Role of the nitric oxide pathway in AMPK-mediated glucose uptake and GLUT4 translocation in heart muscle

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Li, Ji, Xiaoyue Hu, Pradeepa Selvukumar, Raymond R. Russell III, Samuel W. Cushman, Geoffrey D. Holman, and Lawrence H. Young. Role of the nitric oxide pathway in AMPK-mediated glucose uptake and GLUT4 translocation in heart muscle. Am J Physiol Endocrinol Metab 287: E834–E841, 2004. First published July 20, 2004; doi:10.1152/ajpendo.00234.2004.—AMP-activated protein kinase (AMPK) is a serine-threonine kinase that regulates cellular metabolism and has an essential role in activating glucose transport during hypoxia and ischemia. The mechanisms responsible for AMPK stimulation of glucose transport are uncertain, but may involve interaction with other signaling pathways or direct effects on GLUT vesicular trafficking. One potential downstream mediator of AMPK signaling is the nitric oxide pathway. The aim of this study was to examine the extent to which AMPK mediates glucose transport through activation of the nitric oxide (NO)-signaling pathway in isolated heart muscles. Incubation with 1 mM 5-amino-4-imidazole-1-β-carboxamide ribofuranoside (AICAR) activated AMPK (P < 0.01) and stimulated glucose uptake (P < 0.05) and translocation of the cardiac myocyte-glucose transporter GLUT4 to the cell surface (P < 0.05). AICAR treatment increased phosphorylation of endothelial NO synthase (eNOS) ~1.8-fold (P < 0.05). eNOS, but not neuronal NOS, coimmunoprecipitated with both the α2 and α1 AMPK catalytic subunits in heart muscle. NO donors also increased glucose uptake and GLUT4 translocation (P < 0.05). Inhibition of NOS with Nω-nitro-l-arginine and Nω-methyl-l-arginine reduced AICAR-stimulated glucose uptake by 21 ± 3% (P < 0.05) and 25 ± 4% (P < 0.05), respectively. Inhibition of guanylate cyclase with ODQ and LY-83583 reduced AICAR-stimulated glucose uptake by 31 ± 4% (P < 0.05) and 22 ± 3% (P < 0.05), respectively, as well as GLUT4 translocation to the cell surface (P < 0.05). Taken together, these results indicate that activation of the NO-guanylate cyclase pathway contributes to, but is not the sole mediator of, AMPK stimulation of glucose uptake and GLUT4 translocation in heart muscle.

AMP-activated protein kinase; glucose transporter

AMP-activated protein kinase (AMPK) is a serine-threonine kinase that has an important role in the regulation of cellular metabolism (14, 21), ion channels (13), and gene expression (44). AMPK is activated by increases in the AMP/ATP ratio and, as such, is a key signaling pathway during cellular metabolic or energetic stress. AMPK was initially found to be an important regulator of fatty acid oxidation in heart (23, 24) and skeletal muscle (43) but has also emerged as an important mediator of glucose metabolism (37). Specifically, AMPK activation by 5-amino-4-imidazole-1-β-p-carboxamide ribofuranoside (AICAR) increases heart and skeletal muscle glucose uptake (15, 27, 36). AMPK also activates phosphofructokinase-2, which accelerates glycolysis (26). The importance of AMPK during hypoxic conditions is highlighted by recent findings that transgenic mice with deficient AMPK signaling have diminished glucose uptake in both the ischemic (35) and postischemic heart (35, 45) and hypoxic skeletal muscle (29).

The mechanisms through which AMPK modulates glucose uptake are only partially understood and may involve both acute and chronic changes in glucose uptake. AMPK increases glucose transport by stimulating translocation of GLUT4 to the sarcolemma in heart (36) and skeletal muscle (25). In Clone 9 cells, AICAR increases GLUT1 activity without a change in content or distribution (1). Long-term AMPK activation with AICAR also increases the expression of GLUT4 in skeletal muscle (17). However, AMPK is not necessary for GLUT4 expression in muscle tissues, as transgenic mice with AMPK deficiency do not have reduced GLUT4 content (29, 35, 45).

