

Protein kinase A mediates glucagon-like peptide 1-induced nitric oxide production and muscle microvascular recruitment

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Dong Z, Chai W, Wang W, Zhao L, Fu Z, Cao W, Liu Z. Protein kinase A mediates glucagon-like peptide 1-induced nitric oxide production and muscle microvascular recruitment. *Am J Physiol Endocrinol Metab* 304: E222–E228, 2013. First published November 27, 2012; doi:10.1152/ajpendo.00473.2012.—Glucagon-like peptide-1 (GLP-1) causes vasodilation and increases muscle glucose uptake independent of insulin. Recently, we have shown that GLP-1 recruits muscle microvasculature and increases muscle glucose use via a nitric oxide (NO)-dependent mechanism. Protein kinase A (PKA) is a major signaling intermediate downstream of GLP-1 receptors. To examine whether PKA mediates GLP-1's microvascular action in muscle, GLP-1 was infused to overnight-fasted male rats for 120 min in the presence or absence of H89, a PKA inhibitor. Hindleg muscle microvascular recruitment and glucose use were determined. GLP-1 infusion acutely increased muscle microvascular blood volume within 30 min without altering microvascular blood flow velocity or blood pressure. This effect persisted throughout the 120-min infusion period, leading to a significant increase in muscle microvascular blood flow. These changes were paralleled with an approximately twofold increase in plasma NO levels and hindleg glucose extraction. Systemic infusion of H89 completely blocked GLP-1-mediated muscle microvascular recruitment and increases in NO production and muscle glucose extraction. In cultured endothelial cells, GLP-1 acutely increased PKA activity and stimulated endothelial NO synthase phosphorylation at Ser¹¹⁷⁷ and NO production. PKA inhibition abolished these effects. In ex vivo studies, perfusion of the distal saphenous artery with GLP-1 induced significant vasorelaxation that was also abolished by pretreatment of the vessels with PKA inhibitor H89. We conclude that GLP-1 recruits muscle microvasculature by expanding microvascular volume and increases glucose extraction in muscle via a PKA/NO-dependent pathway in the vascular endothelium. This may contribute to postprandial glycemic control and complication prevention in diabetes.

glucagon-like peptide 1; protein kinase A; microvascular recruitment; nitric oxide

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is a major incretin hormone and acts on its 64-kDa G protein-coupled receptor to increase cAMP production and mediates glucose-induced insulin secretion. Recent evidence suggests that GLP-1 is capable of increasing muscle glucose uptake independent of insulin (2). This was most convincingly demonstrated by an observation of increased myocardial uptake of glucose in Langendorff-perfused rat heart preparation (41). In conscious dogs, both GLP-1 and its active metabolite are able to increase myocardial

glucose uptake without altering plasma insulin concentrations (32, 33).

The mechanisms underlying GLP-1-stimulated glucose uptake in muscle are poorly understood. Endothelial cells (ECs) express abundant GLP-1 receptors (35), and GLP-1 has been shown to increase coronary blood flow and myocardial glucose uptake independent of insulin (34, 41). We have reported recently that GLP-1 acutely increases muscle glucose use via increased muscle microvascular recruitment and insulin delivery through a nitric oxide (NO)-dependent mechanism (8). We and others have shown in the past that factors increasing NO production are able to recruit muscle microvasculature, leading to increased endothelial exchange surface area and substrate and hormonal exchanges between plasma and muscle interstitium (4, 5, 12, 24).

Endothelial NO synthase (eNOS) is regulated by signaling pathways involving multiple sites of phosphorylation. The coordinated phosphorylation of eNOS at Ser¹¹⁷⁷ and dephosphorylation at Thr⁴⁹⁷ activate whereas Ser¹¹⁷⁷ dephosphorylation and Thr⁴⁹⁷ phosphorylation deactivate the enzyme. In our previous report, we have speculated that protein kinase A (PKA), a major signaling intermediate downstream of GLP-1 receptors, may have played an important role in GLP-1-mediated NO production and microvascular recruitment, since PKA has been shown to activate eNOS in response to various stimuli (6, 13, 30, 31), and we have demonstrated that GLP-1 potentially increased PKA activity in cultured endothelial cells (8). Because treatment of spontaneously hypertensive rats with sitagliptin for 2 wk improved endothelium-dependent relaxation in renal arteries, restored renal blood flow, and reduced systolic blood pressure, and these effects were prevented by inhibition of either GLP-1 receptor, PKA or NO synthase (27), currently available evidence strongly suggests that GLP-1 exerts its vasodilatory actions via the GLP-1 receptor, PKA, and NO signaling pathways.

In the current study, we examined whether GLP-1-induced PKA activity indeed plays important an role in GLP-1-mediated NO production, microvascular recruitment, and glucose use in muscle. Our results in rats, cultured ECs, and ex vivo arteries indicate that GLP-1 recruits muscle microvasculature by expanding microvascular volume and increases substrate exchange in muscle via a PKA/eNOS-dependent pathway.

