SHORT COMMUNICATION

Liraglutide Preserves Intracellular Calcium Handling in Isolated Murine Myocytes Exposed to Oxidative Stress

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Summary

In ischemic/reperfusion (I/R) injured hearts, severe oxidative stress occurs and is associated with intracellular calcium (Ca^{2+}) overload. Glucagon-Like Peptide-1 (GLP-1) analogues have been shown to exert cardioprotection in I/R heart. However, there is little information regarding the effects of GLP-1 analogue on the intracellular Ca^{2+} regulation in the presence of oxidative stress. Therefore, we investigated the effects of GLP-1 analogue, (liraglutide, 10 μM) applied before or after hydrogen peroxide (H_2O_2 , 50 μM) treatment on intracellular Ca^{2+} regulation in isolated cardiomyocytes. We hypothesized that liraglutide can attenuate intracellular Ca^{2+} overload in cardiomyocytes under H_2O_2 -induced cardiomyocyte injury. Cardiomyocytes were isolated from the hearts of male Wistar rats. Isolated cardiomyocytes were loaded with Fura-2/AM and fluorescence intensity was recorded. Intracellular Ca^{2+} transient decay rate, intracellular Ca^{2+} transient amplitude and intracellular diastolic Ca^{2+} levels were recorded before and after treatment with liraglutide. In H_2O_2 induced severe oxidative stressed cardiomyocytes (which mimic cardiac I/R) injury, liraglutide given prior to or after H_2O_2 administration effectively increased both intracellular Ca^{2+} transient amplitude and intracellular Ca^{2+} transient decay rate, without altering the intracellular diastolic Ca^{2+} level. Liraglutide attenuated intracellular Ca^{2+} overload in H_2O_2 -induced cardiomyocyte injury and may be responsible for cardioprotection during cardiac I/R injury by

preserving physiological levels of calcium handling during the systolic and diastolic phases of myocyte activation.

Key words

Liraglutide • Calcium regulation • Cardiomyocyte • Ischemic/Reperfusion • Cardioprotective

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Since the risk of coronary heart disease is increased 2 to 4 times in type-2 diabetic patients (Beckman *et al.* 2002), anti-diabetic drugs that are associated with the reduction of cardiovascular events may have beneficial effects for this group of patients. Glucagon-Like Peptide-1 (GLP-1) is an incretin peptide secreting from intestinal L-cells, which has a potent effect on glycemic control (Amori *et al.* 2007). The GLP-1 receptors were expressed in ventricular myocytes (Ban *et al.* 2008, Richards *et al.* 2014). Liraglutide is one of a long-acting GLP-1 analogue, which has potent glucose lowering effects for treatment of hyperglycemia in type 2 diabetes patients (Amori *et al.* 2007). Recent studies demonstrated that GLP-1 analogues exert potent cardioprotective effects in both clinical trials

