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Exendin-4, glucagon-like peptide-1 receptor agonist, enhances isoflurane-induced preconditioning against myocardial infarction via caveolin-3 expression

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Abstract. – OBJECTIVE: To investigate the cardioprotective effects of isoflurane and exendin-4 against myocardial ischemia/reperfusion injury and the signaling pathways through which these effects are mediated.

MATERIALS AND METHODS: For infarct size measurements, anesthetized mice were subjected to 30 min of coronary artery occlusion followed by 2 h of reperfusion. Wild-type or caveolin-3 knockout mice received isoflurane, exendin-4, or isoflurane with exendin-4 before ischemia index determination. Caveolin-3 expression in the heart was measured by immunoblotting.

RESULTS: Myocardial infarct size was smaller in the isoflurane- [1.0 minimum alveolar concentration (MAC)] or exendin-4- (30 ng/kg i.v.) treated groups than the controls. Infarct size was not affected by isoflurane at 0.5 MAC or 3 ng/kg i.v. exendin-4, but the combination of these treatments reduced infarct size. Pharmacological preconditioning (isoflurane at 1.0 MAC, 30 ng/kg i.v. exendin-4, or isoflurane at 0.5 MAC with 3 ng/kg i.v. exendin-4) increased caveolin-3 protein expression in the heart after infarct induction. The cardioprotective effects of isoflurane, exendin-4, and isoflurane with exendin-4 were abolished in caveolin-3 knockout mice.

CONCLUSIONS: The combination of isoflurane and exendin-4 reduced infarct size, but it was not more effective than either agent alone, and the cardioprotective effects of these agents are mediated by caveolin-3 expression.

Key Words:
Volatile anesthetic, Isoflurane, Exendin-4, Glucagon-like peptide-1, Preconditioning.

Introduction

Anti-diabetic drugs that could control hyperglycemia and reduce the risk of cardiovascular events are of potential benefit to patients with diabetes mellitus. Glucagon-like peptide-1 (GLP-1), a gut incretin hormone that stimulates insulin secretion, also protects cardiac myocytes against ischemia/reperfusion injury by activating numerous intracellular signaling pathways[1]. Exendin-4 (Ex-4), an exogenous GLP-1 receptor agonist, also produces cardioprotective effects[2, 3].

Caveolae (plasma membrane invaginations) and their structural proteins, the caveolins, have been shown to play a fundamental role in myocardial protection against ischemia/reperfusion injury[4]. Our previous studies revealed that caveolin-3 (Cav-3) knockout (Cav-3 KO) mice[5], which had fewer myocardial caveolae than wild-type mice, lost their susceptibility to the cardioprotective effect of isoflurane (Iso) against ischemia/reperfusion injury[6,7].

Ex-4 is currently approved for the treatment of patients with type 2 diabetes mellitus, in which it is administered daily as a subcutaneous injection. As clinical experience with this drug under general anesthesia has increased, the signaling events through which the cardioprotective effects of Ex-4 are mediated under volatile anesthetic treatment have become a focus of attention. Thus, we tested the hypotheses that the cardioprotective effects of Iso and Ex-4 are dependent on Cav-3 expression, and that their preventative effects against ischemia/reperfusion injury are additive.

Materials and Methods

All animals were treated in compliance with the Guidelines for Proper Conduct of Animal Experiment and Related Activities, and the proto-

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cols were approved by the Animal Care and Use Committee at the University of Tokushima, Japan.

Ischemia/Reperfusion Protocol and Experimental Groups

General preparation was performed as previously described[8]. Briefly, C57BL/6 or Cav-3 KO mice were anesthetized with pentobarbital sodium and mechanically ventilated. Hemodynamic effects were measured via carotid artery cannulation with a pressure transducer and amplifier. After thoracotomy, mice were randomly assigned to receive Iso at 0.5 or 1.0 minimum alveolar concentration (MAC), Ex-4 at 3 ng/kg i.v. or 30 ng/kg i.v., or the combination of Iso and Ex-4. Control mice were subjected to occlusion and reperfusion without pretreatment with Iso or Ex-4. The experimental design is illustrated in Figure 1A.

Lethal ischemia was produced by occluding the coronary artery for 30 min. After 2 h of reperfusion, mice were heparinized, the heart was excised, and the area at risk (AAR) and infarct size (IS) were determined as previously described[9].