MATERIALS AND METHODS

Animal preparations and experimental protocols. Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 220–320 g were studied after an overnight fast. Rats were housed at 22 ± 2°C on a 12:12-h light-dark cycle and fed standard

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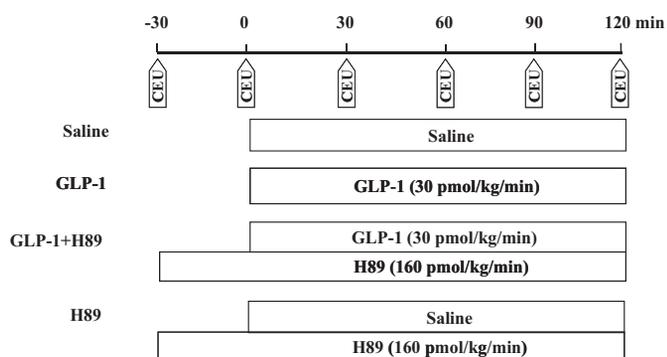


Fig. 1. Experimental protocols. CEU, contrast-enhanced ultrasound; GLP-1, glucagon-like peptide 1.

laboratory chow and water ad libitum before the study. After being anesthetized with pentobarbital sodium (50 mg/kg ip; Abbott Laboratories, North Chicago, IL), rats were placed in a supine position on a heating pad to ensure euthermia and intubated to maintain a patent airway. Polyethylene cannulae (PE-50; Fisher Scientific, Newark, DE) were inserted into the carotid artery and jugular vein for arterial blood pressure monitoring, arterial blood sampling, and various infusions.

After a 30- to 45-min baseline period to ensure hemodynamic stability and a stable level of anesthesia, rats were randomly studied in one of the following four groups (Fig. 1). Each rat received an intravenous infusion of either saline (control) or GLP-1-(7–36) amide (30 pmol·kg⁻¹·min⁻¹; Bachem Americas, Torrance, CA) for 120 min in the presence or absence of systemic infusion of H89 (160 pmol·kg⁻¹·min⁻¹; Sigma-Aldrich, St. Louis, MO). At the doses selected, GLP-1 has been shown to potently recruit muscle microvasculature (8) and H89 to abolish morphine-mediated attenuation of microvascular hyperpermeability via the PKA-dependent pathway (37). H89 infusion was started 30 min before the commencement of saline or GLP-1 infusion and had no significant effect on muscle microvascular recruitment (Fig. 2). Skeletal muscle microvascular blood volume (MBV), microvascular blood flow velocity (MFV), and microvascular blood flow (MBF) were determined using contrast-enhanced ultrasound, as described previously (8–10, 40). Hindleg glucose extraction, plasma NO levels, and plasma insulin concentrations were measured as described below. Rat gastrocnemius muscles were collected for measurement of Akt1 (Ser⁴⁷³), eNOS (Ser¹¹⁷⁷), and ERK1/2 (Thr²⁰²/Tyr²⁰⁴) phosphorylation using Western blotting and PKA activity. Rat hearts and aortae were collected for measurement of PKA activity.

Mean arterial blood pressure (MAP) was monitored via a sensor connected to the carotid arterial catheter (Harvard Apparatus, Holliston, MA, and ADInstruments, Colorado Springs, CO), and pentobarbital sodium was infused at a variable rate to maintain steady levels of anesthesia and blood pressure throughout the study. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (Publication No. 85-23, revised 1996), and the study protocol was approved by the Animal Care and Use Committee of the University of Virginia.

Determination of hindleg muscle glucose extraction. Carotid arterial and femoral venous blood glucose concentrations were determined using an Accu-Chek Advantage blood glucometer (Roche Diagnostics, Indianapolis, IN). Glucose levels were determined four to six times per time point, and the numbers were averaged. Hindleg glucose uptake (mg/dl) was calculated as the arterial-venous glucose difference.

Culture of bovine aortic ECs. Bovine aortic ECs (bAECs) in primary culture were purchased from Lonza (Walkersville, MD) and cultured as described previously (25, 26). After serum starvation for 14 h, cells between passages 3 and 8 were exposed to GLP-1-(7–36) amide (1 ng/ml; Bachem Americas, Torrance, CA) for 20 min in the absence or presence of H89 (10 μM; Sigma-Aldrich) and then used for either Western blotting, measurement of NO production, or PKA activity.

