Exendin-4 Reduces Ventricular Arrhythmia Activity and Calcium Sparks-Mediated Sarcoplasmic Reticulum Ca Leak in Rats with Heart Failure

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Summary

The aim of this study was to investigate the effect of exendin-4 (Ex-4) on ventricular arrhythmias and calcium sparks-mediated calcium leak in a myocardial infarction-heart failure model. We studied the influence of exendin-4 on ventricular arrhythmogenesis in a rat myocardial infarction-heart failure model. In vivo arrhythmia studies (electrocardiogram [ECG] telemetry studies), ex vivo arrhythmia studies calcium sparks tests, and analysis of total and phosphorylated ryanodine receptor (RyR) 2 and CaMK-II were carried out in sham group, myocardial infarction (MI) group, MI + Ex-4 and MI + Ex-4 + Exendin9-39 (Ex9-39) groups.

ECG telemetry studies showed an antiarrhythmic effect of exendin-4 with reduction of spontaneous ventricular arrhythmias. Exendin-4 abbreviated the APD90, which was longer in the heart failure model, and increase the APD alternans thresholds. Exendin-4 also reduced the susceptibility to burst pacing-induced arrhythmia ex vivo. Subcellular sarcoplasmic reticulum (SR) calcium leak characteristics were tested in four groups of rat cardiomyocytes. Exendin-4 reduced calcium spark mass, spark frequency, and calcium leak, which may be due to reduced S2814-RyR2 and CaMK-II phosphorylation. Co-administration of exendin 9-39 with exendin-4 partly abolished the above-mentioned effect of exendin-4.

These findings suggest that exendin-4 exerts an antiarrhythmic effect through decreasing SR calcium leak in spontaneous and burst pacing-induced ventricular arrhythmias, which may be due to reduced RyR2 phosphorylation and suppressed CaMK-II activity. Exendin-4 may act as a novel antiarrhythmic strategy in heart failure.

Key words: Calcium handling, GLP-1R, Ryanodine receptor, CaMK-II

Sudden arrhythmic death is often associated with heart failure, and this accounts for the most common electrophysiological mechanisms leading to sudden cardiac death.1,2 However, its mechanisms remain incompletely illustrated, and therefore hindered the progress of antiarrhythmic therapies. It is believed that a diastolic sarcoplasmic reticulum (SR) calcium leak leads to elevated intracellular calcium, which then activates Na+/Ca2+ exchanger, leading to delayed after-depolarizations and trigger activity.3,4

Exendin-4, a long-acting analog of glucagon-like peptide-1 (GLP-1), is known to be protective in cardiac structure remodeling.5-7 Our and other scholars’ previous studies8,9 demonstrated that exendin-4 and dipeptidyl peptidase-4 inhibition alleviated cardiac apoptosis, myocardial adverse remodeling, inflammation, and fibrosis in a myocardial infarction model and a heart failure model independently of diabetes. However, studies on the electrical remodeling after myocardial infarction are limited. The heart failure ventricular myocardium is characterized by an increased diastolic calcium leak, reflecting an increased probability of ryanodine receptor 2 (RyR2) opening.10,11 Our previous study demonstrated that exendin-4 decreased the phosphorylation of RyR2 at Ser 2814,12 which indicated that exendin-4 may be related to the calcium leak in the heart failure model. In this study, we sought to investigate the effect of exendin-4 on ventricular arrhythmias and calcium sparks-mediated calcium leaks in a myocardial infarction-heart failure model.

Methods

All experimental protocols were approved by The Experimental Animal Care and Institutional Animal Ethi-
In vivo treated every 12 hours. and exendin-9-39 (150 μg/kg/day), which were administered and intraperitoneal injection of exendin-4 (10 μg/kg/day) [GLP-1R antagonists] received coronary artery ligation after surgery, the rats in the sham and MI groups received the same protocol except for the coronary artery ligation. Twenty-four hours after surgery, the rats in the sham and MI groups received an intraperitoneal injection of physiological saline for 4 weeks. Rats in the MI + Ex-4 group underwent coronary artery ligation and intraperitoneal injection of exendin-4 (10 μg/kg/day), which was administered every 12 hours. Rats in the MI + Ex-4 + Ex9-39 group (GLP-1 receptor [GLP-1R antagonists]) received coronary artery ligation and intraperitoneal injection of exendin-4 (10 μg/kg/day) and exendin-9-39 (150 μg/kg/day), which were administered every 12 hours.

