Insulin Stimulates Glucose Transport Via Nitric Oxide/Cyclic GMP Pathway in Human Vascular Smooth Muscle Cells


Objective—In cultured human vascular smooth muscle cells, insulin increases cyclic GMP production by inducing nitric oxide (NO) synthesis. The aim of the present study was to determine whether in these cells the insulin-stimulated NO/cyclic GMP pathway plays a role in the regulation of glucose uptake.

Methods and Results—Glucose transport in human vascular smooth muscle cells was measured as uptake of 2-deoxy-D-[3H]glucose, cyclic GMP synthesis was checked by radioimmunoassay, and GLUT4 recruitment into the plasma membrane was determined by immunofluorescence. Insulin-stimulated glucose transport and GLUT4 recruitment were blocked by an inhibitor of NO synthesis and mimicked by NO-releasing drugs. Insulin- and NO-elicited glucose uptake were blocked by inhibitors of soluble guanylate cyclase and cyclic GMP–dependent protein kinase; furthermore, glucose transport was stimulated by an analog of cyclic GMP.

Conclusions—Our results suggest that insulin-elicited glucose transport (and the corresponding GLUT4 recruitment into the plasma membrane) in human vascular smooth muscle cells is mediated by an increased synthesis of NO, which stimulates the production of cyclic GMP and the subsequent activation of a cyclic GMP–dependent protein kinase. (Arterioscler Thromb Vasc Biol. 2003;23:2215-2221.)

Key Words: insulin signaling ▪ muscle, smooth, vascular ▪ glucose transport ▪ nitric oxide ▪ cyclic GMP

Insulin-stimulated glucose uptake is primarily mediated by the facilitative transporter GLUT4, which after hormonal stimulation is recruited from intracellular membranes to the cell surface, resulting in an enhanced glucose uptake (for review see Foster and Klip1). The precise mechanism by which insulin directs exocytosis of GLUT4-containing vesicles in different cell types has not been completely clarified. Multiple studies have suggested a necessary role of the insulin receptor–catalyzed tyrosine phosphorylation of insulin receptor substrate proteins and the subsequent activation of the p85/p110-type phosphatidylinositol 3-kinase (PI-3K).2 However, several lines of evidence suggest that insulin must generate additional and possibly separate signals to increase glucose transport, such as the activation of the small GTPase TC10,3 and the rearrangement of the microtubule- and actin-based cytoskeleton.4,5

Another cell signaling pathway that appears to markedly stimulate glucose uptake in skeletal muscle involves the nitric oxide (NO)/cyclic GMP (cGMP) pathway. NO stimulates glucose transport in isolated skeletal muscles and mediates in vivo exercise-induced glucose transport.6–8 Part of the in vivo mechanism involves the release of NO from the endothelium, causing enhanced blood flow and glucose delivery to the muscle.9–11 Recent data suggest that NO elicits glucose uptake in skeletal muscle through the activation of an AMP-activated protein kinase (AMPK), a mechanism that is distinct from both insulin and contraction signaling pathways.12,13

It is known that insulin has vasodilator actions in vivo that depend on endothelium-derived NO.14,15 Indeed, insulin stimulates production of NO in human endothelial cells in vitro,16 and the insulin receptor tyrosine kinase, PI-3K, and Akt all play significant roles in insulin-dependent production of NO in endothelium.17

It has been proposed that insulin-induced vasodilation is mediated, at least in part, by the stimulation of endothelial NO production, causing inhibition of contraction of the underlying vascular smooth muscle cells (VSMCs) via activation of the VSMC guanylate cyclase (GC). The relaxant effect exerted by insulin in VSMCs is not necessarily mediated by endothelial cells only. Actually, a direct effect of insulin on cultured VSMCs has been observed in vitro, showing that these cells are target of insulin action (for review, see Trovati and Anfossi18). Indeed, insulin elicits antiapoptotic signaling in VSMCs by inducing rapid tyrosine phosphorylation of the insulin receptor and IRS-1, followed

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by a 3-fold increase of IRS-1–bound PI-3K and a robust enhancement of Akt-3 phosphorylation and activity down-stream of PI-3K. In addition, it has been reported that in cultured VSMCs, insulin increases (1) cGMP production via a NO-dependent mechanism and (2) inducible NOS expression and cGMP generation via the PI-3K pathway.