Sucrose Density Fractionation

Left ventricles (LV) were used for sucrose density membrane fractionation as reported previously[6]. After centrifugation, samples were removed in 1 mL aliquots to yield 12 fractions. We defined fractions 4-6 as buoyant membrane fractions enriched in caveolae and proteins associated with caveolae. Fractions 9-12 were defined as nonbuoyant fractions.

Immunoblot Analysis

Proteins in the membrane fractions were separated by SDS-PAGE in 10% polyacrylamide precast gels (Bio-Rad Laboratories, Hercules, CA, USA) and transferred to a polyvinylidene difluoride membrane by electroelution. Membranes were blocked in phosphate-buffered saline (PBS) containing 2.0% nonfat dry milk and incubated with primary antibodies overnight at 4 °C. Bound primary antibodies were visualized using secondary antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and ECL reagent (Amersham, Buckinghamshire, UK).

Statistical Analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA) with repeated measures, followed by Bonferroni's post-hoc test. All data are expressed as mean ± SD. Statistical significance was defined as $p < 0.05$.

Results

We found no significant differences between the groups in pre-occlusion heart rate, blood pressure, or rate pressure product (data not shown). The AAR was compared with the total LV area, and no significant differences were observed between the groups (Figure 1B and 1C). Treatment with Iso at 0.5 MAC or Ex-4 at 3 ng/kg i.v. had no effect on the IS/AAR ratio in comparison with the control treatment [42 ± 6% (0.5 MAC Iso) or 42 ± 5% (3 ng/kg i.v. Ex-4) *vs.* 45 ± 9% (control); Figure 1B], whereas the combination of these treatments reduced the IS/AAR ratio (24 ± 10%; Figure 1B). Similar infarct size reductions were produced by treatment with Iso at 1.0 MAC or Ex-4 at 30 ng/kg i.v., whereas the combination of these treatments did not result in further infarct size reduction [28 ± 6% (1.0 MAC Iso), 25 ± 8% (30 ng/kg i.v. Ex-4), and 25 ± 9% (1.0 MAC Iso with 30 ng/kg i.v. Ex-4); $p < 0.01$ *vs.* control treatment; Figure 1C].

We assessed the effect of Iso and Ex-4 on cardiac Cav-3 localization. Hearts from mice subjected to Iso (1.0 MAC), Ex-4 (30 ng/kg i.v.), or the combination of Iso (0.5 MAC) and Ex-4 (3 ng/kg i.v.) were fractionated on a discontinuous sucrose gradient, and the distribution of Cav-3 in the buoyant and nonbuoyant fractions was compared to that of control mice (Figure 2A). We assessed signaling components in buoyant fractions which are associated with caveolae. Pretreatment with Iso, Ex-4, or the combination of Iso and Ex-4 significantly increased the amount of Cav-3 protein in the buoyant fractions ($p < 0.05$ *vs.* control; Figure 2B). In comparison with wild-type animals, the cardioprotective effects of Iso and Ex-4 pretreatment against ischemia/reperfusion injury as determined by the IS/AAR ratio were abolished in Cav-3 KO mice, although there was a similar AAR/LV ratio in all groups of animals (Figure 2C), suggesting that Cav-3 is essential for Iso and/or Ex-4 induced cardiac protection.

Discussion

The current investigation confirms our previous finding that exposure to Iso at 1.0 MAC, but