Measurement of plasma NO and insulin levels and endothelial NO production. Plasma NO levels were measured using a 280i Nitric Oxide Analyzer (GE Analytical, Boulder, CO) according to the manufacturer's instructions and as described previously (8, 9). Plasma insulin concentrations were determined using a rat insulin ELISA assay kit (Mercodia, Uppsala, Sweden). For endothelial NO production, bAECs were exposed to GLP-1-(7–36) amide (1 ng/ml) for 20 min in the absence or presence of H89 (10 μM). NO levels in media were measured using the Nitric Oxide Synthase Detection System (Sigma-Aldrich) according to the manufacturer's instructions. Insulin (100 nM) with or without L-NAME (10 mM) was used as positive and negative control for NO production.

Quantification of PKA activity. PKA activities in tissue and ECs were quantified using a PKA assay kit (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, skeletal muscle, heart, aorta, or ECs (5 × 10⁶) were homogenized in cold PKA extraction buffer, and the lysate was centrifuged for 5 min at 4°C at 14,000 g. For aorta, samples from four rats were pooled together. The supernatant was mixed with assay mixture and incubated for 30 min at room temperature, and the

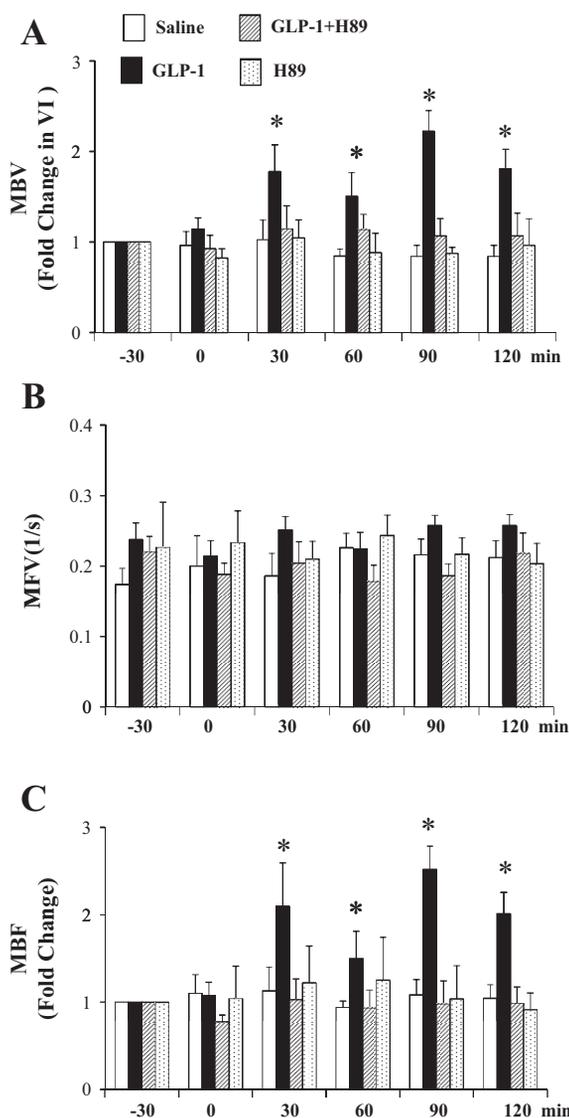


Fig. 2. Inhibition of protein kinase A (PKA) abolishes GLP-1-mediated muscle microvascular recruitment. GLP-1 was infused continuously at 30 pmol·kg⁻¹·min⁻¹ in the absence or presence of H89. *A*: changes in microvascular blood volume (MBV). *B*: changes in microvascular blood flow velocity (MFV). *C*: changes in microvascular blood flow (MBF). **P* < 0.05 compared with -30 or 0 min; *n* = 5–11.

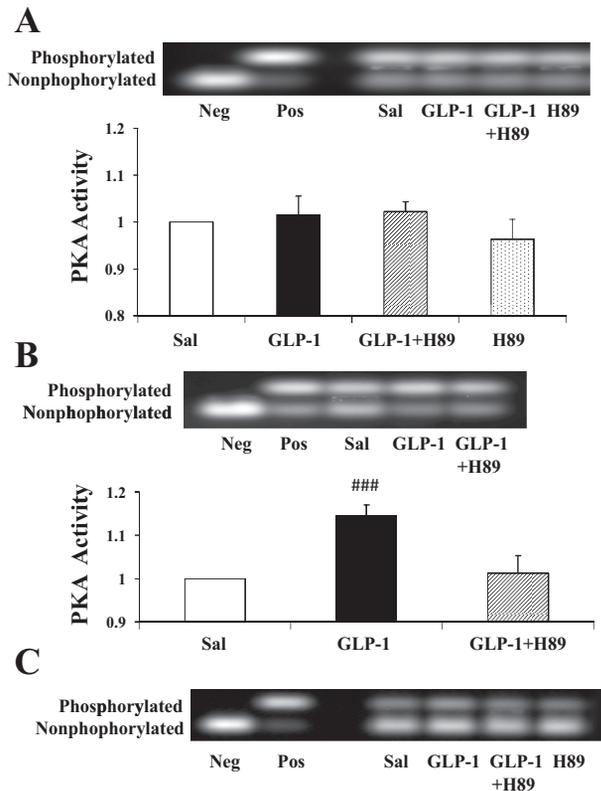


Fig. 3. Effects of H89 on GLP-1-mediated changes in PKA activity. GLP-1 was infused continuously ($30 \text{ pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) for 120 min in the absence or presence of H89 ($160 \text{ pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). A: skeletal muscle. B: heart. C: aorta (samples from 4 rats were pooled together). Neg, negative control; Pos, positive control; Sal, saline. $###P = 0.002$ compared with saline; $n = 4-5$.