The downstream mechanisms through which AMPK mediates the acute activation of glucose transport remain uncertain. However, AMPK may potentially mediate its effect on glucose transport in part through interaction with the nitric oxide pathway. AMPK phosphorylates endothelial nitric oxide synthase (eNOS) on Ser1177 (7, 8, 28), leading to NOS activation in a calcium-independent fashion. AMPK also phosphorylates muscle neuronal NOS (nNOS) on Ser1451 (7), although the functional significance of this interaction remains uncertain. In addition, NOS inhibition with Nω-nitro-l-arginine (1-NAME) reduces AICAR stimulation of glucose transport in skeletal muscle (12), and recent findings indicate that 1-NAME also partially inhibits AICAR stimulation of muscle deoxyglucose uptake in the rat (40). However, the extent to which NOS modulates glucose uptake and GLUT translocation in heart muscle remains uncertain.

Nitric oxide produced in endothelial cells may have an important paracrine role to modulate myocyte metabolism and function in muscle tissues. Nitric oxide has pleiotropic effects, depending in part on its concentration, and both the metabolic and hemodynamic states of the heart (39). In skeletal muscle from eNOS knockout mice, there is diminished insulin-stimulated glucose uptake, indicating that insulin activation of nitric oxide may contribute to the stimulation of glucose transport (5, 10, 42). At the present time, the role of the nitric oxide pathway in mediating AMPK’s action in the heart is unknown.

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The purpose of this study was to determine whether AMPK activation of heart glucose transport and GLUT4 translocation is mediated in part through interaction with and activation of NOS. An isolated rat left ventricular papillary muscle preparation was utilized to assess these effects independently of the hemodynamic and systemic consequences of NOS inhibition. The results indicate that AMPK binds to and phosphorylates eNOS in heart muscle and that nitric oxide and its downstream cGMP pathway modulate in part AMPK activation of glucose uptake and GLUT4 translocation in heart muscle.

**MATERIALS AND METHODS**

**Animal preparations.** Male Sprague-Dawley rats weighing 250–350 g were allowed to have standard chow and water ad libitum before experiments. All procedures were approved by the Yale University Animal Care and Use Committee. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg). Anterior and posterior left ventricular papillary muscles (3–5 mg) were equilibrated in oxygenated phosphate-buffered saline (PBS) containing 1 mM MgCl₂, 1 mM CaCl₂, 5 mM glucose, and 1% BSA, as previously described (36). There was constant oxygen flow through the sealed muscle incubation containers, which were oscillated in a water bath at 37°C. Papillary muscles were then preincubated in buffer containing an inhibitor, or inhibitor vehicle as control, for 30 min before the addition of pharmacological activators for 30–60 min, as designated in results. NOS and guanylate cyclase inhibitors were used in optimally effective concentrations (33).

**Glucose transport.** In experiments designed to assess glucose transport, papillary muscles were incubated as above, and 2-deoxy-[1-³H]glucose (1 µCi/ml) was added during the final 30 min of incubation to measure the rates of glucose transport and phosphorylation. In addition, [U-¹⁴C]mannitol (0.1 µCi/ml) was added to measure the muscle extracellular space to correct for extracellular deoxyglucose (36). After incubations, muscles were washed in ice-cold PBS, blotted dry, weighed, solubilized, and counted by liquid scintillation. In addition, [U-¹⁴C]mannitol (0.1 µCi/ml) was added to measure the muscle extracellular space to correct for extracellular deoxyglucose (36). After incubations, muscles were washed in ice-cold PBS, blotted dry, weighed, solubilized, and counted by liquid scintillation (36).