and animal models (Amori *et al.* 2007, Arturi *et al.* 2016, Chen *et al.* 2016, Kumarathurai *et al.* 2016, Nikolaidis *et al.* 2005, Sonne *et al.* 2008). In animal models, growing evidence demonstrates the cardioprotective effects of GLP-1 in addition to its glycemic control properties (Nikolaidis *et al.* 2005). GLP-1 analogues have been shown to improve cardiac function in ischemic/reperfusion (I/R) injury of porcine model *via* reduced oxidative stress and increased phosphorylated Akt and Bcl-2 expression (Timmers *et al.* 2009) and activate cytoprotective pathways after I/R injury by modulating the expression and activity of cardioprotective genes including Akt, GSK3 β , PPAR β -delta, Nrf-2, and HO-1 (Noyan-Ashraf *et al.* 2009). Recent reports also support these basic studies by demonstrating that GLP-1 analogues have exerted potent cardioprotective effects in clinical trials by improved left ventricular ejection fraction, cardiac output, and left ventricular end-diastolic diameter in patients with myocardial infarction and chronic heart failure (Arturi *et al.* 2016, Chen *et al.* 2016, Chen *et al.* 2015, Kumarathurai *et al.* 2016). Using I/R period, severe oxidative stress occurs and has been shown to be associated with intracellular Ca²⁺ overload, thus facilitating both electrical and mechanical dysfunction in the heart (Shintani-Ishida *et al.* 2012). Therefore, treatment options which prevent intracellular Ca²⁺ overload could potentially be beneficial for I/R hearts. Although currently there is only one study reporting the benefit of GLP-1 on improving intracellular Ca²⁺ homeostasis in HL-1 cells (Huang *et al.* 2016) and one study reporting the neutral effects of liraglutide in cardiac I/R model (Kristensen *et al.* 2009), there is no available information regarding the effects of liraglutide on intracellular Ca²⁺ regulation in the ventricular cardiomyocyte. Therefore, we investigated the effect of liraglutide on the intracellular Ca²⁺ transient in isolated rat cardiomyocytes in this study. Hydrogen peroxide (H₂O₂) was used to induce severe oxidative stress similar to that observed during I/R injury. We hypothesized that liraglutide can attenuate intracellular Ca²⁺ overload in cardiomyocytes under H₂O₂-induced cardiomyocyte injury.

This study was approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Chiang Mai University. All the animals were fed with normal rat chow and water *ad libitum* for two weeks prior to experimentation. Male Wistar rats (8-10-week-old, 250-300 g) were used. The rats were deeply anesthetized with thiopental (0.5 mg/kg; Research institute of antibiotics and biotransformations, Roztoky,

Czech Republic) after which the hearts were removed for single ventricular myocyte isolation (Palee *et al.* 2016, Palee *et al.* 2013).

The isolated cardiomyocytes were used in each study protocol for the measurement of intracellular Ca²⁺ transient. In the first protocol, cardiomyocytes were divided into 3 groups (n=8 cells/rat and 8 rats/group) as shown in Figure 1A. The real-time Ca²⁺ measurements were performed at the beginning of the study (baseline). Then, cardiomyocytes in Group I were treated with normal saline solution (NSS) for 5 min as a control group. Group II's cells were treated with NSS for 2 min and then H₂O₂ for 3 min to simulate I/R injury. Group III's cells were treated with liraglutide (10 μ M) (Novo Nordisk A/S, Denmark) for 5 min. We used liraglutide at a clinically relevant dose; patients receive the maximum clinical dose of 1.8 mg once a day (Margulies *et al.* 2016). The concentration we used for an *in vitro* study in this study was 10 μ M of liraglutide which was approximately similar to the dose used in human (Langlois *et al.* 2016).

In the second protocol, cardiomyocytes were divided into 4 groups (n=8 cells/rat and 8 rats/group) as shown in Figure 2A. The real-time Ca²⁺ measurements were performed at the beginning of the study (baseline). Then, cardiomyocytes in Group I were treated with NSS for 10 min followed by H₂O₂ for 3 min as a control group. Group II's cells were treated with NSS for 5 min followed by liraglutide (10 μ M) for 5 min and then H₂O₂ for 3 min. Group III's cardiomyocytes were treated with NSS for 5 min followed by H₂O₂ for 3 min and then NSS for 5 min as another control group. Group IV were treated with NSS for 5 min followed by H₂O₂ for 3 min and then liraglutide (10 μ M) for 5 min. The real-time Ca²⁺ measurements were performed after drug treatment in all groups (Palee *et al.* 2016).

In this study, we used H₂O₂ (50 μ M) to induce oxidative stress, to simulate the oxidative stress that is generated by ischemia/reperfusion injury. H₂O₂ concentration at 50 μ M has been widely used to trigger oxidative stress-induced intracellular Ca²⁺ dyshomeostasis in cardiomyocytes. H₂O₂ has been shown to decrease sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA) and sodium-calcium exchanger (NCX) activities (Huang *et al.* 2014) by inhibiting protein kinase C (PKC) activities, leading to the alteration of the intracellular Ca²⁺ homeostasis (Goldhaber 1996, Reeves *et al.* 1986).

