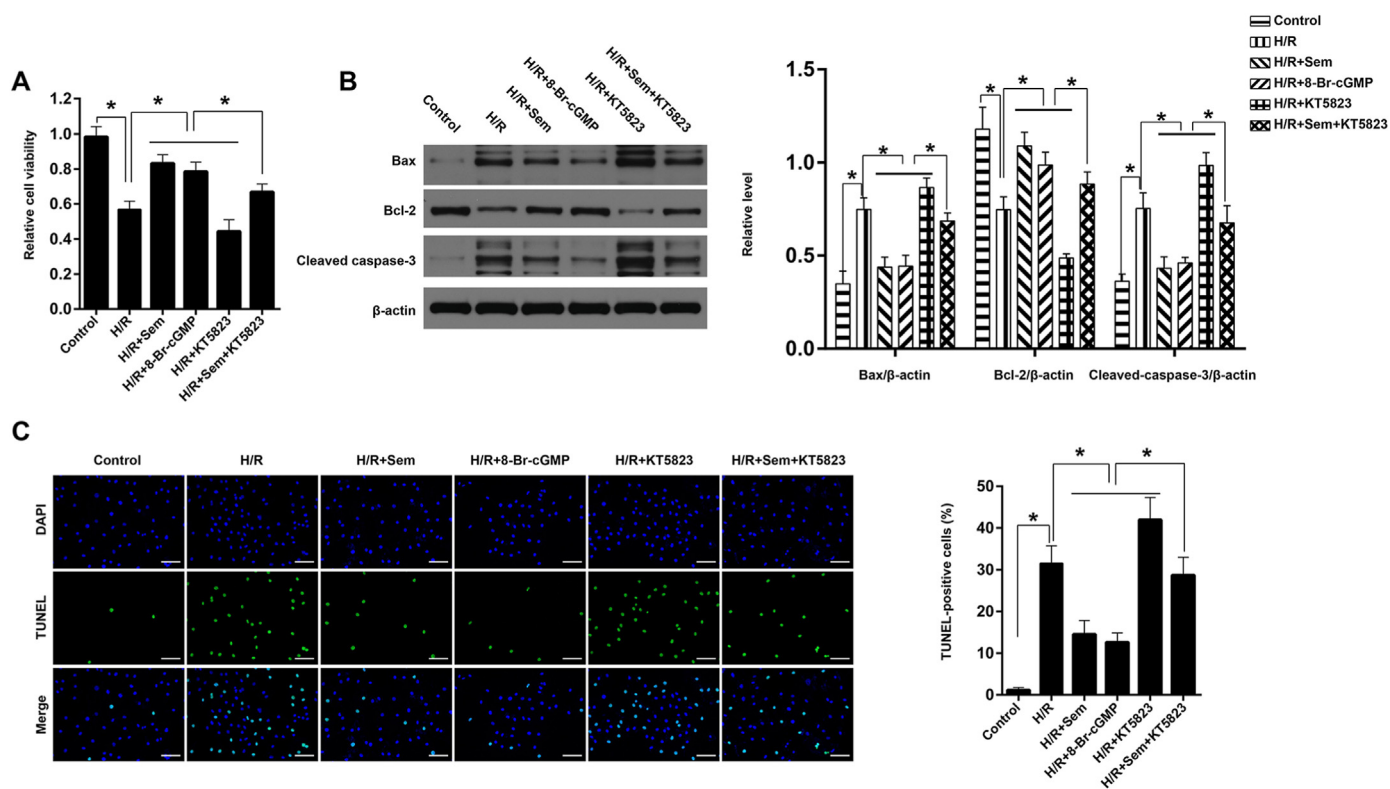


Fig. 4. Semaglutide inhibited apoptosis of H/R H9C2 cells. (A) Cell viability assay in the control, H/R, H/R + Semaglutide, H/R+8-Br-cGMP, H/R + KT-5823, and H/R + Semaglutide + KT-5823 groups. (B) Western blot of Bax, Bcl-2, and cleaved-caspase3 in the control, H/R, H/R + Semaglutide, H/R+8-Br-cGMP, H/R + KT-5823, and H/R + Semaglutide + KT-5823 groups. (C) TUNEL staining of heart tissues in the control, H/R, H/R + Semaglutide, H/R+8-Br-cGMP, H/R + KT-5823, and H/R + Semaglutide + KT-5823 groups (Scale bar = 50 μ m). $n = 6$ per group; one-way ANOVA with Bonferroni post hoc analysis; * indicates $P < 0.05$.

