

ORIGINAL INVESTIGATION

Open Access

Glucagon-like peptide-1 enhances cardiac L-type Ca^{2+} currents via activation of the cAMP-dependent protein kinase A pathway

Yong-Fu Xiao*, Alena Nikolskaya, Deborah A Jaye, Daniel C Sigg

Abstract

Background: Glucagon-like peptide-1 (GLP-1) is a hormone predominately synthesized and secreted by intestinal L-cells. GLP-1 modulates multiple cellular functions and its receptor agonists are now used clinically for diabetic treatment. Interestingly, preclinical and clinical evidence suggests that GLP-1 agonists produce beneficial effects on dysfunctional hearts via acting on myocardial GLP-1 receptors. As the effects of GLP-1 on myocyte electrophysiology are largely unknown, this study was to assess if GLP-1 could affect the cardiac voltage-gated L-type Ca^{2+} current (I_{Ca}).

Methods: The whole-cell patch clamp method was used to record I_{Ca} and action potentials in enzymatically isolated cardiomyocytes from adult canine left ventricles.

Results: Extracellular perfusion of GLP-1 (7-36 amide) at 5 nM increased I_{Ca} by $23 \pm 8\%$ ($p < 0.05$, $n = 7$). Simultaneous bath perfusion of 5 nM GLP-1 plus 100 nM Exendin (9-39), a GLP-1 receptor antagonist, was unable to block the GLP-1-induced increase in I_{Ca} ; however, the increase in I_{Ca} was abolished if Exendin (9-39) was pre-applied 5 min prior to GLP-1 administration. Intracellular dialysis with a protein kinase A inhibitor also blocked the GLP-1-enhanced I_{Ca} . In addition, GLP-1 at 5 nM prolonged the durations of the action potentials by 128 ± 36 ms ($p < 0.01$) and 199 ± 76 ms ($p < 0.05$) at 50% and 90% repolarization ($n = 6$), respectively.

Conclusions: Our data demonstrate that GLP-1 enhances I_{Ca} in canine cardiomyocytes. The enhancement of I_{Ca} is likely via the cAMP-dependent protein kinase A mechanism and may contribute, at least partially, to the prolongation of the action potential duration.

Background

Glucagon-like peptide-1 (GLP-1) is one of the transcription products from the proglucagon gene. GLP-1 is a peptide hormone predominately produced by intestinal endocrine L-cells. GLP-1 has two major isoforms (7-36 amide and 7-37) which are among the most potent stimulators of glucose-dependent insulin secretion. Both peptides are considered equipotent in terms of their biological activity[1,2]. GLP-1 stimulates glucose-dependent insulin secretion and insulin biosynthesis and inhibits glucagon secretion, gastric emptying, and food intake. The N-terminal degradation of GLP-1 by dipeptidyl

peptidase-4 (DPP-4)-mediated cleavage at the position 2 alanine modifies its biological activity[3].

GLP-1 modulates multiple cellular functions believably via acting on GLP-1 receptors (GLP-1Rs)[4], which are expressed in the human pancreas, heart, lung, kidney, stomach and brain[5]. Due to the multiple beneficial effects of GLP-1R agonists in the treatment of diabetes mellitus including weight loss, pharmaceutical companies have developed and introduced GLP-1R agonists as a treatment option for patients with type 2 diabetes mellitus (e.g. Byetta™ (exenatide) and Victoza™ (liraglutide)). Various cardiovascular effects of GLP-1 have been reported. GLP-1 infusion improves glucose uptake [6] and metabolism[7], as well as cardiac function[6] and hemodynamics[8-10] in different species, including in humans[11]. In addition, GLP-1 infusion reduced infarct size in a rodent model of ischemia with no effect

* Correspondence: yong-fu.xiao@medtronic.com
Cardiac Rhythm Disease Management, Medtronic, Inc., 8200 Coral Sea Street
NE, Mounds View, MN 55112, USA

on ventricular function[12,13], but did not alter infarct size in an open-chest, anesthetized porcine model of ischemia[5]. Furthermore, GLP-1R knockout mice (GLP-1R^{-/-}) have lower heart rate and blood pressure with an increase in cardiac mass[14]. In clinical studies using liraglutide (Victoza™), a GLP-1R agonist, a consistent decrease in blood pressure and a reduction of cardiovascular risk markers were observed in a cohort of over 5000 patients[15].

In pancreatic β -cells, GLP-1 inhibits ATP-dependent K⁺ channels via the cAMP-mediated PKA pathway [16-19]. The effects of GLP-1 on myocardium observed in several studies are potentially via its G protein-coupled receptors[10,20]. Recently, a new glucagon-like peptide isolated from the intestine of the eel, *Anguilla japonica*[21] with a structure similar to that of oxyntomodulins has shown an inotropic effect via stimulation of Ca²⁺ influx and a chronotropic effect independent of extracellular Ca²⁺. In addition, GLP-1 and glucose-dependent insulinotropic polypeptide can enhance β -cell cytoplasmic Ca²⁺ oscillation and increase insulin secretion via activation of cAMP-triggered cascades[22,23]. However, the effects of GLP-1 on myocyte electrophysiology have not been carefully assessed. Therefore, in the present study, we investigated the effects of GLP-1 on the voltage-gated L-type Ca²⁺ channel in isolated canine left ventricular myocytes.

Methods

The study was designed and conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* [Department of Health and Human Services Publication No. (NIH) 85-23, Revised 1996]. The animal protocol was approved by the Institutional Animal Care and Use Committee of the Physiological Research Laboratories of Medtronic, Inc.

Isolation of canine left ventricular myocytes

Single left ventricular myocytes were enzymatically isolated from adult canine hearts with a body weight of 30 to 33 kg (n = 13) by the methods described previously [24]. Briefly, left ventricular heart tissue was rapidly excised from canine hearts. Several tissue pieces weighing 3-5 grams each were harvested from the left ventricle and immediately placed in ice-cold Ca²⁺-free dissection buffer ("cardioplegia") solution containing (mM): NaCl 140, KCl 5.4, MgCl₂ 1.2, HEPES 5, glucose 5.5, and 2,3-butanedione monoxime 30, pH 7.4. Tissue pieces were rinsed twice by the dissection solution supplemented with antibiotics and cut into small pieces (~1 mm³) with sharp scissors. Digestion buffer solution was aspirated and replaced with a Ca²⁺-free enzymatic solution containing 0.1% trypsin, 0.075% collagenase type II, 0.05% cyaluronidase, and 0.025% elastase. Tissue was

incubated in Ca²⁺-free enzymatic solution at 4°C overnight and then subjected to a 37°C digestion procedure.