We recently demonstrated that human VSMCs (hVSMCs) express an endothelial-type, calcium/caldesmon-dependent NO synthase (NOS) and exhibit an increased production of both NO and cGMP in response to the calcium ionophore ionomycin. Insulin stimulates hVSMC calcium-dependent NOS activity in a few minutes, and insulin-induced NO and cGMP production is completely blunted by the NOS inhibitor N ω-nitro- L-arginine methyl ester (L-NAME).

The aim of the present study was to determine whether in hVSMC insulin-derived NO plays any role in the regulation of one of the most important effects of insulin, ie, its ability to increase the rate of cellular glucose transport. We additionally investigated which signaling events are required for NO to act as a regulator of insulin-mediated glucose uptake.

Methods

Materials
Dulbecco’s modified Eagle’s medium (DMEM), FCS, and other tissue culture products were obtained from Gibco BRL; plastic for cell culture was from Falcon (Becton Dickinson). 2-deoxy- D-glucose, cytochalasin B, 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ), 8-bromo guanosine-3′,5′-cyclic 1 monophosphate (8-Br-cGMP), sodium nitroprusside (SNP), 5-nitrosoglutathione (GSNO) were obtained from Sigma Chemical. Human recombinant insulin and 8-bromo guanosine-3′,5′-cyclic monophosphorothioate, Rp-isomer (Rp-8-Br-cGMPs) were from Calbiochem-Novabiochem Corporation. 2-Deoxy-D-[ 3 H]glucose (6.1 Ci/mmol) was from NEN Calbiochem-Novabiochem Corporation, and proteins, separated by SDS-PAGE (12%), were transferred to polyvinylidene difluoride filter membrane (Immobilon P, Millipore) and probed with rabbit polyclonal antibodies (diluted 1:500 in PBS-BSA 1%) anti-human GLUT4 (Santa Cruz Biotechnology; catalog no. sc-7938) and anti-human cGMP-dependent protein kinase (PK) Ia (Calbiochem, VWR International; catalog no. 370652), or with the mouse monoclonal antibody 16C2 (1:2000), specific for vasodilator-stimulated phosphoprotein (VASP) phosphorylated at serine 239 (Upstate, D.B.A.; catalog no. 05-611). After an overnight incubation, the membrane was washed with PBS-Tween 0.1% and subjected to a peroxidase-conjugated antibody (diluted 1:1000 in PBS-Tween with Blocker nonfat dry milk 5%, Bio-Rad) anti-rabbit or anti-mouse IgG (donkey; Amersham International; catalog no. NA934V and NA931V). The polyvinylidene difluoride membrane was washed again with PBS-Tween, and proteins were detected by enhanced chemiluminescence (Amersham International).

Immunofluorescence Studies
Cells were grown on polylysine-coated glass coverslips. At the end of the different incubation periods in the presence of agonists and/or inhibitors, cells were rinsed once with glucose-free HEPES-buffered saline solution (mmol/L: 140 NaCl, 20 HEPES/Na, 5 KCl, 2.5 MgSO 4, and 1 CaCl 2 at pH 7.4) and were subsequently incubated in the same buffer for 8 minutes with 10 μmol/L 2-deoxy-D-glucose containing 0.3 μCi/mL 2-deoxy-D-[ 3 H]glucose. Under these conditions the glucose uptake was linear for at least 20 minutes (data not shown). Uptake was stopped by rapid removal of the buffer, followed by 3 washes in ice-cold PBS. Cells were disrupted by adding 50 mmol/L NaOH, and the radioactivity associated with the cells was determined by scintillation counting. Protein concentrations were determined by the BCA kit from Pierce, and the uptake was expressed in pmol 2-deoxy-D-glucose·min -1·mg -1 cell protein. Uptake values were corrected for the noncarrier-mediated transport by measuring hexose uptake in the presence of 10 μmol/L cytochalasin B (a potent inhibitor of facilitated glucose transport). The nonspecific association of deoxy-glucose with the cells was typically <10% of the total uptake.

cGMP Determination
At the end of a 45-minute incubation in 35-mm Petri dishes in the presence of agonists and/or inhibitors, the medium was removed, and 300 μL of absolute ethanol was added to make the cells permeable. After ethanol was completely evaporated, 300 μL of Tris-EDTA buffer (50 mmol/L Tris-HCl, 4 mmol/L EDTA at pH 7.5) was added to each dish. After a 10-minute equilibration period, 100 μL of supernant was tested for the cGMP level with a [ 3 H]cGMP assay kit provided by Immuno Biological Laboratories, and the results were expressed as picomoles cGMP per milligram cell proteins. For the cGMP assay, the discrimination was 0.05 pmol/mL, and the cross-reactivity with cAMP was <0.001%.