reaction was stopped by heating the mixture to 95°C for 10 min. Samples were then separated on a 0.8% agarose gel at 100 V for 15 min. The densities of both the phosphorylated and nonphosphorylated peptides were quantified using Image J software, and the ratios of phosphospecific to total density were calculated.

Western blotting. Phosphorylation of eNOS, Akt1, and ERK1/2 in muscle and cultured ECs was determined by Western blotting, as described previously (8, 9). Primary antibody against phospho-Akt1 (Ser⁴⁷³) and Akt1 was obtained from Upstate Biotechnology (Lake Placid, NY), and primary antibody against phospho-eNOS (Ser¹¹⁷⁷), eNOS, phospho-ERK1/2 (Thr²⁰²/Tyr²⁰⁴), and ERK1/2 was obtained from Cell Signaling Technology (Beverly, MA). Both the total and phosphospecific densities were quantified and the ratios of phosphospecific to total density calculated.

Table 1. Changes in MAP and plasma insulin concentrations

Time (min)	-30	0	30	60	90	120
MAP, mmHg						
Saline		106 ± 4	98 ± 6	94 ± 6#	96 ± 4#	103 ± 3
GLP-1		100 ± 3	105 ± 2	108 ± 2	104 ± 3	103 ± 2
H89 + GLP-1	104 ± 3	94 ± 4*	98 ± 3*	101 ± 3	104 ± 2	106 ± 2
H89	107 ± 2	107 ± 2	99 ± 2	94 ± 4	90 ± 5*	103 ± 4
Insulin concentration, pM						
Saline		75 ± 13	70 ± 11	76 ± 13	92 ± 17	83 ± 12
GLP-1		103 ± 28	101 ± 30	99 ± 36	129 ± 43	157 ± 42
H89 + GLP-1	73 ± 17	64 ± 16	61 ± 16	42 ± 3	58 ± 10	64 ± 5
H89	121 ± 27	111 ± 20	106 ± 23	101 ± 27	120 ± 27	134 ± 37

Values are means ± SE. MAP, mean arterial blood pressure; GLP-1, glucagon-like peptide 1. * $P < 0.05$ compared with -30 min; # $P < 0.05$ compared with 0 min.

Myograph experiments. Distal saphenous artery was dissected from overnight-fasted rats immediately after the rats were euthanized. After the adhering connective tissue was trimmed off, each artery was cut into three ring segments ~ 2 mm in length. Each segment was mounted in a Multi Myograph System (Danish Myo Technology, Aarhus, Denmark). The organ chamber was filled with 6 ml of physiological salt solution buffer (130 mM NaCl, 4.7 mM KCl, 1.6 mM CaCl_2 , 1.17 mM MgSO_4 , 1.18 mM KH_2PO_4 , 14.9 mM NaHCO_3 , 0.026 mM EDTA, and 5.5 mM glucose, pH 7.4), which was constantly bubbled with 95% O_2 -5% CO_2 and maintained at 37°C . Each ring was stretched initially to 5 mN, an optimal tension, and then allowed to stabilize at baseline tone. Relaxant responses to phenylephrine (300 nM) or acetylcholine (1 μM) were performed to test for vessel viability and integrity of endothelium. After precontraction with phenylephrine, GLP-1 (1 ng/ml) was added to the incubation bath with or without H89 (1 μM) pretreatment for 1 h. The relaxant response was expressed as percentage of the contraction induced by phenylephrine.

Statistical analysis. All data are presented as means ± SE. Statistical analyses were performed with SigmaStat 3.1.1 software (Systat Software, Chicago, IL), using either Student's *t*-test or analysis of variance (ANOVA) with post hoc analysis as appropriate. A *P* value of < 0.05 was considered statistically significant.