During 37°C digestion, Ca²⁺-free enzymatic solution containing trypsin was gradually replaced with another enzymatic solution containing 0.1 mM of Ca²⁺, 0.075% collagenase, 0.1% soybean trypsin inhibitor, and 0.2% bovine serum albumin. The tissues were agitated in a heated shaker (~300 rpm at 37°C) for several 15-min intervals. After agitation, the tissue was triturated about 15-20 times with a 10 ml pipette. The tissue was allowed to settle, supernatants were removed, and a fresh aliquot of enzymatic solution was added. The first two supernatants containing mostly cell debris were aspirated and discarded. The subsequent supernatants containing liberated cells were collected into centrifuge tubes, where a cell culture media with 20% fetal bovine serum was added. Collected cell suspensions were centrifuged for 5 min at 300 rpm and the resulting supernatant was discarded. The myocyte pellets were resuspended in a HEPES-buffered cell culture media. Yields from this procedure were about 50% of Ca²⁺-tolerant myocytes. Quiescent, rod-shaped ventricular myocytes with clear striations were chosen for patches to study their electrophysiology and GLP-1 effects.

Electrophysiological recordings

After dissociation, a small amount (30 μ l) of the medium solution with myocytes was transported to a chamber mounted on the stage of a Nikon microscope (Nikon, Japan). The chamber was continuously superfused (~1.0 ml/min) with Tyrode solution. The whole-cell configuration of the patch-clamp technique was applied. Briefly, glass electrodes (World Precision Instruments, Sarasota, FL, USA) with 1 to 3 M Ω resistance were connected via an Ag-AgCl wire to an Axopatch 200A amplifier interfaced with a DigiData-1320 acquisition system (Molecular Devices, Inc., Sunnyvale, CA, USA). After forming a conventional "gigaohm" seal, electrode capacitance was compensated. Additional suction ruptured the patched membrane and formed the whole-cell configuration. Cell membrane capacitance (C_m) was routinely recorded from each patched cell with the pCLAMP program (version 9.2, Molecular Devices, Inc., Sunnyvale, CA, USA). Ca²⁺ currents were recorded with the protocols similar to those previously described[25]. Action potential (AP) was evoked and recorded with the whole-cell current-clamp method[26].

Chemicals and solutions

GLP-1 and its receptor inhibitor Exendin (9-39) were purchased from Bachem Americas, Inc. (Torrance, CA, USA). GLP-1 (7-36 amide) was tested at concentrations ranging from 0.05 nM to 5 nM for its effect on isolated canine left ventricular myocytes. The effects of Exendin

(9-39) or the cAMP-dependent protein kinase A (PKA) inhibitor 4-25 fragment (Sigma, St. Louis, MO, USA) were also assessed in isolated single cardiomyocytes with or without GLP-1 treatment.

For recording Ca^{2+} currents, the bath solution contained (in mM): NaCl 140, CsCl 5, MgCl_2 1, CaCl_2 1.8, glucose 5, and HEPES 10 (pH 7.4 with HCl). The pipette solution contained (in mM): CsCl 100, CsOH 40, MgCl_2 1, CaCl_2 1, EGTA 11, Mg-ATP 5, and HEPES 10 (pH 7.3 with CsOH). ATP and all other chemicals used in this study were obtained from Sigma Aldrich (St. Louis, MO, USA). The bath solution for recording APs contained (mM): NaCl 140; KCl 5.4; CaCl_2 1.8; MgCl_2 1; D-glucose 5; HEPES 10 (pH adjusted to 7.4 with NaOH). The pipette solution for recording APs contained (mM): K-glutamate 130; KCl 15; NaCl 5; MgATP 5; MgCl_2 1; EGTA 5; CaCl_2 1; HEPES 10 (pH adjusted to 7.2 with KOH). A perfusion system was used to change the extracellular solution. Data were collected with pCLAMP (version 9.2, Axon Instruments, CA, USA). All experiments were conducted at room temperature ($\sim 22^\circ\text{C}$).

Data analysis

Peak I_{Ca} was measured in the presence or absence of GLP-1 or other chemical compounds. The parameters of APs were analyzed by using the methods similar to those described in a previous report[26]. The maximal effects of GLP-1 on I_{Ca} and APs were used in the table and figures. All data were presented as mean \pm standard error of the mean unless otherwise stated. The Student's *t*-test and ANOVA were applied for statistical analysis as appropriate. Differences were considered significant if $p < 0.05$.

Results

Effects of GLP-1 and Exendin (9-39) on L-type Ca^{2+} currents

Extracellular perfusion of 5 nM GLP-1 (7-36 amide) solution gradually increased the peak currents and reached the maximal effects around 10 to 15 min. Figure 1A shows the representative traces in the presence or absence of 5 nM GLP-1. The enhancement effect of GLP-1 on I_{Ca} was washable upon washing away of the compound. While GLP-1 at 5 nM significantly increased the peak currents (Fig. 1B), the current-voltage relationship of the activation was not altered and the kinetics of the activation and the steady-state inactivation of I_{Ca} slightly shifted, but not significantly, to the hyperpolarizing direction in the presence of 5 nM GLP-1 (Fig. 1C and 1D, $n = 7$).