Fig. 1. A schematic of study protocol I (**A**) and the effects of liraglutide on intracellular Ca²⁺ transient amplitude (**B**), intracellular Ca²⁺ transient decay rate (**C**), intracellular diastolic Ca²⁺ levels (**D**) and the representative images of Ca²⁺ transient tracing (**E**). *P<0.05 vs. NSS, †P<0.05 vs. H₂O₂ + NSS. NSS = normal saline solution, H₂O₂ = hydrogen peroxide, Ca²⁺ = intracellular calcium measurement.

Fig. 2. A schematic of study protocol II (**A**) and the effects of liraglutide administration before and after H₂O₂ application on intracellular Ca²⁺ transient in cardiomyocytes. Liraglutide significantly increased intracellular Ca²⁺ transient amplitude (**B**) and increased intracellular Ca²⁺ transient decay rate (**C**), but did not alter intracellular diastolic Ca²⁺ levels (**D**), when compared with the H₂O₂ group and the representative images of Ca²⁺ transient tracing (**E**). *P<0.05 vs. NSS + H₂O₂, †P<0.05 vs. H₂O₂ + NSS. NSS=normal saline solution, H₂O₂ = hydrogen peroxide, Ca²⁺ = intracellular calcium measurement.

Cardiomyocytes were isolated from the hearts of male Wistar rats using a method described previously (Palee *et al.* 2016). In brief, under deep anesthesia, the heart was immediately removed and placed into a modified Langendorff apparatus. The hearts were perfused with modified Krebs solution as previously described (Palee *et al.* 2016) for 5 min, followed by calcium-free solution (100 μ M EGTA) for 4 min, Tyrode's solution with collagenase (0.1 mg/ml) for 10 min, and modified Krebs solution containing 100 μ M CaCl_2 and 1 mg/ml type II collagenase for another 8 min. The ventricles were removed from the cannula, cut into small pieces and incubated in 10 ml of collagenase solution gassed with 100 % O_2 for 7 min at 37 °C. A pipette was used to pipette the cell suspension up and down in order to dissociate cardiac tissue into single cells. The cardiomyocytes were separated from undigested ventricular tissues by filtering through cell strainer, and were settling into a loose pellet. Then, the supernatant was removed and replaced with modified Krebs solution containing 1 % BSA and 500 μ M CaCl_2 . This process was repeated with modified Krebs solution containing 1 mM CaCl_2 . After this procedure, the cardiomyocytes were ready for recording. (Palee *et al.* 2013) The isolated cardiomyocytes were placed in a modified Krebs solution containing 1 mM CaCl_2 . Intracellular Ca^{2+} transient were measured using the CELL^R imaging software (Olympus Soft Imaging Solutions GmbH, Germany). The isolated cardiomyocytes were loaded with Fura-2/AM at a final concentration of 5 μ M and fluorescent intensity (excitation wavelengths are 340 nm and 380 nm, and emission wavelength is 510 nm) was recorded during electrical pacing (1 Hz, 10 ms duration, 15 V) (Palee *et al.* 2016). The ratio of the emissions wavelengths (510 nm) is directly related to the amount of intracellular Ca^{2+} . Data are shown as mean $\pm$ SD. Comparisons of variables were performed using the one-way ANOVA followed by LSD *post hoc* test. $P < 0.05$ was considered statistically significant.

We investigated the effects of liraglutide on intracellular Ca^{2+} handling in isolated rat cardiac myocytes exposed to hydrogen peroxide solution to provoke oxidative stress. H_2O_2 significantly decreased both intracellular Ca^{2+} transient amplitude (Fig. 1B) and intracellular Ca^{2+} transient decay rate (Fig. 1C). However, intracellular diastolic Ca^{2+} levels were not altered (Fig. 1D), when compared to the control group (i.e. cardiomyocytes treated with NSS). Moreover, liraglutide

(10 μ M) significantly increased the intracellular Ca^{2+} transient amplitude (Fig. 1B) and Ca^{2+} transient decay rate (Fig. 1C), but did not alter intracellular diastolic Ca^{2+} levels (Fig. 1D), when compared to the control group. The representative Ca^{2+} transient tracings are shown in Figure 1E.