RESULTS

PKA inhibition abolishes GLP-1-induced increase in muscle microvascular perfusion. We have demonstrated previously that GLP-1 recruits muscle microvasculature and increases glucose use via a NO-dependent mechanism and that incubation of bAECs with GLP-1 increases endothelial PKA activity significantly (8). To examine the potential role of PKA in GLP-1's microvascular action in muscle, we infused rats with the selective PKA inhibitor H89 prior to beginning GLP-1 infusion. Control rats received saline or H89 infusion only. Similarly to our previous report (8), GLP-1 potently recruited muscle microvasculature by increasing muscle MBV (~ 2 -fold, $P < 0.001$, ANOVA) without affecting MFV (Fig. 2). This led to an approximately twofold increase in muscle MBF ($P < 0.001$, ANOVA). This was associated with a significant increase in cardiac muscle and aorta PKA activity (Fig. 3). Skeletal muscle PKA activity did not change significantly across any of the groups. Inhibition of PKA activity by coinfusion of H89 completely prevented a GLP-1-induced increase in PKA activity (Fig. 3) and abolished GLP-1-induced microvascular recruitment (Fig. 2). H89 infusion alone did not alter muscle microvascular parameters despite a brief decrease in MAP either alone or with GLP-1 (Table 1).

PKA inhibition blocks GLP-1-induced NO production and muscle glucose extraction. Because microvascular endothelial surface area determines substrate exchange between plasma and muscle interstitium and we have shown previously that GLP-1 increases muscle microvascular recruitment and glucose extraction via NO-dependent mechanism (8), we next assessed whether inhibition of PKA also prevented GLP-1-induced NO production and muscle glucose extraction. As shown in Fig. 4A, GLP-1 infusion increased plasma NO levels approximately threefold within 30 min, which remained elevated for 90 min ($P = 0.001$, ANOVA). This was associated with a two- to threefold increase in muscle glucose extraction ($P < 0.04$; Fig. 4B), as reflected by femoral arterial-venous glucose difference and a significant decrease in arterial plasma glucose levels at 30 and 60 min ($P < 0.001$ and $P < 0.02$, respectively; Fig. 4C). Concurrent H89 infusion completely abolished GLP-1-stimulated increase in both NO production and muscle glucose utilization and prevented a GLP-1-induced

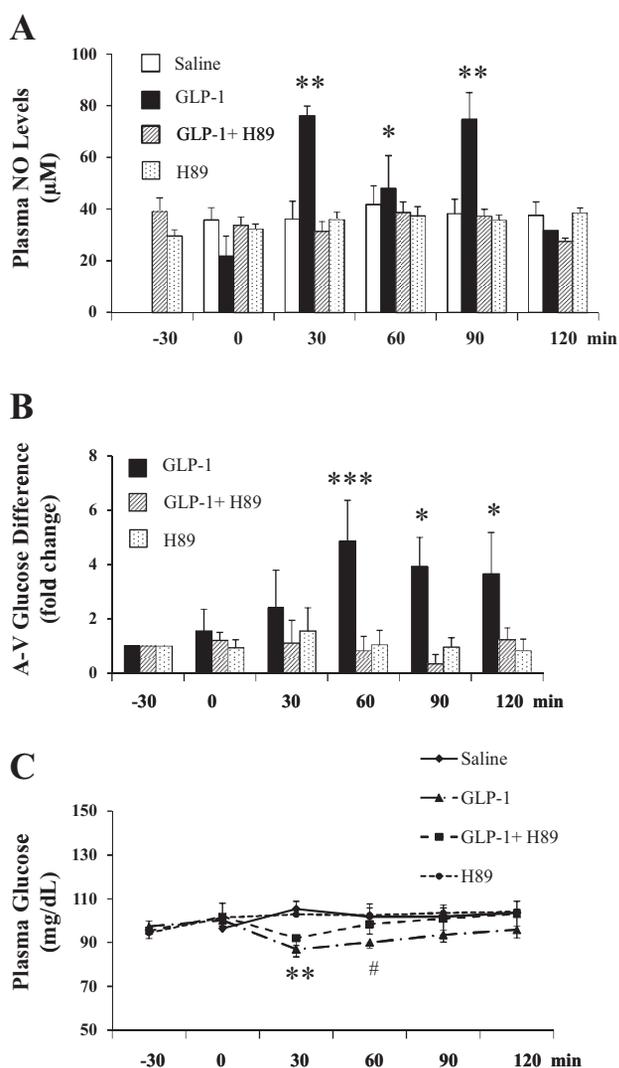


Fig. 4. PKA inhibition blunts GLP-1-mediated nitric oxide (NO) production and muscle glucose extraction. GLP-1 was infused continuously at 30 pmol·kg⁻¹·min⁻¹ in the absence or presence of H89. *A*: changes in arterial plasma NO levels. *B*: changes in muscle glucose extraction. *C*: changes in plasma glucose levels. ** $P < 0.001$, * $P < 0.05$, *** $P = 0.005$, and # $P < 0.02$ compared with -30 or 0 min; $n = 4-8$.