To determine whether the GLP-1-induced enhancement of I_{Ca} was via the activation of membrane GLP-1Rs, the GLP-1R inhibitor Exendin (9-39) was added to

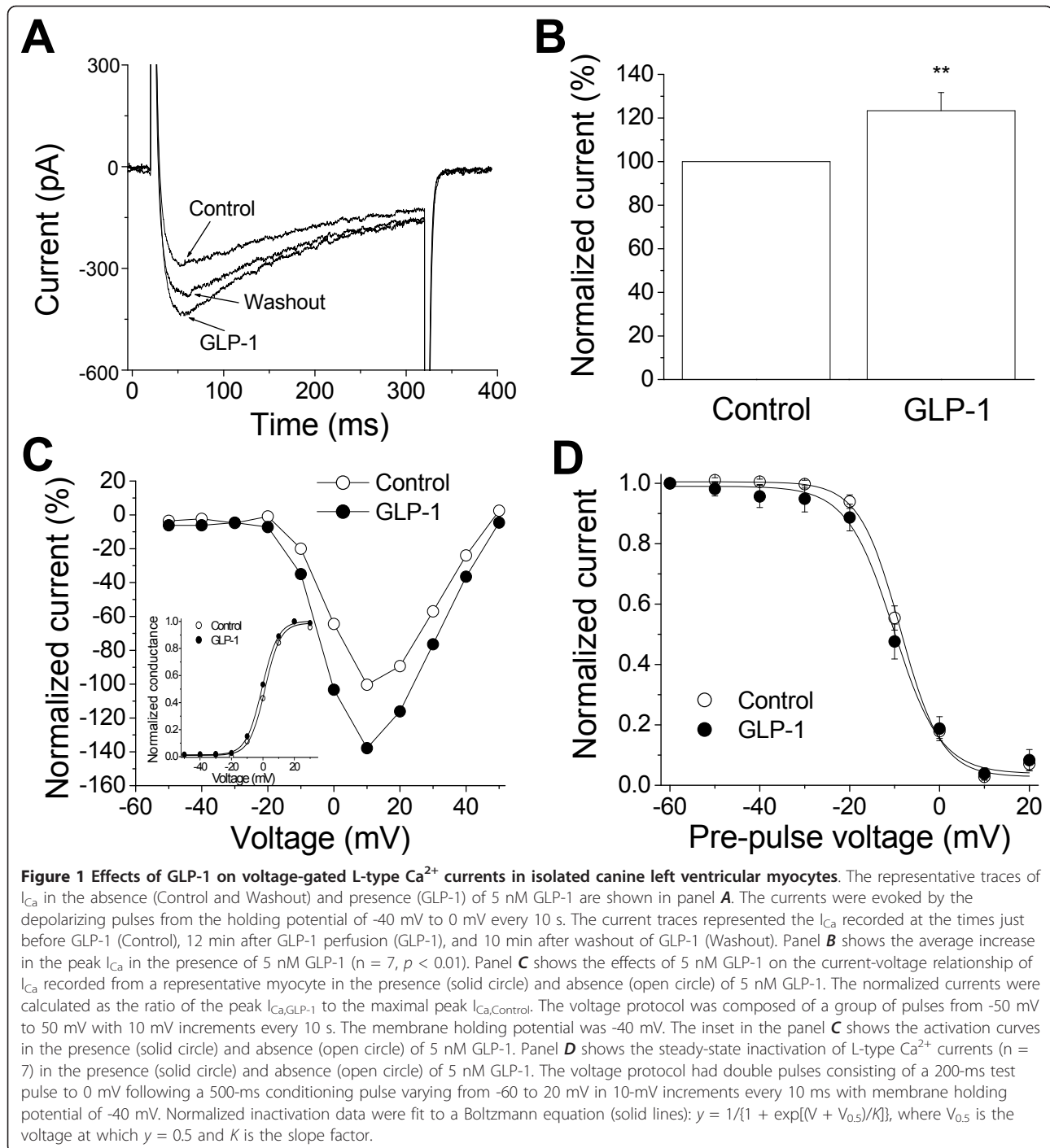
the GLP-1 solution. The non-mammalian peptide, Exendin (9-39 amide), is a specific and competitive antagonist of GLP-1Rs. As Exendin (9-39) has lower binding affinity to GLP-1Rs, 100 nM Exendin (9-39), at a concentration of 20 times higher than that of GLP-1, plus 5 nM GLP-1, was perfused to the canine left ventricular myocytes. Interestingly, Exendin (9-39) at 100 nM was unable to block the GLP-1-induced enhancement of I_{Ca} (Fig. 2A and 2B). These results suggest that when GLP-1 and Exendin (9-39) were applied together at the same time, GLP-1 could activate GLP-1Rs due to its higher affinity for GLP-1 receptors. After the activation of GLP-1Rs, the enhancement of I_{Ca} by GLP-1 was not blocked by 100 nM Exendin (9-39) because the activation process was already beyond the receptor stage. To test this hypothesis, we administered 100 nM Exendin (9-39) 5 min prior to GLP-1. Figure 2C and 2D clearly shows that Exendin (9-39) alone did not significantly alter the currents, but the enhancement of I_{Ca} by 5 nM GLP-1 was abolished by pre-administration of 100 nM Exendin (9-39).

Protein kinase A and GLP-1-induced enhancement of Ca^{2+} currents

To determine whether the GLP-1-induced enhancement of I_{Ca} was via activation of the cAMP/PKA pathway, the PKA inhibitor (PKA-I fragment 4-25 amide, 5 μM) was added to the electrode solution. After forming a whole-cell configuration, I_{Ca} was immediately recorded (Fig. 3, Control). Each cell was dialyzed for 20 min with PKA-I and I_{Ca} was recorded. The peak currents recorded at 20 min after intracellular PKA-I dialysis were not significantly changed (Fig. 3, PKA-I). Extracellular perfusion of 5 nM GLP-1 for 15 min did not induce a significant increase in I_{Ca} (Fig. 3, PKA-I+GLP-1). Compared with the significant increase in I_{Ca} of the cardiomyocytes treated with 5 nM GLP-1 alone ($23 \pm 8\%$, $n = 7$, $p < 0.05$, Fig. 1B), I_{Ca} in the myocytes with intracellular dialysis of PKA-I was not significantly altered by the same concentration of GLP-1 ($8 \pm 13\%$, $n = 5$, $p > 0.05$, Fig. 3, PKA-I+GLP-1). This result suggests that GLP-1-induced enhancement of I_{Ca} was most likely via the activation of the PKA pathway.