Western Blot Analysis
Cells were directly solubilized in boiling Laemmli buffer containing the protease inhibitor cocktail set III (mmol/L: 100 AEBSF, 0.08 aprotinin, 5 benzatin, 1.5 E-64, 2 leupeptin, and 1 pepstatin; Calbiochem-Novabiochem Corporation), and proteins, separated by SDS-PAGE (12%), were transferred to polyvinylidene difluoride filter membrane (Immobilon P, Millipore) and probed with rabbit polyclonal antibodies (diluted 1:500 in PBS-BSA 1%) anti-human GLUT4 (Santa Cruz Biotechnology; catalog no. sc-7938) and anti-human cGMP-dependent protein kinase (PK) Ia (Calbiochem, VWR International; catalog no. 370652), or with the mouse monoclonal antibody 16C2 (1:2000), specific for vasodilator-stimulated phosphoprotein (VASP) phosphorylated at serine 239 (Upstate, D.B.A.; catalog no. 05-611). After an overnight incubation, the membrane was washed with PBS-Tween 0.1% and subjected to a peroxidase-conjugated antibody (diluted 1:1000 in PBS-Tween with Blocker nonfat dry milk 5%, Bio-Rad) anti-rabbit or anti-mouse IgG (donkey; Amersham International; catalog no. NA934V and NA931V). The polyvinylidene difluoride membrane was washed again with PBS-Tween, and proteins were detected by enhanced chemiluminescence (Amersham International).

Glucose Transport Assay
At the end of the different incubation periods in the presence of agonists and/or inhibitors, cells were rinsed once with glucose-free HEPES-buffered saline solution (mmol/L: 140 NaCl, 20 HEPES/Na, 5 KCl, 2.5 MgSO 4, and 1 CaCl 2 at pH 7.4) and were subsequently incubated in the same buffer for 8 minutes with 10 μmol/L 2-deoxy-D-glucose containing 0.3 μCi/mL 2-deoxy-D-[ 3 H]glucose. Under these conditions the glucose uptake was linear for at least 20 minutes (data not shown). Uptake was stopped by rapid removal of the buffer, followed by 3 washes in ice-cold PBS. Cells were disrupted by adding 50 mmol/L NaOH, and the radioactivity associated with the cells was determined by scintillation counting. Protein concentrations were determined by the BCA kit from Pierce, and the uptake was expressed in pmol 2-deoxy-D-glucose·min -1·mg -1 cell protein. Uptake values were corrected for the noncarrier-mediated transport by measuring hexose uptake in the presence of 10 μmol/L cytochalasin B (a potent inhibitor of facilitated glucose transport). The nonspecific association of deoxy-glucose with the cells was typically <10% of the total uptake.

Aerobic Consumption of Glucose
After a 45-minute incubation in the absence or presence of NO donors, the cells were washed, detached, and checked for the rate of mitochondrial respiration by measuring 14CO 2 evolution from [6-14C]glucose, as previously described.

Gluconeogenic Flux
Aerobic glucose consumption was determined by measuring 14CO 2 evolution from [6-14C]glucose, as previously described.

Immunostaining Studies
The immunostaining procedure was as follows: the cells were fixed in 10% formalin for 20 minutes at room temperature, blocked in 1% normal goat serum, and incubated overnight at 4°C with a primary antibody (diluted 1:200 to 1:500 in PBS-BSA 1%). After 4 washes, the cells were incubated for 1 hour with a secondary antibody (diluted 1:500 in PBS-BSA 1%) and a fluorochrome-labeled secondary antibody (diluted 1:500 in PBS-BSA 1%). After a washing step, the cells were examined with a confocal laser scanning microscope equipped with an Argon laser (488/568 nm). All images were taken with the same scanning setting, and fluorescence intensity average of each image was evaluated.
was quantified by Scion Image Software (Scion Corporation) in 20 randomly chosen cellular fields. Fluorescence intensity was expressed as arbitrary units and plotted as a graph.

Statistical Analysis
All data in text and figures are provided as mean±SEM. Statistical analysis has been carried out by means of Student’s t test.