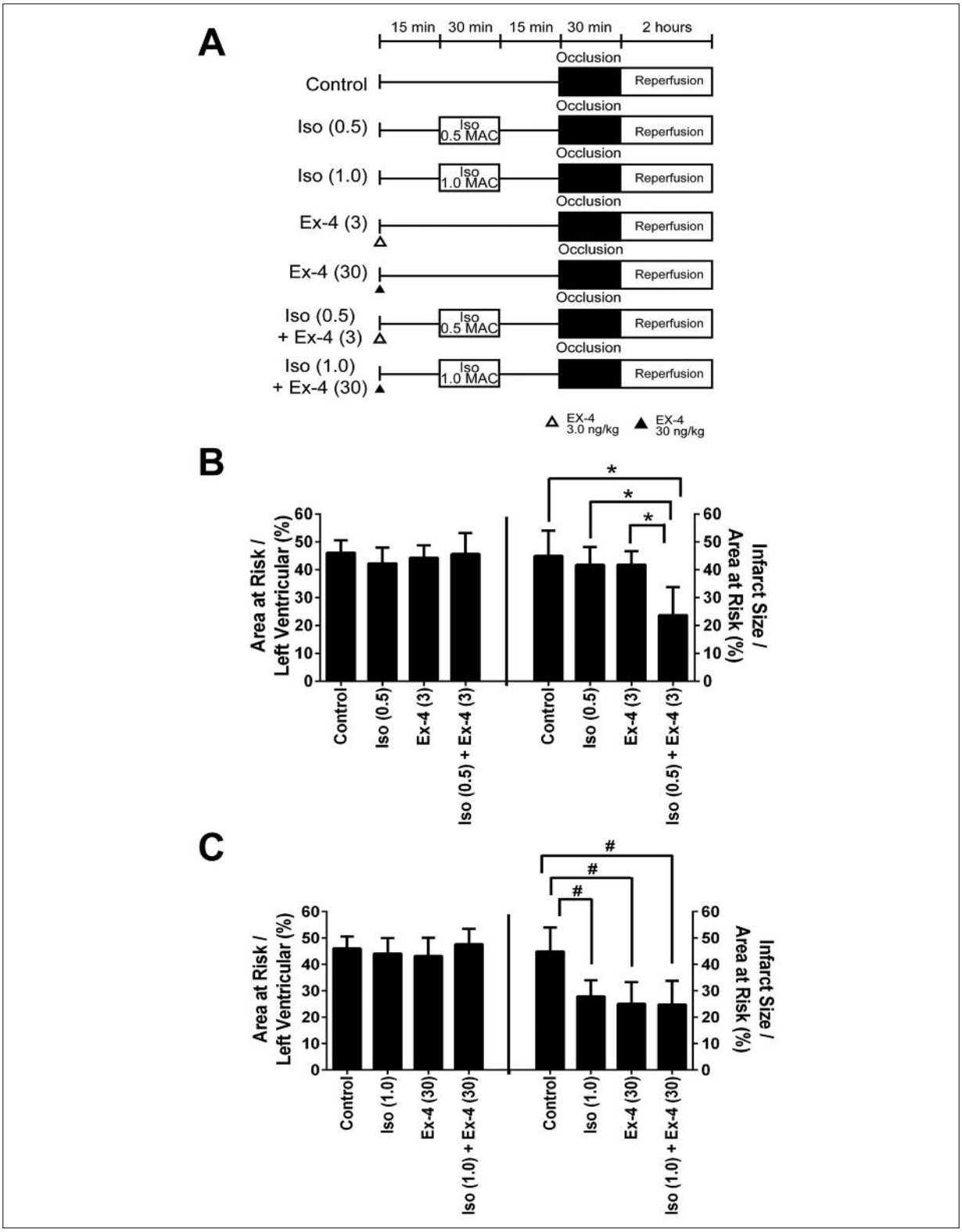

Figure 1. A, Schematic illustration of the experimental protocol. Mice were treated with isoflurane (Iso) and/or exendin-4 (Ex-4). ***B,*** Area at risk as a percentage of left ventricle and infarct size as a percentage of area at risk. n = 7-8 mice per group. *$p < 0.01$ vs. Iso (0.5 MAC) + Ex-4 (3 ng/kg i.v.). ***C,*** Area at risk as a percentage of left ventricle and infarct size as a percentage of area at risk. n = 7-8 mice per group. #$p < 0.01$ vs. control. Iso (0.5) = 0.5 MAC isoflurane; Iso (1.0) = 1.0 MAC isoflurane; Ex-4 (3) = 3 ng/kg i.v. exendin-4; Ex-4 (30) = 30 ng/kg i.v. exendin-4.