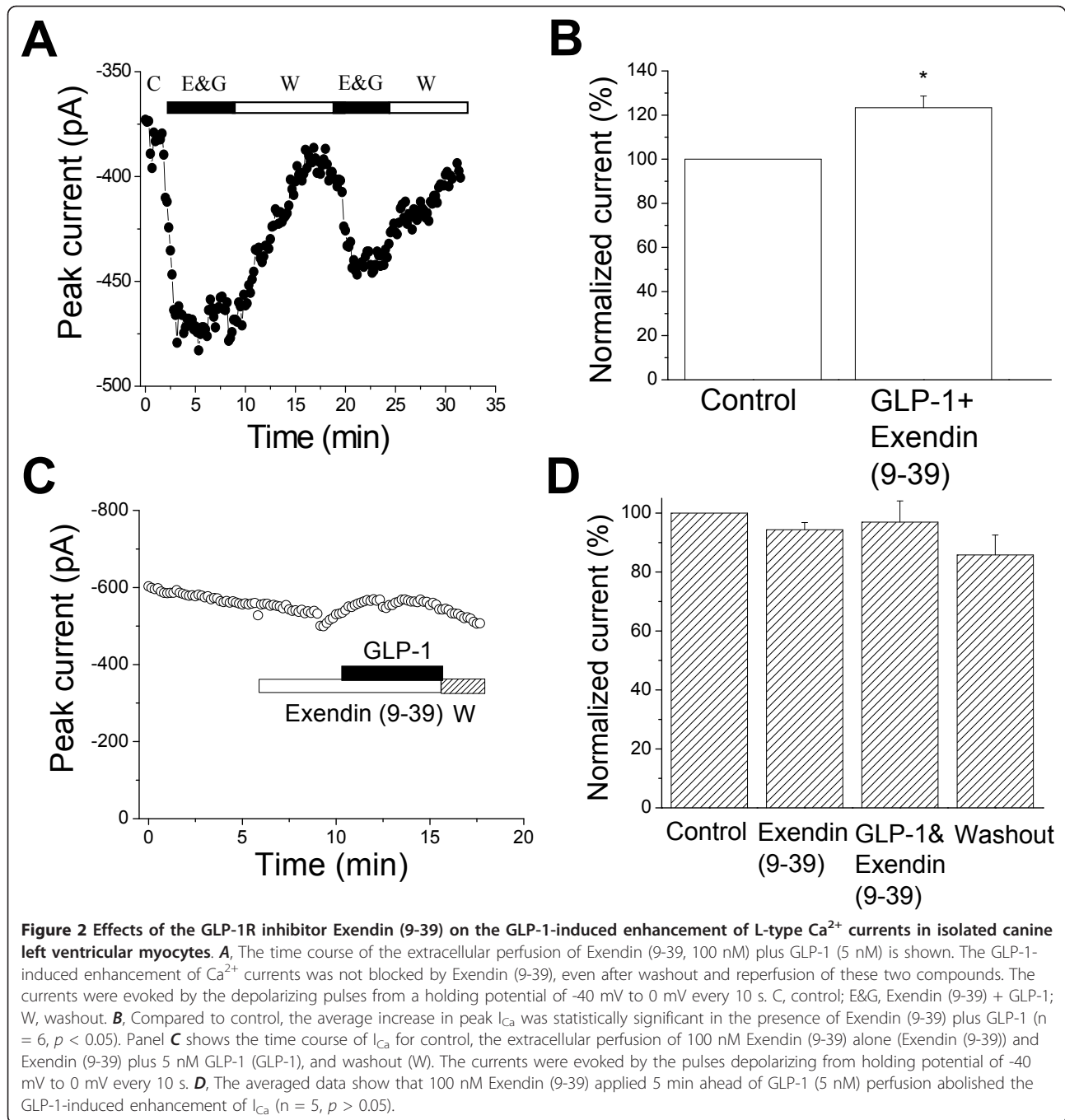
GLP-1-induced prolongation of action potential duration

As L-type Ca^{2+} currents can affect the plateau of a cardiac action potential (AP) and as GLP-1 could enhance I_{Ca} , we next investigated the effects of GLP-1 on the AP duration (APD). Figure 4A shows that extracellular perfusion of 5 nM GLP-1 gradually prolonged the APDs of the patched cardiomyocyte. The prolongation effect was initiated within 2 min after GLP-1 perfusion and reached the maximal level around 10 min. The GLP-1-induced APD prolongation was recoverable after



washing out of the compound (Fig. 4A and Table 1). The durations of the APs measured at 50% and 90% repolarization were significantly prolonged in the presence of 5 nM GLP-1 (Fig. 4B and Table 1). Other AP parameters, such as amplitude, threshold, and maximum upstroke velocity of AP, were not significantly altered in the presence of 5 nM GLP-1. The APD prolongations

showed a concentration-dependent trend. The delta prolongations were 24.8 ± 11.5 ms and 25.7 ± 15.7 ms for 0.05 nM GLP-1, and 42.2 ± 25.1 ms and 48.5 ± 25.2 ms for 0.5 nM GLP-1 measured at 50% and 90% repolarization, respectively, but these changes did not reach statistical significance at both concentrations ($p > 0.05$, $n = 6$).



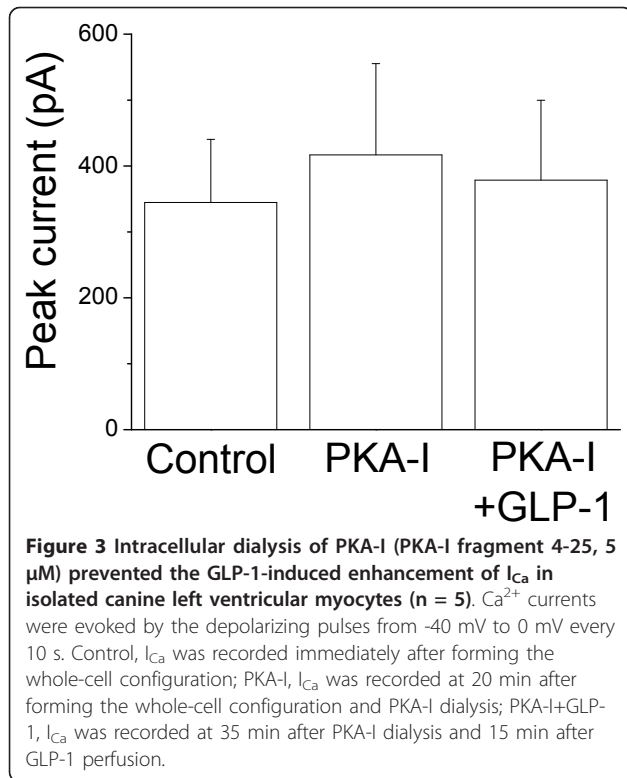
Discussion

The main finding in this study is that GLP-1 (7-36 amide) can enhance voltage-gated L-type Ca^{2+} currents in isolated canine left ventricular myocytes. The GLP-1-induced enhancement of Ca^{2+} currents was significantly attenuated in the presence of the GLP-1R inhibitor Exendin (9-39) (Fig. 2) or by intracellular dialysis with the PKA inhibitor, PKA-I fragment 4-25 (Fig. 3). The effect of GLP-1 on I_{Ca} observed in this study may help

to interpret the chronotropic effect of GLP-1 *in vivo* [8-10], perhaps via the modulation of Ca^{2+} channels of sinoatrial node cells.