**Glucose transporter surface labeling.** Cell surface (sarcolemma and T tubule) glucose transporters were photoaffinity labeled with the cell-impermeant compound 4,4′-O-[2-2-[2-2-[2-6 (bitionylaminohexanoyl)aminooxy]ethoxy]ethoxy]ethoxy]-4-(1-azi-2,2,2-trifluoroethyl)benzoyl]-aminol-1,3-propanediyl]bis-D-mannose (Bio-LC-ATB-BGPA), as previously described (14a, 38). In brief, after experimental incubations, muscles were labeled for 15 min in buffer containing 400 µM Bio-LC-ATB-BGPA at 4°C and then irradiated with ultraviolet light (300 nm) twice for 3 min to cross-link the label with glucose transport proteins. Pooled (3–4) labeled muscles (10–20 mg wet wt) were homogenized in buffer containing 250 mM sucrose, 1 mM EDTA, 20 mM HEPES, and 1 µg/ml of the proteinase inhibitors antipain, aprotinin, pepstatin, leupeptin, and 100 µM 4-(2-aminoethyl)benzenesulfonfonyl fluoride (pH 7.2). Cell membranes were prepared by ultracentrifugation (227,000 g for 50 min at 4°C) and solubilized in PBS containing 2% Thesit and proteinase inhibitors. Membranes underwent centrifugation at 30,000 g for 30 min, and the solubilized membrane proteins were precipitated with streptavidin-agarose (Pierce Chemical). The streptavidin-precipitated surface-labeled proteins were washed several times and then subjected to SDS-PAGE and immunoblotting with GLUT4 or GLUT1 antibody. The surface-labeled GLUT1 and GLUT4 contents were normalized to the amount of these proteins in the total cell membrane fraction.

**AMPK activity.** In experiments designed to assess AMPK activation, papillary muscles were frozen in liquid nitrogen, stored at −80°C, and subsequently homogenized in buffer containing 125 mM Tris, 1 mM EDTA, 1 mM EGTA, 250 mM mannitol, 50 mM sodium fluoride, 5 mM sodium pyrophosphate, 1 mM DTT, 1 mM benzamidine, 0.004% trypsin inhibitor, and 3 mM sodium azide, pH 7.5, at 4°C (9, 36). After centrifugation (13,200 g for 30 min), the supernatant underwent polyethylene glycol (PEG) precipitation, and the 2.5–6% fraction was used for measurement of total AMPK activity by use of the SAMS assay, as previously described (9, 36). Protein concentration was determined spectrophotometrically using the Bio-Rad reagent. The AMPK activity associated with specific catalytic subunits was examined following immunoprecipitation with polyclonal α₁ and α₂ antibodies, as previously described (9).

**AMPK-eNOS communoprecipitation.** After incubation with or without AICAR, heart muscles were homogenized in buffer containing 20 mM HEPES, 50 mM β-glycerol phosphate, 2 mM EGTA, 1 mM DTT, 10 mM NaF, 1 mM sodium orthovanadate, 1% Triton X-100, 10% glycerol, 0.1% PMSF, 0.1 µM leupeptin, and 10 ng/ml aprotonin at 4°C. After low-speed centrifugation, 400 µg of protein were immunoprecipitated with α₁ or α₂ polyclonal antibodies, or nonimmune IgG as a negative control (9). After extensive washing, the immunoprecipitates were resuspended in Laemmli buffer for immunoblotting with eNOS or nNOS antibodies.

**Immunoblotting.** Proteins were combined with Laemmli sample buffer prior to SDS-PAGE. After transfer to PVDF membranes, proteins were immunoblotted, detected with enhanced chemiluminescence, and quantified by densitometry of autoradiographs, as previously described (9). Immunoblots were performed with rabbit pol-α (α₁/α₂) AMPK antibody at 1:10,000 dilution (kind gift from Dr. M. Birnbaum), sheep anti-α₁ AMPK at 1:1,000 dilution (kind gift from Dr. D. G. Hardie), rabbit anti-pThr¹⁷² AMPK antibody at 1:5,000 dilution (Cell Signaling), rabbit anti-pSer¹⁷⁷ eNOS (Cell Signaling) at 1:1,000 dilution, mouse anti-eNOS (BD Transduction Laboratory) at 1:2,500 dilution, rabbit anti-pSer⁵¹⁶ nNOS (Upstate Biotechnology) at 1:2,000 dilution, and mouse anti-nNOS (Santa Cruz Biotechnology) at 1:1,000 dilution.