**In vivo arrhythmia studies:** Four weeks after surgery, the rats from the four groups received implantable electrocardiogram [ECG] telemetry transmitters (Data Sciences International, Saint Paul, MN, USA) for in vivo arrhythmia studies. The animals underwent a continuous 24-hour ECG recording for spontaneous ventricular arrhythmias. In vivo ECG recordings were converted into TXT data format files, and arrhythmia analysis was performed using LabChart 7.0 software (AD instruments, New South Wales, Australia). Most ventricular arrhythmias were ventricular ectopy. Ventricular arrhythmias were categorized as numbers of premature ventricular contractions and incidence of ventricular tachycardia (VT). VT was defined as at least three consecutive PVCs occurred. VTs were categorized as sustained VTs (>30 seconds) and non-sustained VTs (< 30 seconds).

**Ex vivo arrhythmia studies:** Four weeks after surgery, the animals were anesthetized with pentobarbital sodium (40 mg/kg) and heparinized. The hearts were quickly isolated and mounted upon the Langendorff perfusion system (AD Instruments). Hearts were perfused at 37.0°C, pH 7.30, and at a constant pressure of 70-90 cm H₂O with oxygenated Tyrode’s solution (NaCl, 135 mmol/L; KCl, 5.4 mmol/L; CaCl₂, 1.8 mmol/L; MgCl₂, 1 mmol/L; Na₂HPO₄, 0.3 mmol/L; hydroxyethyl piperazine ethanesulfonic acid [HEPES], 10 mmol/L; and glucose, 10) (mmol/L) at 4°C. The aorta was cannulated and mounted upon the Langendorff perfusion system with Ca²⁺-free Tyrode’s solution at 37°C for 5 minutes. Then, the heart was switched to perfusion with Ca²⁺-free Tyrode’s solution containing type II collagen enzyme (12 mg/30 mL; Sigma) until the myocardium was completely digested. The border zone of the left ventricle was carefully excised and agitated to release single cardiomyocytes. The cardiomyocytes were stabilized in Tyrode’s solution with 0.1% bovine serum albumin.

The isolated cardiomyocytes were incubated with 10 μmol/L, Fluo-4 AM (Invitrogen) for 25 minutes at room temperature. The cells were not tested for at least 30 minutes to allow the fluorescent dye to be de-esterified. Fluo-4-loaded myocytes were excited at 488 nm and the emitted fluorescence was collected using a 500-550 nm filter (LCS-SP8-STER-D; Leica Microsystems, Germany). The cardiomyocytes were stimulated at 1 Hz for 1 minute to maintain a stable SR Ca²⁺ load. Then, the cardiomyocytes were scanned at 2-millisecond intervals and 512 pixels wide to record Ca²⁺ sparks. Ca²⁺ sparks were analyzed using Sparkmaster plug-in for Image J with human verification of spark identification. Analysis included spark frequency (number of observed sparks per second and per 1 mm of scanned distance) and spark mass (∆F/F₀). Spark-mediated SR leak was measured as spark mass multiplied by spark frequency.

**Western blotting analysis:** Rats were sacrificed four weeks after surgery and western blot analysis was per-
formed to assess protein expression of phosphorylated and total RyR2 and CaMK-II. The border zones of the left ventricular tissues were used, while in the sham group tissues from the same site were used for western blot analysis. Protein extracts were loaded onto and separated on distinct sodium dodecyl sulfate polyacrylamide gel electrophoresis gels, blotted onto polyvinylidene fluoride membranes, and probed using the following antibodies: anti-CaMK-II (BS5510; Bioworld, USA), anti-Thr-286-phospho-CaMK-II (PA5-37833; Threo, USA), anti-RyR (ab2868; Abcam, USA), and anti-Ser-2814-phospho-RyR (ab59225; Abcam). The primary antibodies were visualized using HRP-linked secondary antibodies for 1 hour at room temperature. The chemiluminescence of the blots was detected using an ECL kit (Beyotime Institute of Biotechnology). The blots were quantified using NIH Image J software.