GLP-1 receptors in cardiomyocytes

The existence of GLP-1Rs has been demonstrated in many human organs and tissues, including the pancreas, heart, lung, kidney, stomach and brain [5]. The data from the current study suggest that canine left



ventricular myocytes functionally express GLP-1Rs, because GLP-1 enhanced I_{Ca} and prolonged APD, and also because the GLP-1-induced enhancement of I_{Ca} was blocked by specific GLP-1R inhibitor Exendin (9-39). However, the concentration of Exendin (9-39)

required to block the GLP-1 effects was relatively high, and the antagonist was also required prior to administration of GLP-1 (Fig. 2). This suggests that compared with GLP-1, Exendin (9-39) is a competitive inhibitor with a lower GLP-1R binding affinity and slower binding kinetics at the cardiac GLP-1R. There is a possibility that the effects of GLP-1 on myocyte electrophysiology is via a GLP-1R-independent mechanism. However, since no report so far has shown that GLP-1 can affect cellular function via a non-receptor approach, and since pre-administration of Exendin (9-39) abolished the increase in I_{Ca} subsequent to GLP-1 administration, we believe that a receptor-independent mechanism of the GLP-1-induced enhancement of I_{Ca} is most unlikely.

GLP-1 and the cAMP/PKA pathway

Voltage-gated L-type Ca^{2+} currents influence the morphology of cardiac action potential, especially the plateau phase, and are also critical for excitation-contraction coupling in the heart. Stimulation of the cardiac β -adrenergic receptor increases Ca^{2+} influx through the voltage-gated L-type Ca^{2+} channel[27]. The cardiac β -adrenergic receptor is coupled to an intracellular signaling cascade via the stimulatory G protein (G_s) which can activate the cAMP-dependent PKA system and enhance Ca^{2+} channel phosphorylation. In cardiomyocytes, the PKA-dependent phosphorylation of L-type Ca^{2+} channels increases I_{Ca} [27-29]. In the current study, we found that the GLP-1-induced increase in I_{Ca} was abolished by intracellular dialysis of a PKA inhibitor (PKA-I fragment 4-25). This result suggests that GLP-1

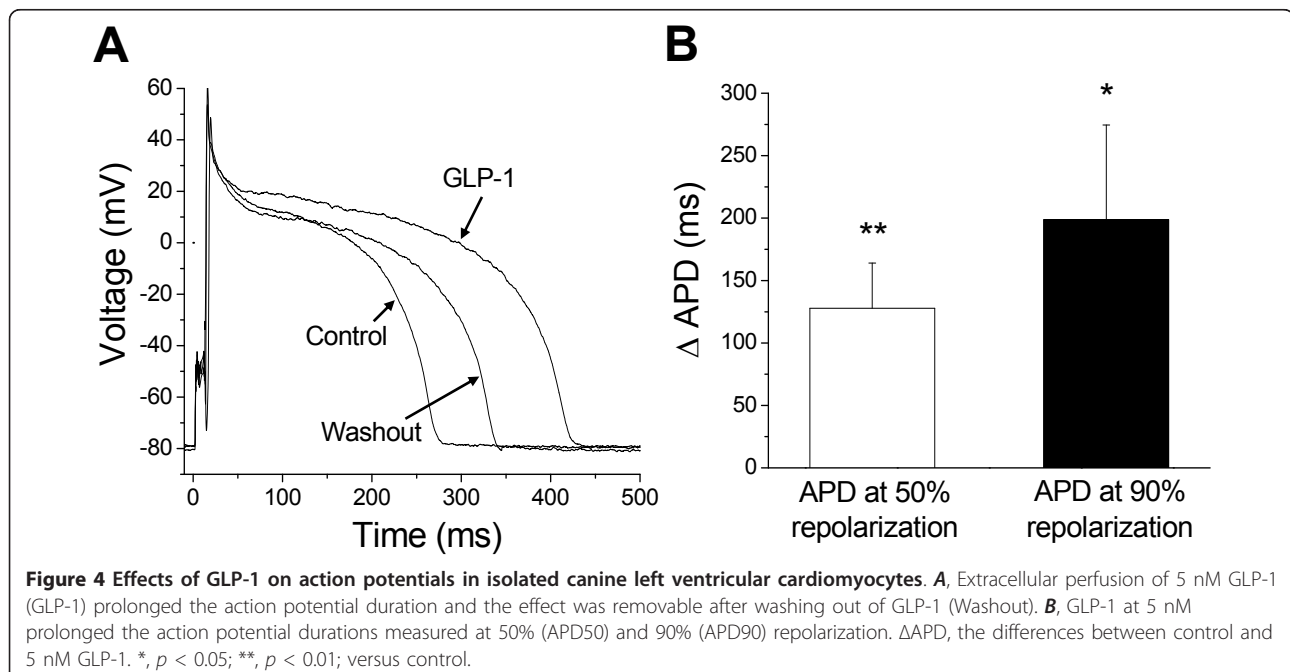


Table 1 Effects of GLP-1 on action potentials of canine left ventricular cardiomyocytes

	MHP mV	AAP mV	APT mV	Vmax V/s	APD ₅₀ ms	APD ₉₀ ms
Control	-80.2 ± 1.9	149.8 ± 4.6	-61.0 ± 2.4	126.6 ± 17.6	124.3 ± 24.4	236.1 ± 17.6
GLP-1	-84.2 ± 2.0	145.3 ± 4.5	-61.8 ± 1.6	120.8 ± 22.7	252.1 ± 30.5**	434.8 ± 79.2*
Washout	-82.4 ± 2.3	142.2 ± 5.8	-62.4 ± 0.9	113.1 ± 25.5	120 ± 27.7	302.8 ± 66.9