**Statistical analysis.** All data are reported as means ± SE. The number of experiments in each group is presented in the text, table, or figure legend. Data were analyzed by two-tailed, unpaired Student’s t-test. Differences were considered significant at P < 0.05.

**RESULTS**

**Effects of AICAR on AMPK activation.** We initially examined the effects of AICAR (1 mM) on AMPK activation in the isolated left ventricular papillary muscles. AICAR increased total AMPK activity threefold (P < 0.01) in PEG-precipitated muscle homogenates (Fig. 1A). Immunoblots with anti-pThr¹⁷² AMPK antibody showed that AICAR also increased AMPKα catalytic subunit phosphorylation at its key regulatory site, reflecting phosphorylation by the upstream AMPK activating protein kinase (Fig. 1B). Finally, AICAR stimulated AMPK activity in both α₁ (P < 0.05) and α₂ (P < 0.01) immunoprecipitates in the heart muscles (Fig. 1C).

**Effects of AICAR on glucose transport.** Incubation with AICAR stimulated heart muscle deoxyglucose transport (P < 0.05; Fig. 2A), confirming our previous results (36). To determine whether this increase in glucose transport was due to translocation of glucose transporters, we assessed cell surface GLUT4 and GLUT1 content with the cell-impermeant biotinylated BGPA compound (38). AICAR increased cell surface GLUT4 (P < 0.05) but did not affect the total cellular content of GLUT4, indicating that AMPK activation led to the glucose transporter translocation (Fig. 2B). AICAR also tended to increase cell surface GLUT1 (Fig. 2B).

**Effects of nitric oxide pathway activation on glucose uptake.** To the extent that AMPK mediates its effect on glucose transport through interaction with the nitric oxide pathway, one would anticipate that nitric oxide donors would stimulate glucose uptake and GLUT4 translocation in heart muscle.
Thus we evaluated whether AICAR stimulated phosphorylation of eNOS and/or nNOS in heart muscles. AICAR incubation for 30 min increased eNOS phosphorylation at pSer1177 by 1.8 ± 0.4-fold (P < 0.05; Fig. 4A). To determine whether AMPK binds directly to eNOS in heart muscles, we immunoblotted α1 and α2 AMPK immunoprecipitates with eNOS antibodies and found coimmunoprecipitation of eNOS with both the α1 and α2 catalytic subunits but no evidence of eNOS in control nonimmune IgG immunoprecipitates (Fig. 4B). These findings were consistent with direct phosphorylation of eNOS by AMPK within heart muscle (8). Although small amounts of nNOS were detectable in papillary muscles, there was no evidence that AICAR stimulated nNOS phosphorylation at Ser1416 or that there was binding of nNOS to AMPK (Fig. 4, C and D).

Effect of nitric oxide pathway inhibition on AICAR-stimulated glucose uptake. To determine the extent to which the nitric oxide pathway might contribute to the stimulation of heart muscle glucose transport by AICAR, muscles were incubated with NOS inhibitors before and during treatment with AICAR. Addition of 1 mM l-NAME (Fig. 5A) or 1 mM sodium nitroprusside (SNP) or L-arginine (Fig. 5B) again indicating glucose transporter translocation (Fig. 3B). Nitric oxide mediates its downstream effects in part through activation of soluble guanylate cyclase and the formation of cGMP, which activates the downstream cGMP-dependent protein kinase pathway (11, 39). To assess whether cGMP might activate heart glucose transport, muscles were incubated with the cell-permeant cGMP analog bromo-cGMP. This compound was also found to increase (P < 0.05) glucose uptake (Fig. 5C). Taken together, these results suggest that nitric oxide, or activation of its downstream cGMP pathway, leads to increased glucose uptake in heart muscle.