Data analysis and statistical methods: Statistical analysis was performed with the SPSS 20.0 software (SPSS Inc, Chicago, IL, USA). The continuous experimental data are expressed as the mean ± SDE and were analyzed by one-way analysis of variance followed by Dunnett’s multiple comparison test. The categorical data are expressed as percentage and were analyzed by Fisher’s exact test. The median PVC and threshold values of the APD alternans and calcium spark parameters were analyzed by nonparametric Kruskal-Wallis test with Dunn’s post-test comparison. A value of $P < 0.05$ was considered to be statistically significant.

Results

Exendin-4 attenuates spontaneous ventricular tachyarrhythmic susceptibility in a HF model: Conscious rats

![Figure 1](image-url)
Figure 2. *In vivo* arrhythmia studies in four groups. A: Examples of *in vivo* ECG recordings in four groups. B: Incidence of ventricular arrhythmia and median of premature ventricular contraction in four groups. $n = 5$ animals/group. ★ $P < 0.05$ versus the sham group. ★★ $P < 0.05$ versus the MI group, ★★★ $P < 0.05$ versus the MI + Ex-4 group.

Figure 3. Effect of exendin-4 on APD in left ventricular border zone. A: Representative recordings of action potentials. B: Quantitative analysis of APD90 of LAA in sham, MI, MI + Ex-4, and MI + Ex-4 + Ex9-39 rat hearts at four weeks after sham or MI surgery ($n = 18$ animals per group). ★★ $P < 0.05$ versus the sham group, ★★★ $P < 0.05$ versus the MI group, ★★★★ $P < 0.05$ versus the MI + Ex-4 group. APD indicates action potential duration; and PCL, pacing cycle length.
in four groups showed similar RR interval, but MI rats showed longer QRS, QT interval, and QTc. Exendin-4 attenuated MI-induced prolongation of the ECG parameters (Figure 1). Conscious MI rats showed frequent ventricular ectopy and high rates of ventricular tachyarrhythmias. Exendin-4 administration reduced both the frequency of premature ventricular contraction and the incidence of VT. However, exendin 9-39 abolished the effect of exendin-4 (Figure 2).

**Exendin-4 decreases induced ventricular tachyarrhythmic susceptibility in a HF model:** APD$_{90}$ was longer in the myocardial infarction group than in the sham group, but it was shorter in the MI + Ex-4 group than in the sham group. Exendin 9-39 neutralized the effect of exendin-4 on APD$_{90}$ (Figure 3). MI increased the maximum PCL of the APD alternans in the left ventricular myocardial infarction border zone, and exendin-4 treatment partially inhibited the destructive effect of MI as indicated by a shorter maximum PCL of APD alternans (Figure 4). The burst pacing protocol induced a high incidence of VT in the MI group. Total VT was triggered in 2/18 sham cases and in 14/18 MI cases. Sustained VTs were absent in the sham group and 11/18 in the MI group. Interestingly, exendin-4 partly abolished burst pacing total and sustained VTs in the failing hearts, whereas exendin 9-39 blocked the protective effect of exendin-4 in total (13/18) and sustained VTs (10/18) on inducing VT (Figure 5).

**Exendin-4 reduces spark-mediated SR Ca$^{2+}$ leak in a HF model:** Ca$^{2+}$ spark frequency was higher in MI myocytes, suggesting an increase in the RyR2 opening frequency. Ca$^{2+}$ spark mass, an indicator reflecting the amount of calcium released with each spark, was significantly increased in MI myocytes and reduced to close to normal levels after exendin-4 treatment. The total spark-mediated SR Ca$^{2+}$ leak was reduced by exendin-4 treatment. Exendin 9-39 inhibited the effect of exendin-4 on spark-mediated SR Ca$^{2+}$ leak (Figure 6).

**Exendin-4 reduces CaMK-II pathway activity and decreases RyR phosphorylation:** To determine whether CaMK-II and RyR were activated in the heart tissues of rats of the MI group as well as the effect of exendin-4, we analyzed the protein levels of phosphorylated and total CaMK-II. P-CaMK-II levels were significantly increased in MI hearts but normalized with exedin-4 treatment (Figure 7A, B). Alteration of CaMK-II phosphorylation could mediate CaMK-II-dependent phosphorylation in RyR2. RyR2-S2814 was a site phosphorylated by CaMK-II, which was increased in the MI group, suggesting an increased calcium release property of RyR2. The alteration was significantly attenuated by exendin-4 treatment (Figure 7C, D). Exendin 9-39 inhibited the effect of exendin-4

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**Figure 4.** The APD alternans in sham, MI, MI + Ex-4, and MI + Ex-4 + Ex9-39 rat hearts. A: Examples of the MAP recordings during the APD alternans in four groups. B: Median of the maximum PCL-induced APD alternans at the left ventricular border zone in each group. n = 18 animals per group. ★P < 0.05 versus the sham group. *P < 0.05 versus the MI group.
on CaMK-II and RyR2 phosphorylation (Figure 7).