Values are expressed as mean ± SEM (n = 6). GLP-1 (7-36 amide) at 5 nM was added to the bath solution. The data were collected at the times just before GLP-1 (Control), 10 to 15 min after GLP-1 perfusion (GLP-1), and 10 to 15 min after washout of GLP-1 (Washout). MHP, membrane holding potential before elicitation of an action potential; AAP, amplitude of action potential; APT, threshold of initiation of an action potential; V_{max}, maximum upstroke velocity of action potential; APD, action potential duration measured at 50% (APD₅₀) and 90% (APD₉₀) of repolarization. *, p < 0.05; **, p < 0.01; versus control and washout.

activates the GLP-1R which couples with the G-protein/adenyl cyclase complex to enhance cAMP production. Increase in intracellular cAMP activates PKA and then enhances Ca²⁺ channel phosphorylation. Phosphorylated Ca²⁺ channels increase their open probability when they are activated by voltage change. This is why the GLP-1-induced enhancement of I_{Ca} was blocked by PKA-I. These results are along with the findings in the previous studies in rodent cardiomyocytes that GLP-1 or the GLP-1R agonist liraglutide significantly increased intracellular cAMP, which was abolished by the GLP-1R antagonist Exendin (9-39)[13,30]. Also, in the pancreatic β-cell, GLP-1 enhances cytoplasmic Ca²⁺ oscillation and insulin secretion via activation of cAMP cascades[22,23].

The effects of GLP-1 on I_{Ca} are most likely via the activation of the GLP-1R and then the cAMP-dependent PKA pathway. Activation of the PKA pathway is widely recognized as a key intracellular signaling mechanism in ischemic and pharmacological preconditioning. Subsequent cardioprotection of the PKA system may be beneficial in both treating/preventing ischemic damage, as well as apoptosis in heart failure. In studies by Bose et al, it has been shown that GLP-1 administration can reduce ischemia-reperfusion injury in part via activation of PKA, as well as via activation of other pro-survival kinases, and can be blocked by PKA inhibitors and inhibitors of pro-survival kinases[12,31,32]. One of the limitations in our experiments is that we did not directly measure intracellular cAMP contents with or without GLP-1 or GLP-1 plus a blocker of GLP-1R. However, one previous study has shown that GLP-1 at 10 nM doubled intracellular cAMP content in rat cardiac myocytes[13]. The GLP-1R agonist liraglutide at 100 nM significantly increased the level of intracellular cAMP in mouse cardiomyocytes and this increase was abolished by the GLP-1R antagonist Exendin (9-39)[30]. In addition, GLP-1 exerts cAMP/PKA-mediated insulinotropic actions in endocrine tissues[19,33,34] and stimulates adenylate cyclase to cause an increase in cAMP in pancreatic islet cells[34,35]. These results strongly suggest that the cellular effects of GLP-1 involve the cAMP-dependent PKA pathway and our data are consistent with the previous findings.

The GLP-1-induced enhancement of I_{Ca} can trigger intracellular Ca²⁺ release. Elevation of intracellular Ca²⁺ level can lead to an improvement of myocardial contractility. Several studies in pancreatic β-cells have shown that GLP-1 synchronizes Ca²⁺ and cAMP oscillations [10,19,36] and such oscillations involves the ryanodine-sensitive Ca²⁺ store[22]. In addition, the experimental evidence has shown that GLP-1 can augment Ca²⁺ influx[37,38] and Ba²⁺ currents[39] through L-type Ca²⁺ channels via the cAMP-dependent PKA pathway in pancreatic β-cells. In addition, GLP-1 increases intracellular cAMP content in adult rat cardiomyocytes[13].

Pharmacological effects of GLP-1

Our data demonstrate that GLP-1 can modulate cardiomyocyte electrophysiology. However, GLP-1 (7-36 amide) in human plasma is normally less than 100 pM [40-42]. GLP-1 administration at 5 nM to obtain a significant effect on APs and I_{Ca} in this study is much higher than a physiological level. Therefore, the effects of GLP-1 on I_{Ca} and APs are most likely pharmacological in this study. Various pharmacological concentrations ranging from 10 to 100 nM of GLP-1 or GLP-1R agonists have been tested in several previous studies. In one clinical trial, GLP-1 (17 nmol) was infused to assess its antidiabetogenic action[40]. GLP-1 (up to 100 nmol/kg) was used to test its effects on insulin secretion, insulin sensitivity, and glucose effectiveness in mice[43]. Moreover, 0.1 to 1000 nM GLP-1 was used to assess the dose-dependent effect of GLP-1 on cAMP production with an EC₅₀ of 10 nM in rat cardiomyocytes[13] and 50 nM GLP-1 to study the role of L-type Ca²⁺ channels in mediating GLP-1-stimulated events in cultured pancreatic β-cell line INS-1[44]. As the GLP-1R agonists, Byetta™ and Victoza™, have been used for the treatment of patients with type II diabetes mellitus, the concentration of GLP-1 used in this study can have pharmacological significance.

The beneficial effects of GLP-1 at various pharmacological concentrations have been shown in several recent animal studies. Chronic treatment with either GLP-1 or AC3174, a peptide analogue with pharmacologic properties similar to the GLP-1R agonist exenatide, showed

cardioprotective effects and improved cardiac function, cardiac remodeling, insulin sensitivity, and exercise capacity in myocardial infarction rats with chronic heart failure[45]. Chronic AC3174 treatment also attenuated salt-induced hypertension, cardiac morbidity, insulin resistance, and renal dysfunction and improved survival in Dahl salt-sensitive rats[46]. In addition, GLP-1 has an antiapoptotic effect on β -cells during oxidative stress probably via blocking the c-Jun-N-terminal kinase (JNK) and glycogen synthasekinase 3β (GSK3 β) mediated apoptotic pathway[47]. So far, GLP-1 and its analogs are only available as injectable dosage forms. Recently, a single dose of the exenatide-based ORMD-0901 formulation was enterically delivered to pigs and beagle canines [48]. The results show that enterically delivered ORMD-0901 was well tolerated by the animals and that GLP-1 (ORMD-0901) was absorbed from the gastrointestinal tracts and retained its biological activity. Hence, development of this drug class in an oral dosage form has the potential to enhance diabetes control and patient compliance.

Conclusions

Our data demonstrate for the first time that GLP-1 (7-36 amide) can enhance voltage-gated L-type Ca^{2+} currents in cardiomyocytes and such enhancement may contribute to the prolongation of APs. These effects are most likely via the activation of the cardiac GLP-1R and then the cAMP-dependent PKA pathway. These findings suggest a novel mechanism of the potential beneficial cardiac effects of GLP-1R agonists on the improvement of myocardial contractility.