8-Br-cGMP groups than in the H/R group (Fig. 4A). H/R H9C2 cells treated with KT-5823 showed lower viability than H/R H9C2 cells treated with semaglutide and 8-Br-cGMP (Fig. 4A). Moreover, the effect of semaglutide on cell viability was inhibited by KT-5823 (Fig. 4A). Bax expression was lower and the higher expression was Bcl-2 in the H/R group than in the control group ($P < 0.05$, Fig. 4B). Both semaglutide and Br-cGMP downregulated Bax and up-regulate Bcl-2 in H/R cells, whereas KT-5823 could up-regulate Bax and cleaved-caspase3 and down-regulate Bcl-2 in H/R cells ($P < 0.05$, Fig. 4B). In addition, the effects of semaglutide on Bax and Bcl-2 expression were inhibited by KT-5823 ($P < 0.05$, Fig. 4B). These data suggest that semaglutide inhibited H/R H9C2 apoptosis, which was verified using TUNEL staining ($P < 0.05$, Fig. 4C).

4. Discussion

Several molecular mechanisms are related to the pathological process in myocardial I/R injury, containing energy depletion, oxidative stress, and activation of programmed cell death [23,24]. Apoptosis, a common form of programmed cell death, is an important pathophysiological change in I/R injury. Therefore, inhibiting apoptosis can be regarded as the dominant cardioprotective approach against this type of injury [25]. For example, Zhang et al. observed obvious apoptosis in H9C2 cells and suggested that melatonin inhibited apoptosis and may be a promising treatment agent against I/R injury. Moreover, Bcl-2 protected cardiomyocytes against apoptosis and was downregulated in I/R mice and H_2O_2 -treated H9C2 cells, and lncRNA H19 acted as a ceRNA of miR-877-3p [26]. Similarly, our study also found upregulation of Bax and downregulation of Bcl-2 in I/R rats and H/R H9C2 cells, implying that apoptosis was observed in myocardial I/R injury

in vivo and in vitro.

ERK1/2 (equal to p44/p42MAPK) are two isoforms of ERK that are involved in regulating different cell movements, including proliferation and migration [27]. ERK1/2 mediates apoptosis through several mechanisms, such as the activation of caspase-8 signaling, regulation of cytochrome *c* release, promotion of p53 stability and activity, and activation of cytosolic phospholipase A2 (cPLA2) by phosphorylation at serine 505 [27]. Recent studies have suggested that ERK1/2-mediated cardiomyocyte apoptosis plays an important role in the development of I/R injury and that activation of ERK1/2 can inhibit cardiomyocyte apoptosis [28,29]. ERK1/2 is activated through kinase signaling pathways, concluding PKG and PKC ϵ , which are regulated in response to chemical stimuli and various cytokines [27,30,31]. I/R injury can be triggered by G protein-coupled receptor-mediated activation of a cascade including PKG, which carries cardioprotective signals from the cytoplasm to the inner mitochondrial membrane through downstream pathways such as PKC ϵ [31,32]. In our study, we used 8-Br-cGMP and KT-5823 to activate and inactivate PKG in I/R rats and found that the PKG/PKC ϵ /ERK1/2 pathway was inactivated in I/R rats, 8-Br-cGMP activated PKG/PKC ϵ /ERK1/2 pathway, and KT-5823 inhibited PKG/PKC ϵ /ERK1/2 pathway in I/R rats. Moreover, 8-Br-cGMP inhibited MI, decreased hs-cTNT levels, increased NT-proBNP levels, and suppressed cardiomyocyte apoptosis. However, KT-5823 had the opposite effect to 8-Br-cGMP. These data suggest that activation of PKG/PKC ϵ /ERK1/2 exerted cardioprotective effects on I/R injury by inhibiting apoptosis, which is consistent with previous research [14].

Although GLP-1/GLP-1R has been identified as a candidate for treating type 2 diabetes, GLP-1/GLP-1R is involved in various physiological and pathological processes, such as apoptosis,