**Discussion**

This study demonstrated that exendin-4 has an effect on ventricular arrhythmias and calcium leak in a HF model. GLP-1R agonists and DPP-4 inhibitors act through the G protein-coupled GLP-1R on multiple organs to exert their actions. GLP-1 and DPP-4 inhibitors have proven to change cardiac electrophysiology and calcium handling, but the mechanism of exendin-4’s effect on cardiac electrophysiology is unknown.

**Reduced arrhythmogenic SR calcium leak:** Increased SR calcium leak is recognized to be proarrhythmic, and is believed to lead to increased intracellular calcium, activating NCX during the diastole, resulting in DADs and trigger activity. In the present study, exendin-4 had effects on intracellular calcium homeostasis through a decrease of the calcium leak. Exendin-4 reduced the calcium spark potential by preventing local calcium elevation to the threshold levels. Stabilization of RyR2 gating is also an antiarrhythmic mechanism. Huang, *et al.* found that GLP-1 significantly decreased phosphorylation of RyR at S2814, and GLP-1 treatment increased calcium transients and SR Ca\(^{2+}\) contents. Exendin-4 reduced the phosphorylation of RyR2-S2814, which has previously been reported to increase the stability of RyR2, decrease spontaneous Ca\(^{2+}\) waves, and delay afterdepolarizations.

Increased RyR2 phosphorylation by CaMK-II could lead to increased calcium leak, which contributes to the occurrence of ventricular arrhythmia and heart failure. RyR2 at S2814 is a target of CaMK-II and activated CaMK-II is generally associated with increased phosphorylation of RyR2 at S2814. In the present study, we found that p-RyR2 (at S2814) is significantly increased in MI hearts, where activated CaMK-II was observed, although no apparent change was found in total RyR2 and CaMK-II. Exendin-4 reduced the phosphorylation of RyR2-S2814, which may be associated with suppressed CaMK-II activity.

**Reduced susceptibility to reentry arrhythmias:** In this study, exendin-4 shortened APD\(_{90}\) and reduced the QT interval, which was prolonged in the HF model. These results suggest that ventricular repolarization was normalized with exendin-4 treatment, which reduced reentry arrhythmias vulnerability in the failing ventricle. Exendin-4 treatment reduced susceptibility to burst pacing-induced reentry arrhythmias, as demonstrated by the lower incidence of VT. Improvement of calcium handling in the HF model by exendin-4 may therefore maintain electrical activity homogeneity, which we showed in our previous study.
APD alternans is a cardiac alternans at the tissue level that is linked to increased risk of ventricular arrhythmias and sudden cardiac death. Although the cause of APD alternans is multifactorial, a great amount of evidence suggests alternans is caused by calcium handling disturbances. The present study showed that exendin-4 decreased the ADP alternans threshold in the HF model, and this may be the underlying mechanism through which exendin-4 decreased the incidence of VT.

**Indirect antiarrhythmic effects from improvement of ventricular remodeling:** We have previously shown that exendin-4 improved adverse ventricular remodeling through increased ejection fraction, ameliorating ventricular and cardiomyocyte hypertrophy, and decreasing interstitial fibrosis. These may indirectly reduce ventricular arrhythmias. However, several lines of evidence from this study suggest that exendin-4 has a direct effect on ventricular arrhythmia generation by affecting electrophysiological characteristics, including ECG parameters, APD duration, the threshold of APD alternans, and SR calcium sparks-mediated calcium leak.

**Conclusion**

The GLP-1R agonist exendin-4 reduced susceptibility to ventricular arrhythmogenesis in spontaneous and burst pacing-induced ventricular arrhythmias in the MI-induced HF rat model. Exendin-4 reduced calcium spark mass,
Disclosures

Conflicts of interest: None.

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