Abbreviations

AP: action potential; APD: action potential duration; cAMP: 3'-5'-cyclic adenosine monophosphate; GLP-1: glucagon-like peptide-1; GLP-1R: glucagon-like peptide-1 receptor; I_{Ca} : voltage-gated L-type Ca^{2+} current; PKA: protein kinase A; PKA-I: protein kinase A inhibitor

Acknowledgements

We are grateful to Corey Leet, Associate Scientist, Physiological Research Laboratories, Division of Medtronic, Inc., for his assistance during cardiac tissue collection.

Authors' contributions

YFX designed the study, conducted the patch clamp experiments, analyzed and interpreted the results, wrote the manuscript. AN collected canine heart tissues and isolated the ventricular cardiomyocytes. AN, DAJ, and DCS participated the discussion of experimental design and manuscript writing and editing. All authors read and approved the final manuscript.

Authors' information

YFX is a principal scientist at Medtronic, Inc. and an adjunct professor at the Department of Pharmacology and Physiology, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, NJ, USA. AN was a research scientist at Medtronic, Inc. DAJ is a senior scientist at Medtronic, Inc. DCS was a senior research manager at Medtronic, Inc. and is currently an adjunct assistant professor at the Department of Integrative Biology and Physiology, University of Minnesota, MN, USA.

Competing interests

All authors were employees and held stock in Medtronic, Inc at the time these experiments were performed.

Received: 17 December 2010 Accepted: 20 January 2011

Published: 20 January 2011

References

1. Kreymann B, Williams G, Ghatei MA, Bloom SR: Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* 1987, **2**:1300-4.
2. Orskov C, Wettergren A, Holst JJ: Biological effects and metabolic rates of glucagonlike peptide-1 7-36 amide and glucagonlike peptide-1 7-37 in healthy subjects are indistinguishable. *Diabetes* 1993, **42**:658-61.
3. Mentlein R, Gallwitz B, Schmidt WE: Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 1993, **214**:829-35.
4. Runge S, Thogersen H, Madsen K, Lau J, Rudolph R: Crystal structure of the ligand-bound glucagon-like peptide-1 receptor extracellular domain. *J Biol Chem* 2008, **283**:11340-7.
5. Kavianiipour M, Ehlers MR, Malmberg K, Ronquist G, Ryden L, Wikstrom G, et al: Glucagon-like peptide-1 (7-36) amide prevents the accumulation of pyruvate and lactate in the ischemic and non-ischemic porcine myocardium. *Peptides* 2003, **24**:569-78.
6. Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelis L, et al: Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. *Circulation* 2004, **110**:955-61.
7. Luque MA, Gonzalez N, Marquez L, Acitores A, Redondo A, Morales M, et al: Glucagon-like peptide-1 (GLP-1) and glucose metabolism in human myocytes. *J Endocrinol* 2002, **173**:465-73.
8. Barragan JM, Rodriguez RE, Blazquez E: Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7-36) amide in rats. *Am J Physiol* 1994, **266**:E459-66.
9. Barragan JM, Rodriguez RE, Eng J, Blazquez E: Interactions of exendin-(9-39) with the effects of glucagon-like peptide-1-(7-36) amide and of exendin-4 on arterial blood pressure and heart rate in rats. *Regul Pept* 1996, **67**:63-8.
10. Holz GG, Heart E, Leech CA: Synchronizing Ca^{2+} and cAMP oscillations in pancreatic beta-cells: a role for glucose metabolism and GLP-1 receptors? Focus on "regulation of cAMP dynamics by Ca^{2+} and G protein-coupled receptors in the pancreatic beta-cell: a computational approach". *Am J Physiol Cell Physiol* 2008, **294**:C4-6.
11. Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, et al: Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation* 2004, **109**:962-5.
12. Bose AK, Mocanu MM, Carr RD, Yellon DM: Glucagon like peptide-1 is protective against myocardial ischemia/reperfusion injury when given either as a preconditioning mimetic or at reperfusion in an isolated rat heart model. *Cardiovasc Drugs Ther* 2005, **19**:9-11.
13. Vila Petroff MG, Egan JM, Wang X, Sollott SJ: Glucagon-like peptide-1 increases cAMP but fails to augment contraction in adult rat cardiac myocytes. *Circ Res* 2001, **89**:445-52.
14. Ban K, Noyan-Ashraf MH, Hoefler 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* 2008, **117**:2340-50.
15. Madsbad S: Clinical Trial Report-Liraglutide Effect and Action in Diabetes (LEAD™) trial. *Expert Review of Endocrinology & Metabolism* 2009, **4**:119-29.
16. McClenaghan NH, Flatt PR, Ball AJ: Actions of glucagon-like peptide-1 on KATP channel-dependent and -independent effects of glucose, sulphonylureas and nateglinide. *J Endocrinol* 2006, **190**:889-96.
17. Yada T, Itoh K, Nakata M: Glucagon-like peptide-1-(7-36)amide and a rise in cyclic adenosine 3',5'-monophosphate increase cytosolic free Ca^{2+} in rat pancreatic beta-cells by enhancing Ca^{2+} channel activity. *Endocrinology* 1993, **133**:1685-92.
18. Sakuta H: ATP-sensitive potassium channel and hormone/neuropeptide. *Nippon Naibunpi Gakkai Zasshi* 1995, **71**:579-86.