AICAR activation and interaction with eNOS. Phosphorylation of eNOS at Ser1177 by AMPK is known to increase its enzymatic activity (7, 8, 28). eNOS is highly expressed in heart capillary endothelial cells, but cardiac myocytes also contain small amounts of eNOS, nNOS, and inducible NOS (iNOS) (30, 39). AMPK is also known to phosphorylate nNOS, although the physiological consequences are uncertain (7, 12).
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Fig. 3. Activation of glucose transport by nitric oxide donors. A: deoxyglucose transport. Isolated heart muscles were incubated without (control) or with the nitric oxide donors sodium nitroprusside (SNP) or 5-nitroso-N-acetyl-L-penicillamine (SNAP) (0.1–10 μM) for 60 min (see MATERIALS AND METHODS). Results indicate the means ± SE for 3–5 experiments, with 2–4 independently analyzed muscles per experiment. *P < 0.05 vs. control. B: GLUT surface labeling. After control, SNP (1 μM), or SNAP (1 μM) incubations, surface GLUT4 and GLUT1 were labeled with Bio-LC-ATB-BGPA, isolated on streptavidin-agarose, and immunoblotted with specific GLUT4 or GLUT1 antibodies. Representative immunoblots are shown, and ratios of surface to total GLUT4 and GLUT1 are expressed as means ± SE for 3 experiments, including 2–3 pooled papillary muscles per experiment. *P < 0.05 vs. control. C: bromo (Br)-cGMP and deoxyglucose transport. Heart muscles were incubated for 60 min with 10 μM Br-cGMP, 1 μM SNP, or 1 μM SNAP (see MATERIALS AND METHODS). Results indicate means ± SE for 3 experiments, including 2–4 independently analyzed muscles per experiment. *P < 0.05 vs. control.

N\textsuperscript{ω}-methyl-L-arginine (1-NMMA; Fig. 5B) inhibited AICAR-stimulated glucose uptake by 21 ± 3% (P < 0.05) or 25 ± 4% (P < 0.05), which is consistent with the hypothesis that NOS has a role in mediating the effect of AMPK to stimulate heart glucose transport. NOS inhibitors had no effect on baseline glucose transport (Fig. 5), suggesting that nitric oxide production is not required, at least acutely, to maintain baseline glucose uptake in heart muscles.

To further distinguish whether AMPK activation of glucose transport is mediated through nitric oxide stimulation of guanylate cyclase or alternatively by non-cGMP-dependent mechanisms, we preincubated papillary muscles with the guanylate cyclase inhibitors 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 25 μM) or LY-83583 (10 μM) before stimulation with AICAR. Both ODQ and LY-83583 inhibited AICAR-stimulated glucose uptake by 31 ± 4% (P < 0.05) and 22 ± 3% (P < 0.05), respectively (Fig. 6, A and C). In addition, ODQ also blunted AICAR-stimulated GLUT4 translocation using the cell surface-labeling technique (Fig. 6B), indicating that downstream activation of guanylate cyclase has a role in mediating the AMPK effect on cardiomyocyte glucose transport. Guanylate cyclase inhibitors had no effect on baseline glucose transport (Fig. 6, A and C), suggesting that cGMP production is not required to maintain baseline glucose uptake in heart muscles.

DISCUSSION

The results of this study indicate that AMPK-mediated activation of the nitric oxide pathway plays a role in mediating the effects of AICAR to stimulate glucose transport and GLUT translocation in heart muscle. AICAR stimulated AMPK activity, which led to increased eNOS Ser\textsuperscript{1177} phosphorylation, as well as to stimulation of heart glucose uptake and translocation of the cardiomyocyte glucose transporter GLUT4. Although AICAR may have additional nonspecific effects, we have previously shown that AICAR-stimulated glucose transport in heart muscles was not inhibited by either phosphatidylinositol 3-kinase inhibitors or adenosine receptor blockers (36). Low concentrations of nitric oxide donors, as well as bromo-cGMP, were found to increase heart muscle glucose uptake and cell surface GLUT4, indicating that the NOS-cGMP pathway activates heart glucose transporter translocation. This hypothesis was supported by the observation that either NOS or guanylate cyclase inhibitors partially blocked AICAR-stimulated (but not basal) glucose uptake. Although these findings demonstrate that NOS and downstream guanylate cyclase activation contribute to the effects of AMPK, there was ~60–80% residual AICAR stimulation of glucose transport despite inhibition of the nitric oxide pathways, indicating that AMPK has additional important effects on glucose transport in heart muscle.