Results
Insulin Stimulates Glucose Transport and a Rapid Increase of GLUT4 at the Cell Surface in hVSMCs: Both Are Mediated by NO
hVSMCs were incubated with different concentrations of insulin for 45 minutes before the assay of 2-deoxy-D-glucose transport (Figure 1, left), and with 100 nmol/L insulin for different time periods up to 1 hour (Figure 1, right). Maximal stimulation of 2-deoxy-D-glucose uptake, corresponding to an ∼1.7- to 1.8-fold increase over basal, was achieved after a 45-minute exposure to 100 nmol/L insulin: this experimental condition was then used in the subsequent experiments. Cytochalasin B decreased basal glucose uptake by >90% (data not shown), which confirmed that glucose uptake resulted from a specific carrier-mediated transport.

To investigate the role of NO in insulin-stimulated glucose transport, hVSMCs were incubated with insulin in the presence of the NOS inhibitor L-NAME, which completely abolished the insulin-mediated 2-deoxy-D-glucose transport (Figure 2). Moreover, the NO donor GS-NO elicited a 2-fold increase in 2-deoxy-D-glucose transport (Figure 2). A similar effect was evoked by SNP (Figure 2). As the mitochondrion has been proposed to be one of the primary cellular targets of the cytotoxic action of NO (for review see Wink and Mitchell27), after exposure to GS-NO and SNP, the cells were permeabilized in order to stain the cell surface only. In the absence of insulin, few transporter molecules were detected on the cell surface; after brief exposure to insulin, the GLUT4 content of the plasma membrane increased (Figure 3) 3.3-fold. The incubation with insulin in the presence of the NOS inhibitor L-NAME reverted the insulin effect (Figure 3). The NO donor GS-NO exerted a remarkable insulin-like effect on GLUT4 subcellular distribution (3.9-fold increase in the plasma membrane), which was not influenced by L-NAME coincubation (Figure 3).

GC and PKG Are Involved in the Insulin-Stimulated Glucose Transport of hVSMCs
When the cells were incubated with insulin in the presence of ODQ, a selective inhibitor of soluble GC (sGC), the insulin-stimulated 2-deoxy-D-glucose uptake was abolished (Figure 4). The inhibitory effect of ODQ was reversed by the coinubcation with the membrane permeable cGMP analog 8-Br-cGMP (Figure 4), which, per se, was a potent inducer of 2-deoxy-D-glucose uptake (Figure 4). Also the GS-NO-elicited 2-deoxy-D-glucose uptake was inhibited by ODQ (Figure 4). We had already shown23 that in hVSMCs, insulin induces a NO-dependent cGMP increase. The stimulating effect of insulin on the cGMP intracellular concentration (Figure 5) was mimicked by SNP and GS-NO (Figure 5).
keeping with the observations on glucose transport (Figure 4), the insulin-, SNP- and GS-NO–induced cGMP increase was completely inhibited by ODQ (Figure 5).

When hVSMCs were incubated with insulin in the presence of Rp-8-Br-cGMPS, a selective inhibitor of PKG, the insulin-stimulated 2-deoxy-ß-glucose uptake was abolished (Figure 6, top). The inhibitor blocked also the activating effect of 8-Br-cGMP on 2-deoxy-ß-glucose uptake (Figure 6, top). The incubation with the PKG inhibitor also resulted in a significant reduction of basal 2-deoxy-ß-glucose transport (Figure 6, top). hVSMCs expressed predominantly PKG Iα, as checked by Western blotting experiments (data not shown). So far, we looked for a direct measurement of PKG activation elicited by insulin, NO donor, and cGMP analog in hVSMCs. The functional effects of PKG are thought to be owing to the phosphorylation of specific target proteins, including VASP, the 46/50-kDa vasodilator-stimulated-phosphoprotein, which is expressed in VSMCs and phosphorylated at serine 239 by PKG.26,28 VASP phosphorylation at serine 239, measured with the monoclonal antibody 16C2,

Figure 3. Confocal microscopy analysis of the GLUT4 transporter concentration at the hVSMC plasma membrane. Cells were incubated for 45 minutes in the absence (ctrl) or presence of 1 mmol/L L-NAME (L-name), 100 nmol/L insulin (ins), 100 nmol/L insulin + 1 mmol/L L-NAME (ins/L-name), 25 µmol/L GS-NO (gs-no), and 25 µmol/L GS-NO + 1 mmol/L L-NAME (gs-no/L-name); GLUT4 was detected as indicated in the Methods section. The figures in the upper panel are representative of 3 similar experiments, giving superimposable results. In the graph the fluorescence intensities, expressed as arbitrary units, are presented as mean±SEM (n=3). *P<0.0001 vs control (ctrl); O P<0.0001 vs insulin (ins).