In the simulated I/R injury protocol, our results demonstrated that cardiomyocytes pretreated with liraglutide significantly increased the intracellular Ca^{2+} transient amplitude (Fig. 2B) and the intracellular Ca^{2+} transient decay rate (Fig. 2C), when compared to the H_2O_2 treated group. However, in all experimental groups, the levels of intracellular diastolic Ca^{2+} levels did not differ (Fig. 2D). The representative Ca^{2+} transient tracings are shown in Figure 2E. Interestingly, we found that when liraglutide was given after H_2O_2 application to cardiomyocytes, it still significantly increased the intracellular Ca^{2+} transient amplitude and intracellular Ca^{2+} transient decay rate, when compared to the H_2O_2 treated group (Fig. 2B, 2C). Similar to the results of pretreatment, liraglutide given after H_2O_2 application did not alter the intracellular diastolic Ca^{2+} levels.

Since patients with type-2 diabetes mellitus have a higher risk (2 to 4 fold) for developing coronary heart disease including myocardial infarction (Beckman *et al.* 2002), anti-diabetic drugs with cardioprotection will be beneficial to these patients. It is known that fatal arrhythmias and LV dysfunction are often observed following acute myocardial infarction (Takamatsu 2008). Importantly, impaired intracellular Ca^{2+} regulation has been shown to be an important factor responsible for these pathological effects (Takamatsu 2008). Therefore, treatment options which can attenuate the impairment of intracellular Ca^{2+} homeostasis could provide cardioprotection for the ischemic heart. In the present study, our results clearly demonstrated that liraglutide exerted cardioprotective effects against H_2O_2 -induced cardiomyocyte injury by attenuating intracellular Ca^{2+} overload.

GLP-1 receptor is expressed in the heart and ventricular myocyte and has a high affinity with a specific GLP-1 receptor agonist liraglutide (Pyke *et al.* 2014, Saraiva *et al.* 2014). Therefore, in this study the cardioprotective effect of liraglutide is mediated by the GLP-1 receptor dependent pathway *via* increased phosphorylation of Akt and GSK3 β which are involved in the reperfusion injury survival kinase (RISK) pathway (Hausenloy *et al.* 2005). This finding was supported by previous studies reported the cardioprotective effects of

GLP-1 in animal models (Bose *et al.* 2005, Bose *et al.* 2007, Kavianipour *et al.* 2003, Nikolaidis *et al.* 2005). Liraglutide pre- and post-treatment in cardiac I/R injury has been shown to provide cardioprotective effects in both animals and clinical studies (Chen *et al.* 2016, McCormick *et al.* 2015, Noyan-Ashraf *et al.* 2009, Salling *et al.* 2012).

In the present study using H₂O₂-induced cardiomyocyte injury, our results demonstrated that intracellular Ca²⁺ transient amplitude was impaired by H₂O₂ and both of liraglutide pre- and post-treatment significantly increased intracellular Ca²⁺ transient amplitude. Our finding consistent with previous studies reported that liraglutide exerts cardioprotective effects by activating GLP-1 receptors in cardiomyocytes by coupled with the G-protein/adenylyl cyclase complex to increase cyclic adenosine monophosphate (cAMP) production. Then, activates protein kinase A (PKA) and Ca²⁺ channel phosphorylation, respectively. Finally, increase Ca²⁺ influx and increasing cardiomyocyte contractility (Kristensen *et al.* 2009). Moreover, cAMP activate sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA2a) activity and then increases Ca²⁺ reuptake into the endoplasmic reticulum (Younce *et al.* 2013), leading to cardiomyocyte relaxation. Moreover, we found that liraglutide increased intracellular Ca²⁺ transient decay rates. This finding is consistent with previous findings, which reported that liraglutide increased intracellular cAMP and activated SERCA2a activity and then increased Ca²⁺ reuptake into the endoplasmic reticulum (Younce *et al.* 2013). This finding also helped to explain the results in a previous report which showed that a GLP-1 analogue improved diastolic functions in liraglutide-treated mice (Noyan-Ashraf *et al.* 2009) and liraglutide also reduced the severity of left ventricular dilation in that study (Noyan-Ashraf *et al.* 2009). Therefore, the ability of liraglutide to attenuate the impairment of physiological Ca²⁺ handling in