autophagy, inflammation, and oxidative stress, through binding with G protein-coupled receptors and calcium release [33–35]. It has been verified to be widely expressed in multiple organizations, such as pancreas, cardiovascular system, central nervous system, afferent neuronal ganglia, liver, and intestines [36]. Several studies have suggested that GLP-1/GLP-1R activation could protect against myocardial I/R injury through different molecular approaches [37,38]. Several studies have also indicated that GLP-1/GLP-1R activates the PKG pathway through multiple mechanisms. For example, Lee et al. concluded that liraglutide, a GLP-1R agonist, increased the expression of eNOS and sGC, and caused further increased synthesis of cGMP in rats with pulmonary arterial hypertension, which is important in the activation of the PKG pathway [39]. Chen et al. found that exendin-4 decreased infarct size and improved heart function in a rat model of heart failure after MI activation of the eNOS/cGMP/PKG pathway [40]. Considering the important roles of PKG, PKC ϵ , and ERK1/2 in myocardial I/R injury-induced cardiomyocyte apoptosis and the fact that GLP-1/GLP-1R activates the PKG pathway, we hypothesized that GLP-1/GLP-1R can activate the PKG/PKC ϵ /ERK1/2 pathway and inhibit cardiomyocyte apoptosis. Various experiments were performed to test this hypothesis. This study found that semaglutide inhibited MI, decreased hs-cTNT levels, increased NT-proBNP levels, and suppressed cardiomyocyte apoptosis in I/R rats and related cells, which was accompanied by activation of the PKG/PKC ϵ /ERK1/2 pathway.