Figure 4. Role of GC in the insulin- and GS-NO–stimulated 2-deoxy-ß-glucose uptake of hVSMCs. Cells were incubated for 45 minutes in the absence (ctrl) or presence of 100 nmol/L insulin (ins), 100 nmol/L insulin+50 µmol/L ODQ (ins/odq), 100 nmol/L insulin+50 µmol/L ODQ followed by a further 45 minutes incubation with 1 mmol/L 8-Br-cGMP (ins/odq) br), 1 mmol/L 8-Br-cGMP (br), 25 µmol/L GS-NO (gs-no), and 25 µmol/L GS-NO +50 µmol/L ODQ (gs-no/odq), and 2-deoxy-ß-glucose transport was assayed as described in the Methods section. Data are presented as mean±SEM (n=8). *P<0.002, **P<0.001, and ***P<0.0001 vs control (ctrl); O P<0.001 versus insulin (ins); ◇ P<0.001 vs insulin+ODQ (ins/odq); ▲ P<0.001 vs GS-NO (gs-no).

Figure 5. cGMP synthesis in hVSMCs after addition of insulin and NO donors. Cells were incubated for 45 minutes in the absence (ctrl) or presence of 100 nmol/L insulin (ins), 100 nmol/L insulin+50 µmol/L ODQ (ins/odq), 25 µmol/L SNP (snp), 25 µmol/L SNP+50 µmol/L ODQ (snp/odq), 25 µmol/L GS-NO (gs-no), and 25 µmol/L GS-NO+50 µmol/L ODQ (gs-no/odq), and cGMP concentration was determined as described in Methods. Data are presented as mean±SEM (n=6). *P<0.002 vs control (ctrl); O P<0.0001 vs insulin (ins), SNP (snp), and GS-NO (gs-no).
hVSMCs owing to its ability to increase endogenous NO production via activation of NOS, as the NOS inhibitor L-NAME completely blocks both insulin-mediated effects. In addition, different NO donors (GS-NO and SNP) behave as insulin mimetics and effectively enhance both glucose transport and GLUT4 translocation in hVSMCs, bypassing the inhibitory effect of L-NAME. These results suggest that in hVSMCs, NO plays an important role in both insulin-dependent relaxation/vasodilation and glucose uptake. VSMCs have metabolic properties that make them peculiarly sensitive to glucose transporter expression and activity: glucose transport is rate-limiting for glucose metabolism in VSMCs,34 as GLUT4 is nearly or completely saturated at most physiological concentrations of glucose.35

To understand further the mechanism whereby NO regulates insulin-mediated glucose transport in hVSMCs, we examined the contribution of GC and cGMP in the insulin effect. NO is known to activate sGC, which is the predominant intracellular NO receptor in VSMCs (for review, see Lincoln and Cornwell36), leading to increased levels of cGMP. We have already shown23 that in hVSMCs, insulin induces a significant enhancement of NO production and a NO-dependent cGMP increase. Our present results show that ODQ, which renders sGC insensitive to activation by NO,37 abolishes the insulin- and the GS-NO-stimulated glucose uptake, and the insulin-, SNP- and GS-NO-induced cGMP increase. So far, both insulin and NO donors require NO-dependent cGMP production to stimulate glucose transport in hVSMCs. Indeed, the membrane permeable cGMP analog 8-Br-cGMP reverses the ODQ inhibitory effect and is a potent inducer of glucose uptake per se.

Our results suggest that the role of NO/cGMP signaling in glucose transport is different between smooth muscle and skeletal muscle. Indeed, in rat skeletal muscle and mouse H-2K b muscle cells,12 the increase in glucose transport is completely blocked by inhibition of NOS only under those conditions that lead to increased AMPK activity, and inhibitors of NOS have no effect on the increase in glucose transport caused by stimuli, such as insulin, that do not activate AMPK. Our data show that in human vascular smooth muscle, the increase in glucose transport is promoted by insulin via a NOS- and GC-dependent mechanism.