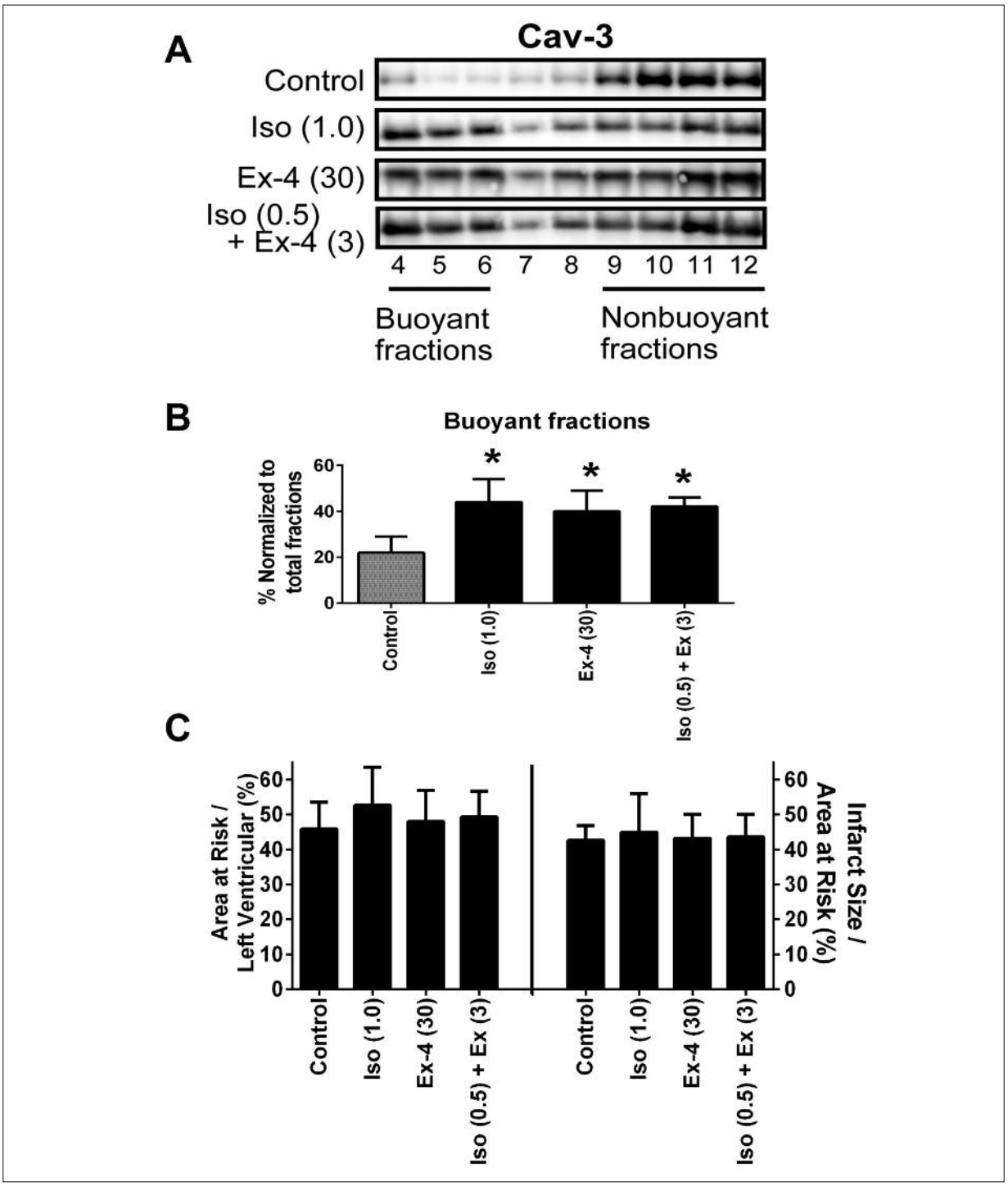

Figure 2. Cardioprotective effects of isoflurane and exendin-4 are associated with caveolin-3 (Cav-3) migration to the buoyant caveolar fractions. ***A,*** Hearts from control mice and mice treated with 1.0 MAC isoflurane [Iso (1.0)], 30 ng/kg i.v. exendin-4 [Ex-4 (30)], or the combination of 0.5 MAC isoflurane and 3 ng/kg i.v. exendin-4 [Iso (0.5) + Ex-4 (3)] were lysed and fractionated on a discontinuous sucrose density gradient after a 2-h recovery period. Fractions were collected and probed for Cav-3. Pharmacological preconditioning induced Cav-3 migration to the buoyant fractions. ***B,*** The protein abundance in each fraction was determined by densitometry and normalized to the total protein in each fraction. n = 4 animals per group. *$p < 0.05$ treated group vs. control. ***C,*** After pharmacological preconditioning, Cav-3 knockout mice underwent a 30-min coronary artery occlusion followed by a 2-h reperfusion. The size of the area at risk as a percentage of the left ventricle area was not different between the groups. The cardioprotective effects of pharmacological preconditioning were abolished in Cav-3 knockout mice, as shown by the lack of a significant decrease in the infarct size/area at risk in comparison with that of control Cav-3 knockout mice. n = 7-8 animals per group.