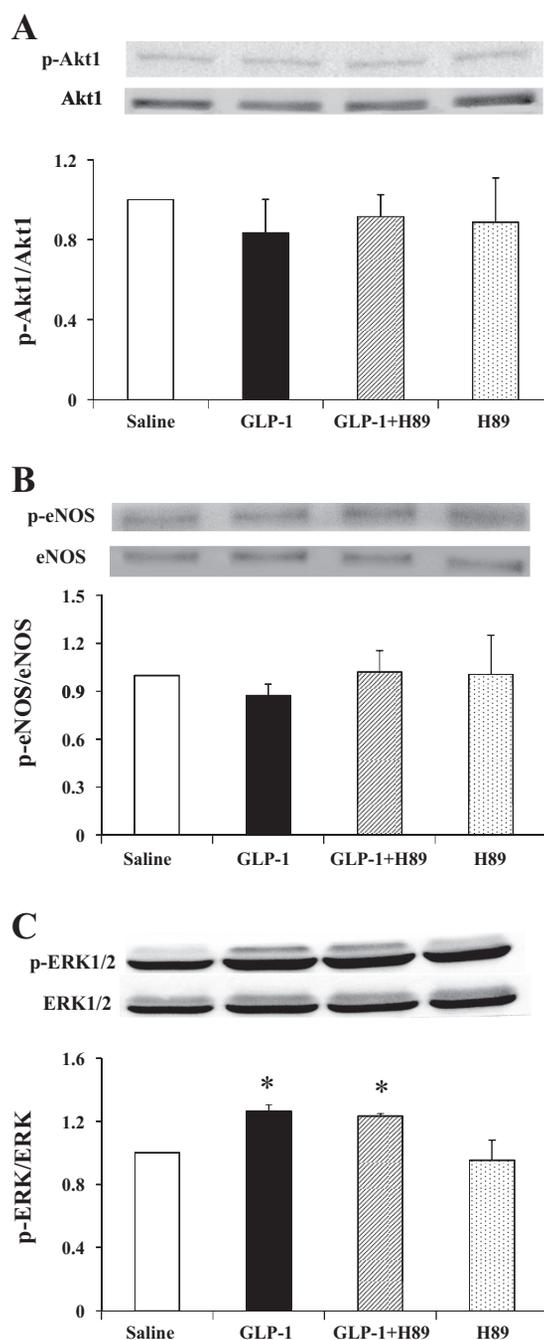


Fig. 5. Effects of PKA inhibition on GLP-1-mediated muscle Akt1 (*A*), endothelial NO synthase (eNOS) (*B*), and ERK1/2 (*C*) phosphorylation. *A*: changes in Akt1 phosphorylation. *B*: changes in eNOS phosphorylation. *C*: changes in ERK1/2 phosphorylation. * $P < 0.05$ compared with saline; $n = 3-5$.

decrease in arterial plasma glucose levels ($P = 0.169$, ANOVA). Plasma insulin concentrations remained stable during the experiments for all groups (Table 1).

GLP-1 effects on Akt1, eNOS, and ERK1/2 phosphorylation in skeletal muscle in vivo. Because we have shown that GLP-1 increased the phosphorylation of Akt1 and eNOS in cultured endothelial cells, we further examined whether GLP-1 also stimulates phosphorylation of Akt1, eNOS, and ERK1/2 in skeletal muscle. As shown in Fig. 5, GLP-1 and H89 had no effect on either Akt1 or eNOS phosphorylation in the skeletal