These results expand our understanding of the mechanisms through which AMPK stimulates glucose transport in the heart and are of interest in light of recent studies showing that AMPK has an important role in the activation of glucose transport in the ischemic heart. Transgenic mice expressing dominant negative α catalytic subunits of AMPK have a reduced glucose uptake response during both ischemia (35) and postischemic reperfusion (35, 45). AMPK deficiency also blocks hypoxia-stimulated glucose transport in skeletal muscle (29). AMPK is also known to be activated during exercise in both heart (9) and skeletal muscle (43), although it remains uncertain how important a role AMPK has in modulating glucose transport during exercise. In isolated skeletal muscle, contraction-stimulated glucose uptake is partially inhibited in AMPK-deficient dominant negative transgenic mice (29), but appears to be normal in muscles from α\textsubscript{2} knockout mice (19).
These latter findings suggest that additional redundant signaling pathways may exist that compensate for AMPK deficiency during contraction. Whether such mechanisms are simply recruited in transgenic mice as the result of developmental and/or chronic AMPK deficiency, or would be operative in normal muscle tissue with acute AMPK inhibition, remains uncertain.

The present results support the hypothesis that activation of the nitric oxide-guanylate cyclase pathway plays a role in mediating the effects of AMPK on glucose transport in heart muscle. These experiments were performed in isolated left ventricular papillary muscles, which contain a variety of cell types, including cardiac myocytes and endothelial cells. However, cardiac myocytes are the only cells in the heart which contain GLUT4 (47) and account for the bulk of glucose transport in heart because they also predominate by mass (46). Thus, taken together, the results from GLUT4 surface labeling and glucose uptake experiments indicate that the eNOS-guanylate cyclase pathway impacts on cardiomyocyte glucose uptake during AICAR stimulation.

We observed not only that AICAR increased eNOS Ser1177 phosphorylation but also that AMPK coimmunoprecipitated with eNOS, suggesting that AMPK directly activated eNOS. These findings are consistent with previous reports in intact heart and skeletal muscle (8, 41). Experiments in isolated mouse H-2Kb muscle cells (12) raised the possibility that AMPK interacts directly with eNOS in muscle cells. However, the current experiments, as well as those in heart and skeletal muscle (8, 41), do not directly define the extent to which AMPK activation of eNOS occurs in the endothelial cells, endocardial cells, or myocytes in the intact muscle tissue.

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Vascular endothelial cell eNOS is known to be activated by AICAR (18), and endothelial cells predominantly express the α1 isoform of AMPK (28). It is interesting in this regard that we found that AICAR activated the α1 isoform and bound to eNOS in the heart muscles, suggesting that AICAR activation of endothelial cell AMPK and eNOS may have an important role in the stimulation of cardiomyocyte glucose transport through a paracrine mechanism. However, additional immunoprecipitation experiments demonstrated that eNOS was also associated with α2 AMPK, the more predominant isoform in cardiac myocytes, which is virtually absent from endothelial cells (28). Although cardiac myocyte expression of eNOS is generally low, there is heterogeneity within the heart with greater eNOS expression in specialized endocardial cells that line the cardiac chambers (6, 30) and epicardial cardiomyocytes (4). Thus these findings suggest that both autocrine and paracrine mechanisms may be involved to some extent in the interaction between the AMPK, eNOS, and glucose transport pathways in heart muscle.

Heart and skeletal muscle cells also express nNOS (7, 12, 30, 39). In skeletal muscle, exercise activates AMPK and increases the phosphorylation of the muscle isoform of nNOS (nNOS-mu) on pSer1451 (7). The physiological effect of nNOS phosphorylation in skeletal muscle remains uncertain. We found a small amount of nNOS present in the heart papillary muscles, but there was no evidence that AICAR treatment increased nNOS phosphorylation or that nNOS was bound to either the α1 or α2 isoform of AMPK. Thus the results of these experiments suggest that AMPK interaction with the nitric oxide-cGMP pathway involves modulation of eNOS, rather than nNOS, in heart muscle.