We next examined the contribution of PKG in the insulin- and cGMP-stimulated glucose transport of hVSMCs. PKG regulates smooth muscle relaxation, platelet aggregation, cell growth, and differentiation (for review, see Pfeifer et al38). The expression of the type I PKG, and particularly the Iα isoform, predominates in VSMCs39 and decreases as VSMCs are passaged in vitro.40 Our results show that in cultured hVSMCs, which express predominantly PKG Iα (data not shown), the stimulating effects of insulin and cGMP on glucose transport require the cGMP-mediated PKG activation, as the pretreatment of hVSMCs with a selective inhibitor of PKG completely blocks the glucose uptake elicited by both insulin and 8-Br-cGMP. VASP, a protein expressed in vascular smooth muscle, is specifically phosphorylated at serine 239 by PKG.28 Insulin elicits the NO/cGMP-dependent activation of PKG, as VASP phosphorylation at serine 239 is significantly increased by insulin, and this effect is almost
completely abolished by the inhibition of sGC and PKG. So far, PKG plays a central role in the insulin-dependent rapid stimulation of glucose transport in hVSMCs, which occurs via the ability of insulin to promote the translocation of intracellular vesicles containing GLUT4 to the plasma membrane. This observation is of importance as in VSMCs, the molecular mechanisms of cGMP signaling distal to PKG I are not yet completely understood and are at present actively investigated. Several targets for PKG have been implicated in mediating the enzyme effects (for review, Piefer10). It is assumed that at least part of the inhibitory responses mediated by PKG depends on the phosphorylation of the inositol triphosphate receptor and on the stimulation of the large conductance K-Ca channel.30 Furthermore, vasorelaxation by NO/cGMP is also associated with an increase in the extent of phosphorylation of small proteins such as heat shock–related proteins that appear to be regulatory components of the actin-based cytoskeleton and seem to interact with intermediate filaments.41 A functional role of the microtubule and actin cytoskeletal systems in the mechanism of insulin action on GLUT4 traffic is hypothesized.4,5 Indeed, GLUT4 translocation has been found to be sensitive to actin depolymerization, and disruption of actin microfilaments results in the localization of GLUT4 in larger, almost immobile vesicles in the juxtanuclear region.42 Actin motors likely coordinate the interaction of GLUT4-enriched vesicles with microtubules, and both actin-based and microtubule-based motors are targets for PKG.43 A very important role of PKG in insulin-induced relaxation of VSMCs has been recently demonstrated.44 The contractile effect of RhoA in VSMCs results from the activation of a Rho-dependent kinase, which phosphorylates the regulatory subunit of the myosin light chain phosphatase (MBP) and thereby inhibits the phosphatase activity. Insulin inhibits Rho signaling by decreasing RhoA translocation via the NO/cGMP/PKG signaling pathway to cause MBP phosphorylation of small proteins such as heat shock proteins. Moreover, it is assumed that at least part of the inhibitory responses mediated by PKG depends on the phosphorylation of the inositol triphosphate receptor and on the stimulation of the large conductance K- Ca channel.30 Furthermore, vasorelaxation by NO/cGMP is also associated with an increase in the extent of phosphorylation of small proteins such as heat shock–related proteins that appear to be regulatory components of the actin-based cytoskeleton and seem to interact with intermediate filaments.41 A functional role of the microtubule and actin cytoskeletal systems in the mechanism of insulin action on GLUT4 traffic is hypothesized.4,5 Indeed, GLUT4 translocation has been found to be sensitive to actin depolymerization, and disruption of actin microfilaments results in the localization of GLUT4 in larger, almost immobile vesicles in the juxtanuclear region.42 Actin motors likely coordinate the interaction of GLUT4-enriched vesicles with microtubules, and both actin-based and microtubule-based motors are targets for PKG.43 A very important role of PKG in insulin-induced relaxation of VSMCs has been recently demonstrated.44 The contractile effect of RhoA in VSMCs results from the activation of a Rho-dependent kinase, which phosphorylates the regulatory subunit of the myosin light chain phosphatase (MBP) and thereby inhibits the phosphatase activity. Insulin inhibits Rho signaling by decreasing RhoA translocation via the NO/cGMP/PKG signaling pathway to cause MBP phosphorylation via site-specific dephosphorylation of the regulatory subunit. PKG Iα has also been shown to phosphorylate and inactivate RhoA, blocking Rho kinase–dependent inhibition of MBP in VSMCs.44

Our results about the PKG dependence of the insulin- and cGMP-stimulated glucose transport (and even of the basal glucose transport) in hVSMCs indicate that the major role exerted by endogenous NO in both insulin-dependent relaxation/vasodilation and glucose uptake is mediated by PKG activation. Interestingly, pretreating cells with the PKG inhibitor resulted in a significant reduction of basal 2-deoxy-D-glucose transport, suggesting that PKG activity is needed for basal glucose transport. So far, physiopathological events in which NO has been shown to exert an effect have grown exponentially: such effects include now the insulin-stimulated glucose transport in hVSMC.

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References


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