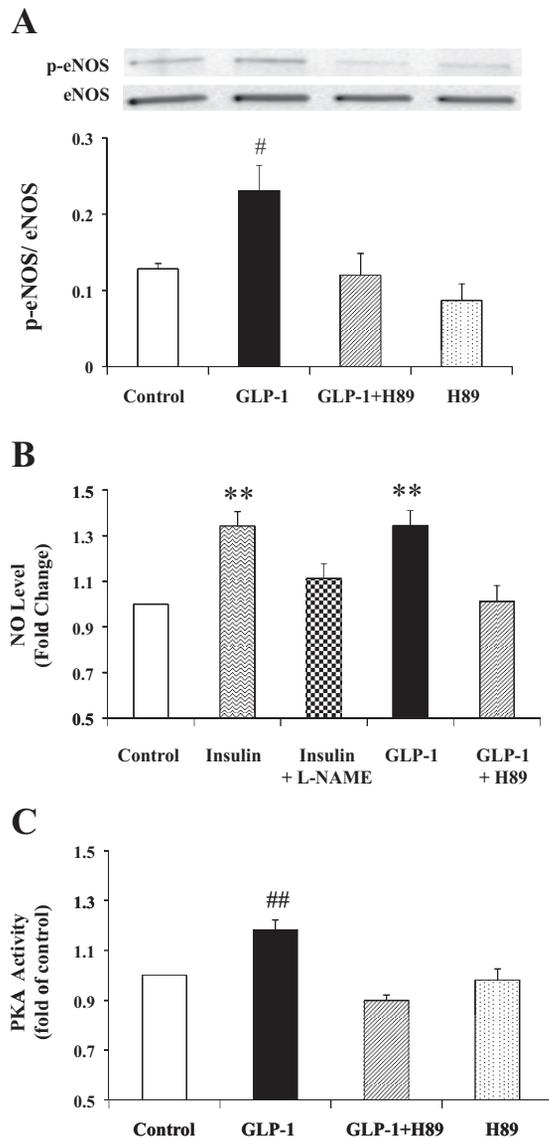


Fig. 6. GLP-1 increases NO production in cultured endothelial cells via PKA-dependent pathway. Cells were incubated with or without L-NAME (10 mM), GLP-1 (1 ng/ml), and/or H89 (10 μ M). *A*: changes in eNOS phosphorylation. *B*: changes in endothelial NO production. Insulin (100 nM) was used as positive control. *C*: changes in PKA activity. [#] $P < 0.02$, ^{**} $P < 0.001$, and ^{##} $P = 0.001$ compared with control; $n = 3-9$.

muscle. On the contrary, muscle ERK1/2 phosphorylation increased significantly during GLP-1 infusion ($P = 0.027$, ANOVA), and pretreatment with H89 did not block this effect ($P = 0.762$, ANOVA).

PKA mediates GLP-1-induced NO production in cultured bAECs. Because plasma NO increased but muscle eNOS phosphorylation did not increase, the increase in plasma NO concentrations were most likely secondary to endothelial production of NO. Figure 6 shows that incubation of bAECs with GLP-1 for 20 min potentially increased endothelial eNOS phosphorylation at Ser¹¹⁷⁷ ($P = 0.019$, ANOVA) and NO production (by $\sim 30\%$, $P < 0.001$), which was associated with a significant increase in PKA activity ($P = 0.001$, ANOVA). Incubation of cells with H89 completely abolished GLP-1-induced increases in PKA activity, eNOS phosphorylation, and

NO production, confirming that GLP-1-mediated endothelial NO production is PKA-dependent.

PKA mediates GLP-1-induced vasodilation in isolated saphenous artery communicating branch. Because we observed increased PKA activity in heart and aorta but not in the skeletal muscle, we finally used an ex vivo approach to examine the role of PKA activation in isolated rat saphenous (Fig. 7). Incubation of the artery rings with GLP-1 potentially increased vasorelaxation ($\sim 30\%$, $P < 0.001$, ANOVA), and this effect was once again prevented by pretreatment of the vessels with H89. Thus, GLP-1-mediated PKA activity within the vessel wall is indeed responsible for GLP-1-induced vasorelaxation.

DISCUSSION

The current study demonstrates that GLP-1-induced PKA activity plays a pivotal role in GLP-1-mediated microvascular recruitment and glucose use in muscle. Whereas systemic infusion of GLP-1 increased tissue PKA activity in vivo and incubation of ECs with GLP-1 potentially increased PKA activity in vitro, administration of H89, a selective inhibitor of PKA, completely abolished GLP-1-induced NO production, microvascular recruitment, and glucose use in muscle and ex vivo vasodilation. Thus, our data strongly suggest that PKA signaling is required in GLP-1's microvascular actions and the associated metabolic consequences.

Although ex vivo studies using rat arterial rings or pulmonary arteries have shown that vascular endothelium and NO production are essential in the vasorelaxant effect of GLP-1 (16, 38), and incubation of ECs with GLP-1 analog liraglutide dose and time dependently increases NO production (19), our recent report demonstrated a direct stimulatory effect of GLP-1 on eNOS phosphorylation and that endothelial production of NO is crucial in GLP-1-induced microvascular recruitment and glucose use in muscle, as inhibition of NO production completely abolished the microvascular effects of GLP-1 (8). The current study further extends our previous findings by demonstrating that these effects are mediated via the PKA signaling pathway. This is consistent with the fact that activation of the GLP-1 receptor triggers the generation of second-messenger

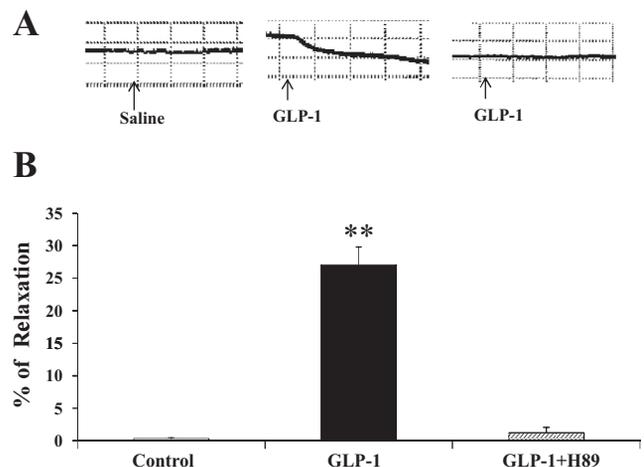


Fig. 7. PKA mediates GLP-1-induced arterial vasodilation ex vivo. Rat saphenous artery was isolated, precontracted with phenylephrine, and incubated with GLP-1 (1 ng/ml) for 30 min with or without H89 (1 μ M) pretreatment for 1 h. ^{**} $P < 0.001$ compared with control; $n = 3$.

cAMP via a direct action on the adenylate cyclase (18) and leads to the activation of PKA (14) and that eNOS is colocalized with the catalytic subunit of PKA in EC junctions (20).