a H₂O₂-induced cardiomyocyte injury model by increasing intracellular Ca²⁺ amplitude and decay rates, is a cardioprotective effect, in addition to its glycemic control, which is responsible for the improvement of cardiac function observed in previous reports. In addition, our results showed that liraglutide did not alter the intracellular diastolic Ca²⁺ level. Even though there is a high level of intracellular Ca²⁺ transient amplitude which reflect an increased intracellular Ca²⁺ during systolic period, there was a high rate of Ca²⁺ elimination which represented by intracellular Ca²⁺ transient decay rate. The balance on this intracellular calcium regulation could be contributed to the unaltered intracellular diastolic calcium level as seen in this study. Although we did not assess the oxidative stress parameters, previous studies demonstrated that liraglutide activated of PI3K-Akt-eNOS-NO signaling pathway and inhibited of oxidative stress (Inoue *et al.* 2015, Liu *et al.* 2016, Noyan-Ashraf *et al.* 2009).

Conflict of Interest

There is no conflict of interest.

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Abbreviations

Ca²⁺ – calcium, GLP-1 – glucagon-like peptide-1, H₂O₂ – hydrogen peroxide, I/R – ischemic/reperfusion, NCX – sodium-calcium exchanger, NSS – normal saline solution, PKC – protein kinase C, SERCA – sarco/endoplasmic reticulum Ca²⁺-ATPase

References

- AMORI RE, LAU J, PITTAS AG: Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. *JAMA* **298**: 194-206, 2007.
- ARTURI F, SUCCURRO E, MICELI S, CLORO C, RUFFO M, MAIO R, PERTICONE M, SESTI G, PERTICONE F: Liraglutide improves cardiac function in patients with type 2 diabetes and chronic heart failure. *Endocrine* **57**: 464-473, 2016.
- BAN K, NOYAN-ASHRAF MH, HOEFER J, BOLZ SS, DRUCKER DJ, HUSAIN M: Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation* **117**: 2340-2350, 2008.