19. Holz GG, Leech CA, Habener JF: Activation of a cAMP-regulated Ca(2+) signaling pathway in pancreatic beta-cells by the insulinotropic hormone glucagon-like peptide-1. *J Biol Chem* 1995, **270**:17749-57.
20. Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, et al: Direct effects of glucagon-like peptide-1 on myocardial contractility and glucose uptake in normal and postischemic isolated rat hearts. *J Pharmacol Exp Ther* 2006, **317**:1106-13.
21. Uesaka T, Yano K, Sugimoto S, Ando M: Glucagon-like peptide isolated from the eel intestine: effects on atrial beating. *J Exp Biol* 2001, **204**:3019-26.
22. Sasaki S, Nakagaki I, Kondo H, Hori S: Involvement of the ryanodine-sensitive Ca2+ store in GLP-1-induced Ca2+ oscillations in insulin-secreting HIT cells. *Pflügers Arch* 2002, **445**:342-51.
23. Kang G, Chepurny OG, Holz GG: cAMP-regulated guanine nucleotide exchange factor II (Epac2) mediates Ca2+-induced Ca2+ release in INS-1 pancreatic beta-cells. *J Physiol* 2001, **536**:375-85.
24. Cigaina V, Hirschberg AL: Gastric pacing for morbid obesity: plasma levels of gastrointestinal peptides and leptin. *Obes Res* 2003, **11**:1456-62.
25. Xiao YF, Gomez AM, Morgan JP, Lederer WJ, Leaf A: Suppression of voltage-gated L-type Ca2+ currents by polyunsaturated fatty acids in adult and neonatal rat ventricular myocytes. *Proc Natl Acad Sci USA* 1997, **94**:4182-7.
26. Ke Q, Xiao YF, Bradbury JA, Graves JP, Degraff LM, Seubert JM, et al: Electrophysiological properties of cardiomyocytes isolated from CYP2J2 transgenic mice. *Mol Pharmacol* 2007, **72**:1063-73.
27. Reuter H: Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature* 1983, **301**:569-74.
28. McDonald TF, Pelzer S, Trautwein W, Pelzer DJ: Regulation and modulation of calcium channels in cardiac, skeletal, and smooth muscle cells. *Physiol Rev* 1994, **74**:365-507.
29. Keef KD, Hume JR, Zhong J: Regulation of cardiac and smooth muscle Ca (2+) channels (Ca(V)1.2a,b) by protein kinases. *Am J Physiol Cell Physiol* 2001, **281**:C1743-56.
30. Noyan-Ashraf MH, Momen MA, Ban K, Sadi AM, Zhou YQ, Riazi AM, et al: GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. *Diabetes* 2009, **58**:975-83.
31. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM: Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes* 2005, **54**:146-51.
32. 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* 2007, **21**:253-6.
33. Howell SL, Jones PM, Persaud SJ: Regulation of insulin secretion: the role of second messengers. *Diabetologia* 1994, **37**(Suppl 2):S30-5.
34. Ramos LS, Zippin JH, Kamenetsky M, Buck J, Levin LR: Glucose and GLP-1 stimulate cAMP production via distinct adenylyl cyclases in INS-1E insulinoma cells. *J Gen Physiol* 2008, **132**:329-38.
35. Leibowitz MD, Biswas C, Brady EJ, Conti M, Cullinan CA, Hayes NS, et al: A novel insulin secretagogue is a phosphodiesterase inhibitor. *Diabetes* 1995, **44**:67-74.
36. Holz GG, Leech CA, Heller RS, Castonguay M, Habener JF: cAMP-dependent mobilization of intracellular Ca2+ stores by activation of ryanodine receptors in pancreatic beta-cells. A Ca2+ signaling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7-37). *J Biol Chem* 1999, **274**:14147-56.
37. Gomez E, Pritchard C, Herbert TP: cAMP-dependent protein kinase and Ca2+ influx through L-type voltage-gated calcium channels mediate Raf-independent activation of extracellular regulated kinase in response to glucagon-like peptide-1 in pancreatic beta-cells. *J Biol Chem* 2002, **277**:48146-51.
38. Britsch S, Krippeit-Drews P, Lang F, Gregor M, Drews G: Glucagon-like peptide-1 modulates Ca2+ current but not K+ATP current in intact mouse pancreatic B-cells. *Biochem Biophys Res Commun* 1995, **207**:33-9.
39. Suga S, Kanno T, Nakano K, Takeo T, Dobashi Y, Wakui M: GLP-I-(7-36) amide augments Ba2+ current through L-type Ca2+ channel of rat pancreatic beta-cell in a cAMP-dependent manner. *Diabetes* 1997, **46**:1755-60.
40. Gutniak MK, Svartberg J, Hellstrom PM, Holst JJ, Adner N, Ahren B: Antidiabetogenic action of glucagon-like peptide-1 related to administration relative to meal intake in subjects with type 2 diabetes. *J Intern Med* 2001, **250**:81-7.
41. Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S: Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 1992, **326**:1316-22.
42. Svartberg J, Holst JJ, Gutniak M, Adner N: The ethanol augmentation of glucose-induced insulin secretion is abolished by calcium antagonism with nifedipine: no evidence for a role of glucagon-like peptide-1 (GLP-1). *Pancreas* 1998, **16**:66-71.
43. Ahren B, Pacini G: Dose-related effects of GLP-1 on insulin secretion, insulin sensitivity, and glucose effectiveness in mice. *Am J Physiol* 1999, **277**:E996-E1004.
44. Jacobo SM, Guerra ML, Hockerman GH: Cav1.2 and Cav1.3 are differentially coupled to glucagon-like peptide-1 potentiation of glucose-stimulated insulin secretion in the pancreatic beta-cell line INS-1. *J Pharmacol Exp Ther* 2009, **331**:724-32.
45. Liu Q, Anderson C, Brody A, Polizzi C, Fernandez R, Baron A, et al: Glucagon-like peptide-1 and the exenatide analogue AC3174 improve cardiac function, cardiac remodeling, and survival in rats with chronic heart failure. *Cardiovasc Diabetol* 2010, **9**:76.
46. Liu Q, Adams L, Brody A, Fernandez R, Baron AD, Parkes DG: The exenatide analogue AC3174 attenuates hypertension, insulin resistance, and renal dysfunction in Dahl salt-sensitive rats. *Cardiovasc Diabetol* 2010, **9**:32.
47. Kim JY, Lim DM, Moon CI, Jo KJ, Lee SK, Baik HW, et al: Exenidin-4 protects oxidative stress-induced beta-cell apoptosis through reduced JNK and GSK3beta activity. *J Korean Med Sci* 2010, **25**:1626-32.
48. Eldor R, Kidron M, Greenberg-Shushlav Y, Arbit E: Novel glucagon-like peptide-1 analog delivered orally reduces postprandial glucose excursions in porcine and canine models. *J Diabetes Sci Technol* 2010, **4**:1516-23.

doi:10.1186/1475-2840-10-6

Cite this article as: Xiao et al.: Glucagon-like peptide-1 enhances cardiac L-type Ca²⁺ currents via activation of the cAMP-dependent protein kinase A pathway. *Cardiovascular Diabetology* 2011 **10**:6.

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