not at 0.5 MAC, produces myocardial protection against ischemia/reperfusion injury by inducing Cav-3 expression[7]. Our results also show that administration of Ex-4 (30 ng/kg i.v.) reduces infarct size through a Cav-3-dependent mechanism. However, at lower concentrations of Ex-4 (3 ng/kg i.v.), cardiac protection was not observed. Consistent with these findings, the thresholds for the induction of these pharmacological preconditioning effects are located between 0.5 and 1.0 MAC for Iso and between 3 and 30 ng/kg i.v. for Ex-4. The current findings further demonstrate that Ex-4, when administered at a dose that does not alter infarct size alone, enhances the cardioprotective effect of Iso preconditioning against infarction. The cardioprotective effect of the combination of subthreshold doses of Iso and Ex-4 was abolished in Cav-3KO mice, indicating that the observed decrease in the extent of infarction was mediated by Cav-3 expression. Additionally, treatment with the combination of Iso (1.0 MAC) + Ex-4 (30 ng/kg i.v.) reduced infarct size, but not more than treatment with either agent alone.

Although there is little evidence regarding the relationship between volatile anesthetics and GLP-1 within the heart, other organ systems, including pancreatic cells, have been investigated in this context[10]. Using a patch-clamp technique in pancreatic cells, GLP-1 receptor signaling was shown to restore glucose-induced depolarization suppressed by Iso. Additionally, Iso inhibited glucose-induced insulin secretion, but did not affect the insulinotropic action of exogenous GLP-1, suggesting that GLP-1 may be a useful agent for intraoperative glycemic control[10]. Furthermore, another study demonstrated that diabetes attenuated the cardioprotective effects of Iso preconditioning, and that the extent of infarction was dependent on blood glucose concentration[11]. Taken together with these findings, the present investigation of Iso and exogenous GLP-1 receptor agonist Ex-4 suggest that combined treatment with these agents could provide benefits for diabetic patients with an increased risk of myocardial ischemia.

Ex-4 has been shown to produce a dose-dependent increase in blood pressure and heart rate in rats[12]. In our studies, however, there were no hemodynamic changes among the groups at the pre-occlusion time (data not shown). This may be due to the dose and the timing of administration. Yamamoto et al[12] showed that hemodynamic parameters returned to basal levels within 60-70 min after a dose of 30 ng i.v. Ex-4, but found that hemodynamics remained significantly increased for up to 2 h after treatment with 300 or 3000 ng i.v. Ex-4. As the ideal dose and optimal time period at which to administer Ex-4 to produce cardiac protection in mice are unknown, we selected the Ex-4 dose for the assessment of hemodynamic differences based on the study above, and chose the pre-occlusion period as the time of drug administration.

We evaluated the GLP-1 receptor-dependent effects of Ex-4, an exogenous GLP-1 receptor agonist, in experiments that investigated ischemia/reperfusion injury. Recent studies show that GLP-1 (9-36), a GLP-1 metabolite generated by dipeptidyl peptidase-IV that has 1000-fold lower affinity for the GLP-1 receptor[13], improves LV contractile function and post-ischemic myocardial injury[14]. In addition, GLP-1 receptor knockout mice have slower heart rates, increased blood pressure, and greater cardiac mass, and GLP-1 has been shown to protect perfused hearts from ischemia in rodents lacking the GLP-1 receptor[3]. These findings suggest that GLP-1 and its metabolite GLP-1 (9-36) may be capable of exerting GLP-1 receptor-independent effects on the cardiovascular system[15]. Thus, determination of the specific role of GLP-1 receptor-independent signaling in the cardioprotective effects of GLP-1 ligands will require additional investigation.

Conclusions

The results of this investigation show that exposure to isoflurane at 1.0 MAC and/or 30 ng/kg i.v. exendin-4 reduced infarct size after coronary artery occlusion in mice. The current study also demonstrates that combined administration of isoflurane and exendin-4 at doses that do not alter infarct size alone significantly reduces myocardial infarct size. Furthermore, the cardioprotective effects of isoflurane and exendin-4 are mediated by the activation of signal transduction pathways that converge on caveolin-3 expression.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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