- BECKMAN JA, CREAGER MA, LIBBY P: Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* **287**: 2570-2581, 2002.
- BOSE AK, MOCANU MM, CARR RD, BRAND CL, YELLON DM: Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes* **54**: 146-151, 2005.
- BOSE AK, MOCANU MM, CARR RD, YELLON DM: Myocardial ischaemia-reperfusion injury is attenuated by intact glucagon like peptide-1 (GLP-1) in the in vitro rat heart and may involve the p70s6K pathway. *Cardiovasc Drugs Ther* **21**: 253-256, 2007.
- CHEN WR, CHEN YD, TIAN F, YANG N, CHENG LQ, HU SY, WANG J, YANG JJ, WANG SF, GU XF: Effects of liraglutide on reperfusion injury in patients with ST-segment-elevation myocardial infarction. *Circ Cardiovasc Imaging* **9**: e005146, 2016.
- CHEN WR, HU SY, CHEN YD, ZHANG Y, QIAN G, WANG J, YANG JJ, WANG ZF, TIAN F, NING QX: Effects of liraglutide on left ventricular function in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Am Heart J* **170**: 845-854, 2015.
- CHEN WR, TIAN F, CHEN YD, WANG J, YANG JJ, WANG ZF, DA WANG J, NING QX: Effects of liraglutide on no-reflow in patients with acute ST-segment elevation myocardial infarction. *Int J Cardiol* **208**: 109-114, 2016.
- GOLDHABER JI: Free radicals enhance $\text{Na}^+/\text{Ca}^{2+}$ exchange in ventricular myocytes. *Am J Physiol* **271**: H823-H833, 1996.
- HAUSENLOY DJ, TSANG A, YELLON DM: The reperfusion injury salvage kinase pathway: a common target for both ischemic preconditioning and postconditioning. *Trends Cardiovasc Med* **65**: 69-75, 2005.
- HUANG JH, CHEN YC, LEE TI, KAO YH, CHAZO TF, CHEN SA, CHEN YJ: Glucagon-like peptide-1 regulates calcium homeostasis and electrophysiological activities of HL-1 cardiomyocytes. *Peptides* **78**: 91-98, 2016.
- HUANG SY, LU YY, CHEN YC, CHEN WT, LIN YK, CHEN SA, CHEN YJ: Hydrogen peroxide modulates electrophysiological characteristics of left atrial myocytes. *Acta Cardiol Sin* **30**: 38-45, 2014.
- INOUE T, INOUCHI T, SONODA N, HENDARTO H, MAKIMURA H, SASAKI S, YOKOMIZO H, FUJIMURA Y, MIURA D, TAKAYANAGI R: GLP-1 analog liraglutide protects against cardiac steatosis, oxidative stress and apoptosis in streptozotocin-induced diabetic rats. *Atherosclerosis* **240**: 250-259, 2015.
- KAVIANIPOUR M, EHLERS MR, MALMBERG K, RONQUIST G, RYDEN L, WIKSTROM G, GUTNIAK M: Glucagon-like peptide-1 (7-36) amide prevents the accumulation of pyruvate and lactate in the ischemic and non-ischemic porcine myocardium. *Peptides* **24**: 569-578, 2003.
- KRISTENSEN J, MORTENSEN UM, SCHMIDT M, NIELSEN PH, NIELSEN TT, MAENG M: Lack of cardioprotection from subcutaneously and preischemic administered liraglutide in a closed chest porcine ischemia reperfusion model. *BMC Cardiovasc Disord* **9**: 31, 2009.
- KUMARATHURAI P, ANHOLM C, NIELSEN OW, KRISTIANSEN OP, MOLVIG J, MADSBAD S, HAUGAARD SB, SAJADIEH A: Effects of the glucagon-like peptide-1 receptor agonist liraglutide on systolic function in patients with coronary artery disease and type 2 diabetes: a randomized double-blind placebo-controlled crossover study. *Cardiovasc Diabetol* **15**: 105, 2016.
- LANGLOIS A, DAL S, VIVOT K, MURA C, SEYFRITZ E, BIETIGER W, DOLLINGER C, PERONET C, MAILLARD E, PINGET M, JEANDIDIER N, SIGRIST S: Improvement of islet graft function using liraglutide is correlated with its anti-inflammatory properties. *Br J Pharmacol* **173**: 3443-3453, 2016.
- LIU MM, SHEN YF, CHEN C, LAI XY, ZHANG MY, YU R: Protective effect of glucagon-like peptide-1 analogue on cardiomyocytes injury induced by hypoxia/reoxygenation. *Zhonghua Nei Ke Za Zhi* **55**: 311-316, 2016.
- MARGULIES KB, HERNANDEZ AF, REDFIELD MM, GIVERTZ MM, OLIVEIRA GH, COLE R, MANN DL, WHELLAN DJ, KIERNAN MS, FELKER GM, ET AL.: Effects of liraglutide on clinical stability among patients with advanced heart failure and reduced ejection fraction: A randomized clinical trial. *JAMA* **316**: 500-508, 2016.
- MCCORMICK LM, HOOLE SP, WHITE PA, READ PA, AXELL RG, CLARKE SJ, O'SULLIVAN M, WEST NE, DUTKA DP: Pre-treatment with glucagon-like Peptide-1 protects against ischemic left ventricular dysfunction and stunning without a detected difference in myocardial substrate utilization. *JACC Cardiovasc Interv* **8**: 292-301, 2015.