The role of PKA in GLP-1-mediated glucose-stimulated insulin secretion is well characterized in pancreatic β -cells (3, 14). Binding of GLP-1 to its receptors activates adenylate cyclase and increases intracellular cAMP levels, leading to activation of PKA (21). Our observations that PKA inhibition completely prevented GLP-1-induced NO production, microvascular recruitment, and muscle extraction of glucose confirm that PKA signaling plays a key role in GLP-1's microvascular actions. That administration of PKA inhibitor H89 completely blocked GLP-1-induced PKA activity in the aorta and abolished GLP-1-induced vasodilatation ex vivo in the rat saphenous artery strongly suggests that PKA mediates GLP-1 actions in conduit artery and resistance arterioles as well.

In the current study, we observed that GLP-1 infusion increased the phosphorylation of ERK1/2 but not Akt1 in muscle. Although the increased phosphorylation of ERK1/2 was consistent with a previous report (1), the same study showed that incubation of isolated rat soleus muscles with GLP-1 for 3 min potently increased phosphatidylinositol 3-kinase (PI 3-kinase) activity and Akt phosphorylation. This discrepancy may be explained by differences in GLP-1 exposure time (2 h vs. 3 min), variations in Akt isoforms (the antibody we used did not cross-react with other Akt isoforms), and study setting (in vivo vs. in vitro). Indeed, in isolated rat hearts GLP-1 markedly increased cardiac muscle glucose uptake without altering Akt1 phosphorylation (41).

Inasmuch as both PKA and the PI 3-kinase are able to phosphorylate eNOS and increase NO production and GLP-1 has been shown to activate the PI 3-kinase pathway in muscle (1) and β -cells (7), our study findings suggest that the PI 3-kinase pathway is likely uninvolved in GLP-1-induced NO production and microvascular recruitment since inhibition of PKA alone with H89 completely blocked GLP-1-induced NO production and vascular responses both in vivo and ex vivo. Our findings are in agreement with prior reports in cultured ECs that PKA but not PI 3-kinase mediates GLP-1-induced attenuation on ROS-induced senescence (36).

H89 is a potent and selective inhibitor of PKA (11, 23) and has been widely used to inhibit PKA in biochemical studies. It blocks PKA actions through competitive inhibition of the adenosine triphosphate site on the PKA catalytic subunit. A limitation of the current study is that H89 at high concentrations also inhibits several other kinases, such as p70S6K, MSK1, ROCK11, and MAPKAP-K1b (28, 29). However, it is less likely that these kinases contributed to GLP-1-mediated NO production since inhibition of PKA activity with another specific inhibitor, Rp-cAMPS, also abolished exendin-4-induced activation of eNOS and cell proliferation (15). Rp-cAMPS is a cell-permeable cAMP analog that specifically inhibits PKA by interacting with cAMP-binding sites on the regulatory subunits (39).

It seems contradictory that we observed that GLP-1 increased muscle microvascular perfusion via the PKA-dependent pathway but not PKA activity in the skeletal muscle. However, PKA activity did increase significantly in both heart and aorta after GLP-1 infusion. This is not surprising since skeletal muscle per se expresses much lower GLP-1 receptors than heart (8-fold less) and aorta (4-fold less) (17). Together

with our findings in cultured cells and ex vivo saphenous artery study, our findings are entirely consistent with a direct action of GLP-1 on its receptors to activate PKA in the vasculature.

In conclusion, GLP-1 recruits muscle microvasculature, which leads to expansion of the microvascular blood volume and increased glucose use in muscle through a process that involves PKA activation and NO production. Given that in the resting state only ~30% of muscle capillaries are perfused (22) and an increase in muscle endothelial surface area could significantly increase insulin delivery and action and substrate exchange in muscle (4, 12), GLP-1's microvascular actions in muscle may contribute significantly to postprandial glycemic control and diabetes complication prevention in diabetes.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Z.D., W. Chai, L.Z., and Z.F. performed the experiments; Z.D., W. Chai, W.W., L.Z., Z.F., and Z.L. analyzed the data; Z.D. edited and revised the manuscript; Z.D., W. Chai, W.W., L.Z., Z.F., W. Cao, and Z.L. approved the final version of the manuscript; W.W., W. Cao, and Z.L. interpreted the results of the experiments; Z.L. did the conception and design of the research; Z.L. prepared the figures; Z.L. drafted the manuscript.

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