5. Conclusion

In summary, we found that semaglutide inhibited I/R-induced cardiomyocyte apoptosis by activating the PKG/PKC ϵ /ERK1/2 pathway. The beneficial effect of GLP-1/GLP-1R, involved in the activation of the PKG/PKC ϵ /ERK1/2 pathway, may provide a novel treatment method for such I/R injury.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] W.H. Organization, Cardiovascular Diseases, CVDs, 2011.
- [2] M. Hayashi, W. Shimizu, C.M. Albert, The spectrum of epidemiology underlying sudden cardiac death, *Circ. Res.* 116 (2015) 1887–1906.
- [3] W. Zasada, B. Bobrowska, K. Plens, A. Dziewierz, Z. Siudak, A. Surdacki, D. Dudek, S. Bartus, Acute myocardial infarction in young patients, *Kardiolog. Pol.* 79 (2021) 1093–1098.
- [4] S.S. Virani, A. Alonso, E.J. Benjamin, M.S. Bittencourt, C.W. Callaway, A.P. Carson, A.M. Chamberlain, A.R. Chang, S. Cheng, F.N. Delling, L. Djousse, M. Elkind, J.F. Ferguson, M. Fornage, S.S. Khan, B.M. Kissela, K.L. Knutson, T.W. Kwan, D.T. Lackland, T.T. Lewis, J.H. Lichtman, C.T. Longenecker, M.S. Loop, P.L. Lutsey, S.S. Martin, K. Matsushita, A.E. Moran, M.E. Mussolino, A.M. Perak, W.D. Rosamond, G.A. Roth, U. Sampson, G.M. Satou, E.B. Schroeder, S.H. Shah, C.M. Shay, N.L. Spartano, A. Stokes, D.L. Tirschwell, L.B. VanWagner, C.W. Tsao, Heart disease and stroke statistics-2020 update: a report from the American heart association, *Circulation* 141 (2020) e139–e596.
- [5] B. Shin, D.B. Cowan, S.M. Emani, P.J. Del Nido, J.D. McCully, Mitochondrial transplantation in myocardial ischemia and reperfusion injury, *Adv. Exp. Med. Biol.* 982 (2017) 595–619.
- [6] L. Yao, H. Chen, Q. Wu, K. Xie, Hydrogen-rich saline alleviates inflammation and apoptosis in myocardial I/R injury via PINK-mediated autophagy, *Int. J. Mol. Med.* 44 (2019) 1048–1062.
- [7] K. Konstantinidis, R.S. Whelan, R.N. Kitsis, Mechanisms of cell death in heart disease, *Arterioscler. Thromb. Vasc. Biol.* 32 (2012) 1552–1562.
- [8] Z. Lang, X. Fan, H. Lin, L. Qiu, J. Zhang, C. Gao, Silencing of SNHG6 alleviates hypoxia/reoxygenation-induced cardiomyocyte apoptosis by modulating miR-135a-5p/HIF1AN to activate Shh/Gli1 signalling pathway, *J. Pharm. Pharmacol.* 73 (2021) 22–31.
- [9] M. Zhang, K. Cheng, H. Chen, J. Tu, Y. Shen, L. Pang, W. Wu, Galectin-3 knock down inhibits cardiac ischemia-reperfusion injury through interacting with bcl-2 and modulating cell apoptosis, *Arch. Biochem. Biophys.* 694 (2020), 108602.
- [10] H.T. Shi, Z.H. Huang, T.Z. Xu, A.J. Sun, J.B. Ge, New diagnostic and therapeutic strategies for myocardial infarction via nanomaterials, *EBioMedicine* 78 (2022), 103968.
- [11] T. Nakamura, K. Tsujita, Current trends and future perspectives for heart failure treatment leveraging cGMP modifiers and the practical effector PKG, *J. Cardiol.* 78 (2021) 261–268.
- [12] L.M. Yu, X. Dong, J. Zhang, Z. Li, X.D. Xue, H.J. Wu, Z.L. Yang, Y. Yang, H.S. Wang, Naringenin attenuates myocardial ischemia-reperfusion injury via cGMP-PKG signaling and in vivo and in vitro studies, 2019, *Oxid. Med. Cell. Longev.* (2019), 7670854.
- [13] X. Wan, K. Belanger, S.G. Widen, M.N. Kuyumcu-Martinez, N.J. Garg, Genes of the cGMP-PKG-Ca(2+) signaling pathway are alternatively spliced in cardiomyopathy: role of RBFOX2, *Biochim. Biophys. Acta, Mol. Basis Dis.* 1866 (2020), 165620.
- [14] T. Angelone, E. Filice, T. Pasqua, N. Amodio, M. Galluccio, G. Montesanti, A.M. Quintieri, M.C. Cerra, Nesfatin-1 as a novel cardiac peptide: identification, functional characterization, and protection against ischemia/reperfusion injury, *Cell. Mol. Life Sci.* 70 (2013) 495–509.
- [15] D.M. Zhang, Y. Chai, J.R. Erickson, J.H. Brown, D.M. Bers, Y.F. Lin, Intracellular signalling mechanism responsible for modulation of sarcolemmal ATP-sensitive potassium channels by nitric oxide in ventricular cardiomyocytes, *J. Physiol. (Lond.)* 592 (2014) 971–990.
- [16] D. Nathanson, O. Erdogdu, J. Pernow, Q. Zhang, T. Nyström, Endothelial dysfunction induced by triglycerides is not restored by exenatide in rat conduit arteries ex vivo, *Regul. Pept.* 157 (2009) 8–13.
- [17] L.A. Nikolaidis, D. Elahi, Y.T. Shen, R.P. Shannon, Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy, *Am. J. Physiol. Heart Circ. Physiol.* 289 (2005) H2401–H2408.
- [18] S. Hall, D. Isaacs, J.N. Clements, Pharmacokinetics and clinical implications of semaglutide: a new glucagon-like peptide (GLP)-1 receptor agonist, *Clin. Pharmacokinet.* 57 (2018) 1529–1538.
- [19] J. Huang, W. Qing, Y. Pan, NPAS2 ameliorates myocardial ischaemia/reperfusion injury in rats via CX3CL1 pathways and regulating autophagy, *Aging (Albany NY)* 13 (2021) 20569–20584.
- [20] Q. Li, X. Tuo, B. Li, Z. Deng, Y. Qiu, H. Xie, Semaglutide attenuates excessive exercise-induced myocardial injury through inhibiting oxidative stress and inflammation in rats, *Life Sci.* 250 (2020), 117531.
- [21] Z. Shi, F. Fu, L. Yu, W. Xing, F. Su, X. Liang, R. Tie, L. Ji, M. Zhu, J. Yu, H. Zhang, Vasonatin peptide attenuates myocardial ischemia-reperfusion injury in diabetic rats and underlying mechanisms, *Am. J. Physiol. Heart Circ. Physiol.* 308 (2015) H281–H290.
- [22] P. Chang, M. Zhang, X. Zhang, G. Li, H. Hu, J. Wu, X. Wang, Z. Yang, J. Zhang, W. Chen, M. Ren, X. Li, M. Zhu, B. Chen, J. Yu, B-type natriuretic peptide attenuates endoplasmic reticulum stress in H9c2 cardiomyocytes underwent hypoxia/reoxygenation injury under high glucose/high fat conditions, *Peptides* 111 (2019) 103–111.
- [23] A. Lochner, E. Marais, B. Huisamen, Melatonin and cardioprotection against ischaemia/reperfusion injury: What's new? A review, *J. Pineal Res.* 65 (2018), e12490.
- [24] J. Wang, H. Zhou, Mitochondrial quality control mechanisms as molecular targets in cardiac ischemia-reperfusion injury, *Acta Pharm. Sin. B* 10 (2020) 1866–1879.
- [25] R. Badalzadeh, B. Mokhtari, R. Yavari, Contribution of apoptosis in myocardial reperfusion injury and loss of cardioprotection in diabetes mellitus, *J. Physiol. Sci.* 65 (2015) 201–215.
- [26] X. Li, S. Luo, J. Zhang, Y. Yuan, W. Jiang, H. Zhu, X. Ding, L. Zhan, H. Wu, Y. Xie, R. Song, Z. Pan, Y. Lu, lncRNA H19 alleviated myocardial I/R via suppressing miR-877-3p/bcl-2-mediated mitochondrial apoptosis, *Mol. Ther. Nucleic Acids* 17 (2019) 297–309.
- [27] S. Cagnol, J.C. Chambard, ERK and cell death: mechanisms of ERK-induced cell death—apoptosis, autophagy and senescence, *FEBS J.* 277 (2010) 2–21.
- [28] S. Zhang, H. Li, S.J. Yang, Tribulosin suppresses apoptosis via PKC epsilon and ERK1/2 signaling pathway during hypoxia/reoxygenation in neonatal rat ventricular cardiac myocytes, *J. Asian Nat. Prod. Res.* 13 (2011) 1135–1145.
- [29] Y. Yin, Y. Guan, J. Duan, G. Wei, Y. Zhu, W. Quan, C. Guo, D. Zhou, Y. Wang, M. Xi, A. Wen, Cardioprotective effect of Danshensu against myocardial ischemia/reperfusion injury and inhibits apoptosis of H9c2 cardiomyocytes via Akt and ERK1/2 phosphorylation, *Eur. J. Pharmacol.* 699 (2013) 219–226.
- [30] J. Šrámek, V. Němcová-Fürstová, J. Kovár, Kinase signaling in apoptosis induced by saturated fatty acids in pancreatic β -cells, *Int. J. Mol. Sci.* 17 (2016).
- [31] A.D. Costa, K.D. Garlid, I.C. West, T.M. Lincoln, J.M. Downey, M.V. Cohen, S.D. Critz, Protein kinase G transmits the cardioprotective signal from cytosol to mitochondria, *Circ. Res.* 97 (2005) 329–336.
- [32] M.V. Cohen, X.M. Yang, Y. Liu, N.V. Solenkova, J.M. Downey, Cardioprotective