- NIKOLAIDIS LA, DOVERSPIKE A, HENTOSZ T, ZOURELIAS L, SHEN YT, ELAHI D, SHANNON RP: Glucagon-like peptide-1 limits myocardial stunning following brief coronary occlusion and reperfusion in conscious canines. *J Pharmacol Exp Ther* **312**: 303-308, 2005.
- NOYAN-ASHRAF MH, MOMEN MA, BAN K, SADI AM, ZHOU YQ, RIAZI AM, BAGGIO LL, HENKELMAN RM, HUSAIN M, DRUCKER DJ: GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. *Diabetes* **58**: 975-983, 2009.
- PALEE S, APAIJAI N, SHINLAPAWITTAYATORN K, CHATTIPAKORN SC, CHATTIPAKORN N: Acetylcholine attenuates hydrogen peroxide-induced intracellular calcium dyshomeostasis through both muscarinic and nicotinic receptors in cardiomyocytes. *Cell Physiol Biochem* **39**: 341-349, 2016.
- PALEE S, WEERATEERANGKUL P, CHINDA K, CHATTIPAKORN SC, CHATTIPAKORN N: Mechanisms responsible for beneficial and adverse effects of rosiglitazone in a rat model of acute cardiac ischaemia-reperfusion. *Exp Physiol* **98**: 1028-1037, 2013.
- PYKE C, HELLER RS, KIRK RK, ORSKOV C, REEDTZ-RUNGE S, KAASTRUP P, HVELPLUND A, BARDRAM L, CALATAYUD D, KNUDSEN LB: GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology* **155**: 1280-1290, 2014.
- REEVES JP, BAILEY CA, HALE CC: Redox modification of sodium-calcium exchange activity in cardiac sarcolemmal vesicles. *J Biol Chem* **261**: 4948-4955, 1986.
- RICHARDS P, PARKER HE, ADRIAENSSENS AE, HODGSON JM, CORK SC, TRAPP S, GRIBBLE FM, REIMANN F: Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. *Diabetes* **63**: 1224-1233, 2014.
- SALLING HK, DOHLER KD, ENGSTROM T, TREIMAN M: Postconditioning with curaglutide, a novel GLP-1 analog, protects against heart ischemia-reperfusion injury in an isolated rat heart. *Regul Pept* **178**: 51-55, 2012.
- SARAIVA FK, SPOSITO AC: Cardiovascular effects of glucagon-like peptide 1 (GLP-1) receptor agonists. *Cardiovasc Diabetol* **13**: 142, 2014.
- SHINTANI-ISHIDA K, INUI M, YOSHIDA K: Ischemia-reperfusion induces myocardial infarction through mitochondrial Ca(2)(+) overload. *J Mol Cell Cardiol* **53**: 233-239, 2012.
- SONNE DP, ENGSTROM T, TREIMAN M: Protective effects of GLP-1 analogues exendin-4 and GLP-1(9-36) amide against ischemia-reperfusion injury in rat heart. *Regul Pept* **146**: 243-249, 2008.
- TAKAMATSU T: Arrhythmogenic substrates in myocardial infarct. *Pathol Int* **58**: 533-543, 2008.
- TIMMERS L, HENRIQUES JP, DE KLEIJN DP, DEVRIES JH, KEMPERMAN H, STEENDIJK P, VERLAAN CW, KERVER M, PIEK JJ, DOEVENDANS PA, ET AL.: Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. *J Am Coll Cardiol* **53**: 501-510, 2009.
- YOUNCE CW, BURMEISTER MA, AYALA JE: Exendin-4 attenuates high glucose-induced cardiomyocyte apoptosis via inhibition of endoplasmic reticulum stress and activation of SERCA2a. *Am J Physiol Cell Physiol* **304**: C508-C518, 2013.