Nitric oxide stimulates glucose transport in isolated skeletal muscle (2, 48), where it is thought to have a role in mediating both insulin (20, 22) and exercise-stimulated glucose uptake (34). In skeletal muscle and other tissues from eNOS knockout mice, there is diminished insulin-stimulated glucose uptake, indicating that insulin activation of NOS may contribute to the stimulation of glucose transport (5, 10, 42). In this study, we observed that relatively low concentrations of nitric oxide donors (0.1 μM) increased glucose uptake and stimulated GLUT4 translocation in isolated heart papillary muscles. The effects of nitric oxide on heart GLUT4 translocation have not been previously examined. Prior studies in working hearts have suggested that nitric oxide may activate fatty acid metabolism and inhibit overall glucose utilization (32), leading to the postulate that deficient eNOS activity in heart failure may reduce fatty acid oxidation and increase glucose metabolism (31). However, nitric oxide modulates cardiac contractility and oxidative metabolism in vivo, which may counterbalance the direct effects of nitric oxide to activate glucose transport. In the present experiments, the utilization of an isolated, quiescent papillary muscle preparation enabled us to examine more directly the role of nitric oxide and NOS inhibition on glucose transport and GLUT4 translocation. Although increased nitric oxide activates GLUT4 translocation, NOS inhibition experiments had no effect on basal glucose uptake, indicating that nitric oxide is not required to maintain basal glucose uptake in isolated heart muscles.

The findings that NOS inhibitors decrease AICAR-stimulated glucose transport in heart muscles are consistent overall with findings in skeletal muscle-derived mouse H-2Kb cells and isolated rat skeletal muscles (12), as well as in rat skeletal muscle in vivo (40). In isolated skeletal muscles, there was virtually complete inhibition of AICAR-stimulated glucose uptake by NOS inhibitors (12). In recent in vivo studies, L-NAME decreased AICAR-stimulated deoxyglucose uptake but also decreased basal uptake (40). There was partial (20–40%) inhibition of AICAR-stimulated glucose transport by NOS inhibitors in the present study in heart muscles. These results indicate that the majority of AMPK-mediated glucose transport in the heart is mediated either through additional downstream signaling pathways or by the direct effects of AMPK on GLUT4 vesicular trafficking. Although additional pathways that might mediate AMPK’s effects on glucose transport in heart are unknown, the phosphatidylinositol-3-kinase pathway, which plays an essential role in insulin signaling, does not appear to be involved (3, 16, 36). In addition, the partial inhibition of glucose transport and GLUT4 translocation seen with guanylate cyclase inhibitors is also consistent with prior studies in skeletal muscle cells (12). These findings indicate that guanylate cyclase is an important downstream mediator of glucose transport activation by nitric oxide, al-

Fig. 6. Guanylate cyclase inhibition and AICAR-stimulated deoxyglucose uptake. Isolated heart muscles were preincubated for 30 min with 25 μM ODQ (A) or 10 μM LY-83583 (C), before incubation with or without AICAR (1 mM) for 60 min (see MATERIALS AND METHODS). Results indicate means ± SE for 3 experiments, including 3–4 independently analyzed muscles per experiment. *P < 0.01 vs. control, †P < 0.05 vs. control, ‡P < 0.05 vs. AICAR. B: guanylate cyclase inhibition and AICAR-stimulated GLUT4 translocation. After control or AICAR incubation with or without ODQ (25 μM), cell surface GLUT4 was labeled with Bio-LC-ATB-BGPA, isolated on streptavidin-agarose, and immunoblotted with specific GLUT4 antibodies. Representative immunoblots are shown, and ratios of surface to total GLUT4 are expressed as means ± SE for 3 experiments, including 2–3 pooled muscles per experiment. *P < 0.05 vs. control, †P < 0.05 vs. AICAR.
though the mechanism by which cGMP activates glucose transport in muscle remains uncertain.

Thus these findings indicate that the nitric oxide-guanulate cyclase pathway partially contributes to the AMPK stimulation of glucose transport in heart muscle. The importance of this pathway in mediating the activation of glucose transport in the heart during exercise and ischemia is of interest for future studies.

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