- PKG-independent NO signaling at reperfusion, *Am. J. Physiol. Heart Circ. Physiol.* 299 (2010) H2028–H2036.
- [33] M. Correale, O. Lamacchia, M. Ciccarelli, G. Dattilo, L. Tricarico, N.D. Brunetti, Vascular and Metabolic Effects of SGLT2i and GLP-1 in Heart Failure Patients, *Heart Fail Rev*, 2021.
- [34] E. Candeias, I. Sebastião, S. Cardoso, C. Carvalho, M.S. Santos, C.R. Oliveira, P.I. Moreira, A.I. Duarte, Brain GLP-1/IGF-1 signaling and autophagy mediate exendin-4 protection against apoptosis in type 2 diabetic rats, *Mol. Neurobiol.* 55 (2018) 4030–4050.
- [35] A. Winiarska, M. Knysak, K. Nabrdalik, J. Gumprecht, T. Stompór, Inflammation and oxidative stress in diabetic kidney disease: the targets for SGLT2 inhibitors and GLP-1 receptor agonists, *Int. J. Mol. Sci.* 22 (2021).
- [36] P. Richards, H.E. Parker, A.E. Adriaenssens, J.M. Hodgson, S.C. Cork, S. Trapp, F.M. Gribble, F. Reimann, Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model, *Diabetes* 63 (2014) 1224–1233.
- [37] H.C. Ku, W.P. Chen, M.J. Su, DPP4 deficiency preserves cardiac function via GLP-1 signaling in rats subjected to myocardial ischemia/reperfusion, *Nauyn-Schmiedeberg's Arch. Pharmacol.* 384 (2011) 197–207.
- [38] J. Chen, S. Xu, L. Wang, W. Zhou, P. Li, N. Deng, Q. Tang, Y. Li, L. Wu, J. Chen, W. Li, Exendin-4 inhibits atrial arrhythmogenesis in a model of myocardial infarction-induced heart failure via the GLP-1 receptor signaling pathway, *Exp. Ther. Med.* 20 (2020) 3669–3678.
- [39] M.Y. Lee, K.B. Tsai, J.H. Hsu, S.J. Shin, J.R. Wu, J.L. Yeh, Liraglutide prevents and reverses monocrotaline-induced pulmonary arterial hypertension by suppressing ET-1 and enhancing eNOS/sGC/PKG pathways, *Sci. Rep.* 6 (2016), 31788.
- [40] J. Chen, D. Wang, F. Wang, S. Shi, Y. Chen, B. Yang, Y. Tang, C. Huang, Exendin-4 inhibits structural remodeling and improves Ca(2+) homeostasis in rats with heart failure via the GLP-1 receptor through the eNOS/cGMP/PKG pathway, *Peptides* 90 (2017) 69–77.